DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF CANINE RESPIRATORY AFFECTIONS

Thesis

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

MASTER OF VETERINARY SCIENCE
in
VETERINARY MEDICINE
(Minor Subject: Veterinary Surgery and Radiology)

By

Jasleen Kaur
(L-2017-V-40-M)

Department of Veterinary Medicine
College of Veterinary Science

Ludhiana-141 004

2019

CERTIFICATE – I

This is to certify that the thesis entitled, "**DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF CANINE RESPIRATORY AFFECTIONS**" submitted for the degree of **M.V.Sc.** in the subject of **Veterinary Medicine (Minor subject: Veterinary Surgery and Radiology)** of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, is a bonafide research work carried out by **Jasleen Kaur (L-2017-V-40-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

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

(Dr. Swaran Singh Randhawa)
Major Advisor
Professor
Department of Veterinary Medicine,
COVS, Guru Angad Dev Veterinary and
Animal Sciences University, Ludhiana,
141004 (India)

CERTIFICATE – II

This is to certify that the thesis entitled "**DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF CANINE RESPIRATORY AFFECTIONS**" submitted by **Jasleen Kaur (L-2017-V-40-M)** to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in the partial fulfillment of the requirements for the degree of **M.V.Sc.** in the subject of **Veterinary Medicine** (Minor subject**: Veterinary Surgery and Radiology)** has been approved by Student's Advisory Committee after an oral examination on the same, in collaboration with an external examiner.

(Dr. Swaran Singh Randhawa)
Major Advisor

(Dr. Jaswinder Singh Soodan)
External Examiner
Professor-cum-Head
Division of Teaching Veterinary Clinical Complex
Faculty of Veterinary Sciences & A.H.
SKUAST, R.S. Pura, Jammu–181 102

(Dr. Charanjit Singh Randhawa)
Head of the Department

(Dr. Sanjeev Kumar Uppal)
Dean, Postgraduate Studies
Guru Angad Dev Veterinary and Animal Sciences University
Ludhiana, Punjab

ACKNOWLEDGEMENT

Fore mostly with folded hands, profound sense of obligations, I bow my head with reverence and dedicatedly award my recondite gratitude to the 'ALMIGHTY', for showering immense blessings upon me due to which I could be able to accomplish the academic endeavour. I take this humble opportunity to express my sincere and heartfelt gratitude to my esteemed ***Major Advisor Dr. Swaran Singh Randhawa, Professor, Department of Veterinary Medicine,*** *for his invaluable guidance, constructive suggestions, persistent encouragement and continuous moral support throughout the course of this study and, above all, for inculcating in me a spirit of scientific research. With all sincerity, I bestow my deep sense of appreciation and gratitude to him, for his expert planning, sagacious supervision, unflinching help and prudent and valuable suggestions.*

It is my sole prerogative to express my sincere thanks and gratefulness to ***Dr. C.S. Randhawa*** *(Dean PGs Nominee), Professor-cum-Head, Department of Veterinary Medicine and* ***Dr. Sanjeev Kumar Uppal****, Dean Post Graduate Studies, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India.*

I extend my special thanks to the members of my advisory committee ***Dr. Sushma Chhabra, Professor, Department of Veterinary Medicine*** *and* ***Dr. Shashi Kant Mahajan****, Professor, Department of Veterinary Surgery and Radiology for their invaluable suggestions, constructive counsel and help.*

I convey my sincere obligation to the faculty members of the department ***Dr. B.K, Bansal, Dr. Kirti Dua, Dr. Shukriti Sharma, Dr. S.T. Singh, Dr. Neetu Saini , Dr. D. K, Gupta, Dr. Sujata Turkar*** *and* ***Dr. Randhir Singh*** *for their full cooperation and assistance.*

I am highly thankful to ***Dr. Raj Sukhbir Singh,*** *Associate Professor, Department of Teaching Veterinary Clinical Complex for his untiring help with Statistics for data analysis of my research work. I am greatly indebted to* ***Dr. Asmita Narang,*** *Assistant Professor, Department of Veterinary Medicine for her help and supportive guidance.*

I would also like to thank ***Dr. Kuldip Gupta,*** *Professor, Department of Veterinary Pathology and* ***Dr. Arun Anand,*** *Professor, Department of Veterinary Surgery and Radiology and* ***Dr. Mudit Chandra,*** *Professor, Department of Veterinary Microbiology for help and technical input provided for this work.*

My special thanks to my seniors, ***Dr. Shabnam Sidhu, Dr.Darshan, Dr.Iqra, Dr. Rajnish, Dr. Sheetal, Dr. Harneet,*** *my batch mates* ***Kanu, Ishan, Omran, Nitika, Kanchan, Pallavi, Kiran, Preeti, Shubham, Zinnia*** *and* ***Supriya*** *for their wholehearted support, help and encouragement during the course of the study. I cannot end this section without mention of* ***Dr. Gurpreet Singh Preet, Dr. Puneet Singh*** *and* ***Dr.Lakhan Sachdeva*** *without whose help I would have never been able to complete this task.*

It is a pleasure to mention my best friends ***Drs. Isha, Gurleen, Harnoor, Gulgul, Harleen, Rajapartap****,* ***Harsimran*** *and* ***Aman*** *for their invaluable suggestions, support to get through the difficult times and for creating a fun-filled, pleasant atmosphere for me here. I convey my special acknowledgement to* ***Dr(s). Ravneet*** *and* ***Chan*** *for helping me in my research,*

for all the emotional support during the stressful and difficult moments and encourage me to work harder.

I am ever indebted to my father ***Dr. Jatinder Pal Singh*** *and mother* ***Mrs. Balvinder Kaur*** *who deserve special mention for their indissoluble support, love, prayers and sacrifices for educating and preparing me for my future. My brother* ***Mr. Manvir Singh*** *is heartily appreciated for his love, wishes and moral support during this challenging phase of my life. I am fortunate and lucky to have the ceaseless encouragement and love from my family members specially my grandparents.*

I am indebted to ***Mr. Pritpal Singh, Mr. S P Singh and Mrs. Harpreet Kaur****Lab technician and nonteaching staff of our department* ***Mini Mahajan, Chotelalji, Ganga Prasad, Anita Sharma, Kewalji, Baljinderji*** *and* ***Satinder Pal*** *and office staff members for their support and help.*

At last, I express my sympathy to all those affected dogs to which I dealt and treated for the sake of science and knowledge.

I duly acknowledge and appreciate ***Mr. Gurdeep Singh (Alps)*** *for making the manuscript presentable.*

Before I finish, I offer my regards and blessings to all of those people who supported me and offered me their gracious help in any respect during the completion of this research work; with my sincere apology, if I was not able to mention them all individually.

Place: Ludhiana

Date: ***(Jasleen Kaur)***

Title of the Thesis	**:**	Diagnosis and therapeutic management of canine respiratory affections
Name of the student	**:**	Jasleen Kaur
Admission No.	**:**	L-2017-V-40-M
Major Subject	**:**	Veterinary Medicine
Minor Subject	**:**	Veterinary Surgery and Radiology
Name and Designation of Major Advisor	**:**	Dr. Swaran Singh Randhawa Professor
Degree to be Awarded	**:**	Master of Veterinary Science
Year of award of Degree	**:**	2019
Total Pages of Thesis	**:**	139 + VITA
Name of University	**:**	Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004, Punjab, India.

ABSTRACT

Forty dogs with clinical signs of respiratory disease, presented to small animal clinics of the university were included in the study. Ten clinically healthy dogs without any evidence of respiratory disease were kept as healthy controls. Thorough physical examination was carried out in all the animals. Blood (5 ml) was collected via cephalic vein for hemato-biochemistry. Thoracic radiography (right lateral and ventro-dorsal view) was performed in all the animals. Ultrasonography of chest was undertaken wherever required. Transtracheal wash (TTW) was collected for cytological examination, bacterial isolation and culture sensitivity testing. Arterial blood was collected for blood gas analysis. Faecal examination was done by faecal floatation method in all diseased dogs. Transtracheal wash (TTW) method was standardized using disposable 14gauge over the needle cannula and 4 FG dog tube via cricothyroid ligament. Out of 40 patients, 4 were diagnosed for upper respiratory tract affections and 36 with the lower respiratory tract affections. Squamous cell carcinoma was common nasal cavity tumour present in 3 dogs having history of epistaxis. Allergic rhinitis was confirmed in one dog based on absolute eosinophilia on haematology and nasal swab cytology. Chronic bronchitis is most common respiratory affection followed by non-specific interstitial pneumonia, bacterial bronchopneumonia and lung neoplasia/nodular lung pattern, anthracosis and eosinophilic bronchopneumopathy (EBP). Chronic bronchitis (CB) was diagnosed primarily, on the basis of bronchial pattern on thoracic radiography along with bronchial thickening and multiple donuts. TTW cytology revealed increased cellularity along with moderate increase in neutrophils, presence of mucus and hyperplastic epithelial cells in all CB dogs. Bacterial bronchopneumonia was confirmed by mixed interstitial and alveolar pattern on thoracic radiography and markedly increased cellularity and percentage of degenerative and non-degenerative neutrophils in TTW cytology. Histiocytic sarcoma, bronchioloalveolar carcinoma and pulmonary abscessation were diagnosed in one case each, based on FNAC, along with undiagnosed nodular interstitial pneumonia in 3 dogs. Interstitial lung diseases were recognised in 12 dogs, including anthracosis in 2, EBP in 1 and non-specific interstitial pneumonia in 9 dogs.TTW cytology in anthracosis dog revealed increased number of neutrophils and macrophages with engulfed blackish pigment and lymphoid cells; and severe eosinophilia in EBP. Rare presence of giant cells in TTW fluid was also noticed in ILDs. Overall, a five-fold increase in neutrophils were appreciated in TTW ($P<0.05$) of diseased dogs as compared to healthy animals. Absolute neutrophilia, hyperfibrinogenemia ($P<0.05$) and hypoxemia were the most common findings in dogs with respiratory infections. *Staphylococcus aureus, E. Coli* and *Klebsiella* sp. are the organisms isolated from the TTW fluid in majority of the dogs. Treatment with Amoxicillin and Clavulanic acid combination @ 15 mg/kg PO or IM q12h for first 3 days followed by antibiotic selected on the basis of CST up to 2 weeks along with bronchodilators terbutaline @ 1.25-5 mg/dog PO q8-12h, was found effective in chronic bronchitis and interstitial lung diseases, however, prognosis was guarded in lung tumours, undiagnosed nodular interstitial pneumonia and bacterial bronchopneumonia dogs.

Keywords: Bronchopneumonia, bronchitis, cytology, dog, radiography, squamous cell carcinoma, TTW fluid

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

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

%	:	Per cent
<	:	Less than
±	:	Plus minus
Alb	:	Albumin
AnGap	:	Anionic gap
APPs	:	Acute phase proteins
BAC	:	Bronchioloalveolar carcinoma
BAL	:	Bronchioalveolar lavage
BE	:	Base excess
BHI	:	Brain heart infusion
BID	:	Bis in a day
BP	:	Bacterial bronchopneumonia/pneumonia
bpm	:	Beats per minute
CBC	:	Complete Blood Count
CCB	:	Canine chronic bronchitis
CH^+	:	Concentration of hydrogen ions
Cl^-	:	Chlorine
DLC	:	Differential Leukocyte Count
DV	:	Dorsoventral
E.coli	:	*Escherichia coli*
EBP	:	Eosinophilic Bronchopneumopathy
EDTA	:	Ethylenediaminetetraacetic Acid
EMB	:	Eosin methylene blue
et al	:	and co worker
Fig.	:	Figure
FNAC	:	Fine needle aspirate cytology
g	:	Gram
g/dl	:	Gram per decilitre
g/l	:	Gram per liter
Hb	:	Haemoglobin
HCO_3	:	Bicarbonate
HPF	:	High power field
HS	:	Histiocytic Sarcoma
i.e.	:	That is
ICS	:	Intercostal Space
IIPs	:	Idiopathic interstitial pneumonias
ILDs	:	Interstitial lung diseases
IM	:	Intramuscular
IPF	:	Idiopathic pulmonary fibrosis
IU	:	International Unit
IU/l	:	International Units Per litre
IV	:	Intravenous
K^+	:	Potassium
kg	:	Kilogram

L	:	Litre
meq/l	:	Milliequivalents per litre
mg	:	Milligram
mg/dl	:	Milligram per decilitre
min	:	Minute
ml	:	Millilitre
MLA	:	MacConkey's lactose agar
mmHg	:	millimeter of mercury
mmol/l	:	Millimoles per litre
n	:	Number
Na^+	:	Sodium
NSIP	:	Non-specific interstitial pneumonia
^{o} C	:	Degree Celsius
OD	:	Once a day
^{o}F	:	Degree Fahrenheit
$PaCO_2$	:	Partial pressure of carbon dioxide in arterial blood
PaO_2	:	Partial pressure of oxygen in arterial blood
PCV	:	Packed Cell Volume
pH	:	Potential of hydrogen
PMN	:	Polymorphonuclear neutrophils
RBC	:	Red Blood Cells
RT	:	Rectal temperature
SAS	:	Statistical Analysis System
SCC	:	Squamous cell carcinoma
SD	:	Standard deviation
SE	:	Standard Error
SO_2	:	Oxygen saturation
Spp.	:	Species
$stHCO_3$	:	Standard biocarbonate
TB	:	Total Bilirubin
tCO_2	:	Total CO_2
TEC	:	Total Erythrocyte Count
TLC	:	Total Leukocyte count
TNCC	:	Total nucleated cell count
TP	:	Total Protein
TTW	:	Transtracheal wash
TW	:	Tracheal wash
USG	:	Ultrasonography
VD	:	Ventrodorsal
vs	:	Versus
WBC	:	White Blood Cells
µg/dl	:	Microgram per decilitre
µg/ml	:	Microgram per millilitre
µmol/l	:	Micromole per liter

CHAPTER – I

INTRODUCTION

Dogs are performing a wide variety of roles for humans including sheep herding, sniffing out drugs and explosives, hunting of prey, security, breeding purpose and companionship. Respiratory infection is a common clinical problem in dogs especially those housed in pet shops, breeding and boarding kennels, shelters, research facilities or veterinary clinics.

The respiratory system is one of the four major systems of the body. It consists of the upper airways (larynx, pharynx and trachea) and the lower airways (bronchi, bronchiole and alveoli). The invasion of the respiratory tract by the harmful pathogens is normally prevented by physical, chemical and immunologic barriers including mucus and mucociliary clearance, various innate antimicrobial factors, alveolar macrophages and the pulmonary immune response. The whole respiratory tree is lined by ciliated epithelial cells which actively move the overlying blanket of mucus by a ciliary beat toward oropharynx and esophagus. Only the bronchioles are not lined with cilia, instead they contain non-ciliated granular secretary cells called *Clara cells*, which play an important role in detoxification of foreign substances. The main goal of respiratory system is to provide oxygen to the tissues and to remove carbon dioxide. To fulfill this goal, respiration is divided into four major functions which includes pulmonary ventilation, diffusion of oxygen and carbon dioxide between the alveoli and the blood, transport of oxygen and carbon dioxide in the blood and body fluids to and from the body's tissue cells and regulation of ventilation and other factors of respiration (Reece 2004, Guyton and Hall 2006). The pulmonary alveoli are the principle sites for gas diffusion between the air and the blood. The alveoli are very delicate structures, susceptible to injury if defense mechanisms are impaired (Vegad and Katiyar 2004). This indicates a need for an emergency treatment for respiratory diseases.

Constant exposure of the respiratory tract to the infectious agents, that can reach the respiratory tract through aerogenous or hematogenous route, makes the respiratory system vulnerable to injury. The continuous passage of large volume of air into the lungs leads to the vulnerability of the respiratory system to the aerogenous injury. Irrespective of whether upper or the lower respiratory tract is affected, the host

response will vary depending on the severity of infection, its pathogenesis and immune status of the host. Consequently, the morphological changes will vary from mild circulatory changes such as mucosal or pulmonary congestion/edema to severe mucosal or pulmonary inflammation. These pathological changes will impair the normal homeostasis and manifest clinically as respiratory dysfunction.

A variety of syndromes such as rhinitis, sinusitis, tracheal collapse, infectious tracheobronchitis, bacterial and viral pneumonia, lung lobe torsion and sinonasal tumor have been observed in dogs. Clinical signs in chronic respiratory diseases range from dyspnoea, costal or abdominal respiration, cough, nasal discharge and congestion, edema, consolidation of lungs, lethargy and weight loss (Ayodhya *et al* 2013). Coughing is an important physiological function used to expel harmful substances, such as foreign bodies, mucus or debris, from the airways and preserve the normal health of the respiratory tract (Mazzone 2005). Cough can be elicited by stimulation of coughing receptors present in the larynx, trachea or bronchi, which are not there in smaller bronchi, bronchioles and alveoli, where the luminal flow would also be too low to generate enough shear forces to clear airway mucus and debris (Widdicombe 2003). In a way, pulmonary edema should never be an expected cause of cough until severe fluid accumulation is there, producing a soft moist cough accompanied by blood-tinged sputum (Farzan 1990). On the whole, respiratory signs can be vague, varying from mild unproductive cough to severe pneumonia accompanied with systemic changes (Vieson *et al* 2012). Other non-infectious causes of chronic cough in dogs include airway collapse and inflammatory airway diseases, such as chronic bronchitis and eosinophilic bronchopneumopathy.

Diseases of the nasal cavity and paranasal sinuses characteristically cause nasal discharge, sneezing, facial deformity, stertor, lethargy, inappetence, weight loss and rarely, central nervous system signs. Brachycephalic breeds are more predisposed to upper respiratory tract (URT) disorders due to stenotic nares, enlarged tonsils, elongated soft palate, everted laryngeal saccules, narrowed rima glottides, collapse of the larynx and tracheal hypoplasia (Roedler *et al* 2013). Though, rhinitis is a primary inflammatory disease but chronic nasal disease can arise from fungal infection, neoplasia, remnant foreign body or oral disease (Cooke 2005). Common diagnoses in canine nasal diseases are the inflammatory and neoplastic diseases (Pietra *et al* 2010).

Tracheal collapse is a progressive disease of middle-aged, small breed dogs. Clinical signs are proportional to the degree of collapse, ranging from mild airway irritation and paroxysmal "goose-honking" coughing to respiratory distress and dyspnoea (Tappin 2016).

Infectious tracheobronchitis (ITB) is an acute contagious respiratory disease in dogs affecting the larynx, trachea, bronchi and occasionally the lower respiratory tract. Multiple agents have been reported as potential aetiological agents (Buonavoglia and Martella 2007). Viral-bacterial co-infections are well supported by the epidemiological data and the laboratory studies. It has been concluded that respiratory viruses predispose to the development of secondary bacterial infections by destroying the respiratory epithelium and facilitating bacterial adhesion, up-regulating expression of molecules that act as receptors for bacteria and inducing immunosuppression thus promoting bacterial infection (Peltola and McCullers 2004, Joseph *et al* 2013). Diagnosis of bacterial tracheobronchitis is usually based on the presence of acute or chronic cough and a positive bacterial culture in BAL fluid in the absence of alveolar consolidation on thoracic radiographs as well as other concurrent respiratory diseases. Negative bacterial culture and absence of intracellular bacteria in BAL fluid was the criteria for inclusion of dogs with chronic bronchitis, eosinophilic bronchopneumopathy and canine idiopathic pulmonary fibrosis (Viitanen *et al* 2014). About 80% of sinonasal tumors, occurring in older dogs of more than 8 years, are malignant and carry a poor long-term prognosis (Wilson and Dungworth 2002).

Bacterial pneumonia is characterized by colonization of the airways or pulmonary parenchyma with bacteria, resulting in exudation and lung consolidation. Common causes of bacterial pneumonia include aspiration of gastrointestinal tract contents, decreased ciliary function and infection with opportunistic pathogens secondary to immune-suppression (Ford 2009). The common bacteria involved in respiratory infections in dogs and cats include *Bordetella bronchiseptica, Escherichia coli*, *Klebsiella*, *Streptococcus* and *Staphylococcus* species. Severe respiratory infections were seen in *Staphylococcus* spp. involvement, which sometimes become fatal in pups (Adaszek *et al* 2009, Attili *et al* 2012 and Ayodhya *et al* 2013). The opportunistic infections of the lower respiratory tract were observed in viral or parasitic infection, inflammation, trauma, aspiration, neoplasia, systemic immuno-

deficiency or any other cause leading to impaired local defence (Vieson *et al* 2012). Aspiration pneumonia is a serious life-threatening inflammatory lung process. Pathologic damage to the lungs leads to ventilation-perfusion mismatch and hypoxemia. Eosinophilic bronchopneumopathy (EBP) is characterized by eosinophilic infiltration of lung and bronchial mucosa that was referred as pulmonary infiltrates with eosinophilia (PIE) earlier (Clercx *et al* 2000).

Lung lobe torsion is the rotation of a lung lobe along its long axis with twisting of the bronchovascular pedicle at the hilus. Right middle lobe has been seen most affected in dogs due to its long narrow shape and loose attachment to the mediastinum, thoracic wall and adjacent lobes. Male dogs of pug breed are seen more predisposed to develop lung lobe torsion (Murphy and Brisson 2006).

The diagnosis in chronic respiratory diseases is always challenging. In veterinary practice, evaluation of animals for respiratory diseases is limited to chest auscultation, complete blood count, serum biochemistry, thoracic radiography, arterial blood gas analysis, fecal examination, nasal swabs culture, bronchoscopy, transtracheal wash and BAL fluid cytology and culture. However, in India, transtracheal wash and BAL fluid cytology and culture and bronchoscopy are hardly used. The results of nasal swabs and sputum culture are not much reliable as they included nasal and oropharyngeal contamination. Thoracic radiographs provides important information related to cardiac size, gross chamber abnormalities, alteration in the size and appearance of great vessels, abnormalities of the chest wall, pleural space and the lung itself particularly the bronchi, interstitial spaces and the alveolar tissues (Fox 2007). Radiographs help in differentiating primary cardiac disease from respiratory problems in coughing dogs (Spier 2011). Though, radiography is a good diagnostic tool but has less utility for soft tissue pathology and requires expertise. The trachea and main stem bronchi constitute a central site of exposure to all inhaled materials and addition of central airway sampling could enhance the confirmation of airway inflammation (Zhu *et al* 2015). Tracheal wash aspiration is the minimally invasive procedure which allows the blind sampling of the larger airways for cytologic and culture analysis (Finke 2013). The predominant normal cells in transtracheal wash fluid are respiratory epithelial cells and macrophages (Cowell *et al* 1989).

The diversity of microbial organisms that colonizes/invades the respiratory tract during disease process poses great challenge during its therapeutic management. In emergency situations the selection of antimicrobial agents for the first line treatment must be based on empirical data on bacterial prevalence and then based on the culture sensitivity results (Ford 2009). Very few studies are available on respiratory tract affections of dogs in India (Ayodhya *et al* 2013, Patil 2014). The need of the time to elucidate the etiology of respiratory affections in dogs presented to the university hospital. In addition, diagnostic protocols need to be standardized to undertake specific treatment of different respiratory affections. There is utmost need to add transtracheal wash to the diagnostic protocol for respiratory disorders. So the present study was planned with the following objectives:

1. Identification of different respiratory affections in dogs.
2. Therapeutic management of different respiratory affections in dogs.

CHAPTER – II

REVIEW OF LITERATURE

Respiratory infection is a common clinical problem in dogs. Disease is comparatively more frequent in dogs kept in breeding kennels, pet shops, shelters and research facilities. Very young and older dogs have higher risk of respiratory infections as compared to healthy adult dogs. The syndrome may vary from merely rhinitis, sinusitis to tracheal collapse, infectious tracheobronchitis, bacterial and viral pneumonia, lung lobe torsion and sinonasal tumors. Clinical signs usually include laboured breathing and coughing however, they may range from dyspnoea, costal or abdominal respirations, cough, nasal discharge and congestion, edema to consolidation of lungs, lethargy and weight loss. Till date, diagnosis had been based on chest auscultation, haemato-biochemistry, radiography, arterial blood gas analysis, fecal examination and nasal swab culture in India. Now, the advanced techniques like transtracheal wash, bronchoalveolar lavage and bronchoscopy are being explored for specific identification of the aetiology. In this review, we are particularly focussing on the diagnosis and therapeutic management of the canine respiratory affections.

2.1 Anatomy of the respiratory tract in dogs

The upper airway consists of respiratory structures that include nasal cavity, pharynx and larynx (Schwartz *et al* 1994). The respiratory tract begins with the nares which provide the external openings for the paired nasal cavities. Its main function is to humidify, filter and warm the inspired air. Both the nasal cavities are separated by the nasal septum and each of the cavities contains the mucosa covered turbinate bones known as conchae. The caudal portion of each of the nasal cavity contains the olfactory epithelium.

The pharynx begins at the level of the choana and is a common pathway for respiratory as well gastrointestinal system. The overlapping function of the pharynx elucidates the relatively common occurrence of the aspiration pneumonia.

The larynx is a complex musculo-cartilaginous structure which is cranially bounded by the arytenoid cartilages, vocal folds (dorsal and lateral) and the epiglottis (ventral) and caudally by the thyroid and cricoid cartilages. It provides primary protection against the aspiration of food, water, secretions, or other debris into the

trachea and lower airways. During swallowing the rima glottis is usually protected by the folding over of the epiglottis and by the adduction of the arytenoid cartilages (Hedlund 1997, Harpster 2004). The larynx also functions as an organ of phonation in cats and dogs. Mineralization of the laryngeal cartilages on radiography of larynx in clinically normal dogs is taken as the reference to describe the variations within the normality (Gaskell 1974).

Trachea is a semirigid flexible tube that connects larynx to the bronchi and consists of 35 to 45 C-shaped cartilages (Ettinger and Feldman 2010). These cartilages are attached by rings of an elastic annular ligament. Free ends of the C-shaped tracheal rings are joined dorsally by trachealis muscles (Reece 2004) and their contraction regulates the diameter of the trachea. Bifurcation of trachea takes place at the level of the fourth or fifth thoracic vertebra. A continuous division of trachea creates many subdivisions from largest to smallest including bronchi, bronchioles, terminal bronchioles, respiratory bronchioles, alveolar duct, alveolar sac and alveoli. The trachea is bifurcated into the right and the left principal bronchi, which have similar structure as that of the trachea except the cartilage rings are complete and smaller. Each of the bronchus enters into its associated lung and divides into the smaller secondary bronchi. Each bronchi divides into the smaller and smaller bronchioles within the lung tissue. Bronchioles are not kept open by the cartilage rings but are lined with the ciliated mucous membrane. This arrangement makes 'the bronchial tree' (Aspinall 2004).

2.1.1 The lungs and the pleura

Lungs are the principal structures of the respiratory system occupying the thorax. The expansion of lungs along with the expansion of the thorax provides the airflow into the lungs. The lungs appear pale pink and feel firm and spongy when they are inflated with air and become dark red when they are collapsed or consolidated. Both lungs slightly differ in the shape and size. The right lung is larger than the left lung and is consisted of an apical or cranial lobe, a cardiac lobe, a caudal or diaphragmatic lobe and a small accessory lobe lying in between both the lungs. The left lung is made up of cranial (further divided into apical and cardiac) and diaphragmatic lobes. In lungs, the smallest bronchioles lead into respiratory bronchioles which are lined with ciliated mucous membranes and each divides further

to form two or three smaller alveolar ducts ending into alveolar sacs or alveoli, which have a grape-like appearance. The pulmonary membrane forms the alveoli and alveolar ducts (Aspinall 2004).

Pleura, a smooth serous membrane provides the friction free movement of the lungs within the thorax. This membrane consists of a single layer of cells which are fused to the surface of a connective tissue layer. The visceral pleura envelop both the lungs and the costal pleura provide its lining on the inner thoracic wall. The mediastinal space is the space between the respective visceral pleural layers as they ascend towards the dorsal wall. This space contains the vena cavae, thoracic lymph duct, oesophagus, aorta and trachea. The pressure changes in the intrapleural space are accompanied with the similar changes in the mediastinal space because they both are intimately associated (Reece 2015).

The blood supply to the lungs is known as pulmonary circulation. Each alveolus is surrounded by the capillary networks. The branches of pulmonary artery brings deoxygenated blood from the right ventricle of the heart to the lungs and the capillaries which leads away from the alveoli, in a network of the larger vessels, ultimately combines to form the pulmonary vein**,** which drains the oxygenated blood into the left atrium of the heart (Aspinall 2004).

The main sites of gas exchange are the respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli (Guyton and Hall 1996). The exchange of O_2 and CO_2 occurs by the means of passive diffusion generated by a pressure gradient. The conditions leading to hypoxemia include low inspired oxygen fraction, hypoventilation, thickening of the respiratory barrier and ventilation perfusion disparity (West 1990, Rozanski and Chan 2005). Shunting of pulmonary blood occurs in conditions like atelectatic lung and alveolar edema when ventilation is not enough to fully oxygenate the blood flowing through the alveolar capillaries (Miller 2007). Physiologic dead space occurs in conditions like pulmonary thromboembolism (PTE) and congenital cardiac shunts, when alveolar ventilation is normal but there is low blood flow to the alveoli, leading to inadequate oxygenation of the alveolar blood (Miller 2007).

A two to three fold increase in the thickness of the respiratory barrier either due to edema or fibrosis of the alveolar interstitium impairs the O_2 diffusion. Oxygen in blood is mostly carried in bound form and only 3 percent of O_2 is present in the

dissolved state. CO_2 in blood is carried out by different chemical forms including HCO_3 (70%), CO_2 (7%) and CO_2 bound to haemoglobin (23%). The lungs play an important role in acid-base regulation through the diffusion of CO_2 out of the respiratory tract (Guyton and Hall 1996, Rose and Post 2001).

2.1.2 The Thoracic cavity

Thoracic cavity is one of the three body cavities and is outlined by the cranial thoracic inlet, sternum, thoracic vertebrae and the ribs. The diaphragm, which is a musculo-tendinous structure projecting cranially into the thoracic cavity like a dome, fills the caudal thoracic inlet. It attaches to the last few ribs, to the xiphoid cartilage of sternum and to the ventral surface of the first few lumbar vertebrae. It contains three foramina, which allows the structures such as the oesophagus, caudal vena cava and the aorta to run between the thorax and the abdomen.

The movement of the diaphragm is controlled by the phrenic nerve, which arises from the cervical spinal cord and runs down the neck. The sides of thoracic cavity are formed by the ribs. Between the ribs are the two layers of the intercoastal muscles. The external intercostals (the outer layer) are thicker of the two muscles and runs from the caudal border of each rib in a caudoventral direction. During respiration, the external intercoastal muscle contracts and lift the ribcage outwards, thus bringing inspiration. The internal intercostals are thinner and runs from the cranial border of each rib in a cranioventral direction and they co assist in expiration, mainly a passive process. Intercostal nerves from the spinal cord innervate the both sets of muscles (Aspinall 2004).

2.2 Diseases affecting upper respiratory tract in dogs

Approximately 1 percent of the all canine tumours are intranasal with comparatively more incidence in dolichocephalic breeds and dogs from urban areas (Rief *et al* 1998, Wilson and Dungworth 2002). Among the all sinonasal tumors, one third are the sarcomas whereas two third are carcinomas (Wilson and Dungworth 2002, Turek and Lana 2007). The average age of the dogs with sinonasal tumors is between 8.7 to 10.7 years (older dogs) (Wilson and Dungworth 2002). Common clinical signs in sinonasal tumours include epistaxis, nasal discharge (hemorrhagic or mucopurulent), sneezing, dyspnea, stertor, ocular discharge and neurological signs in case of metastasis of tumor into the cribriform plate (Olgilvie and Moore 2006, Turek

and Lana 2007). Squamous cell carcinoma, adenocarcinoma and the undifferentiated carcinoma are the common intranasal tumors in dogs (Lana and Withrow 2001). Lana and Withrow (2001) stated that after the diagnosis of nasal carcinoma with no further treatment, the mean survival time was 3 to 6 months due to progression of local disease. Elliot and Mayer (2009) concluded that sinonasal tumors in dogs are rarely cured but the median survival time can be significantly improved by radiation therapy and is the treatment of choice. In other study conducted by Lana and Withrow (2001) radiation therapy was found the most effective treatment available.

Chronic inflammatory rhinitis is often present in dogs with chronic nasal disease and is mainly characterized by the lymphoplasmacytic infiltrates in the nasal mucosa and absence of any obvious etiological process (Windsor and Johnson 2006). Common clinical signs in this disease are nasal discharge, sneezing, coughing, epistaxis and stertor.

Infectious rhinitis can be caused by bacterial, viral and mycotic infections. Primary bacterial rhinitis is very rare in dogs (Roudebush 1990, Nelson and Couto 2014). It occurs mostly as a secondary complication to viral, parasitic or fungal rhinitis, nasal trauma or foreign body, nasal aspiration of food or liquid, neoplasia, dental disease, oronasal fistula or bacterial bronchopneumonia. The most abundant virus in the respiratory exudates of the infected patients is canine distemper virus (CDV) that often spreads by aerosol exposure (Greene and Appel 1990). Other viruses involved in canine viral rhinitis include adenovirus, reovirus, herpesvirus and influenza virus. Focal areas of necrosis are commonly seen in the nasal, tracheal and the bronchial mucosa as these viruses are epitheliotropic (Lane 1977). Clinical signs are usually minimal with the uncomplicated viral infections. *Aspergillus* and *Penicillium* species (Mathews 2004) are the fungal causes of canine rhinitis, although canine nasal aspergillosis is seen more frequent (Peeters and Clercx 2007). Dolichocephalic and mesocephalic breeds are found to be more susceptible to the infection, while brachycephalic breeds are very rarely affected (Zonderland *et al* 2002, Peeters *et al* 2005). All age dogs between 1 and 7 years are commonly affected, however disease is uncommon in dogs less than 1 year of age and older patients (Peeters and Clercx 2007).

Allergic rhinitis is associated with a hypersensitivity reaction to the airborne antigens present in the nasal cavity and sinuses. Clinical signs include sneezing, nasal

congestion and serous or mucopurulent nasal discharge which can be seasonal, continuous or intermittent and/or acute or chronic (Nelson and Couto 2014). Allergic rhinitis was reported in an adult male Afghan hound with history of recurrent epistaxis, which responded very well to the hyposensitization therapy with an alum-precipitated antigenic extract (McDougal 1977). Improvement in the clinical signs after the removal of the suspected antigen is suggestive of this disorder. The nonspecific inflammation is the most common finding on rhinoscopic examination whereas eosinophilic inflammation is usual in nasal mucosal biopsy (Nelson and Couto 2014).

Parasitic rhinitis is rare in dogs and the common nasal parasites include mites (*Pneumonyssoides caninum)* and nematodes (*Capillaria boehmi*). Dogs affected with nasal mites can be asymptomatic but sometimes can exhibit clinical signs such as sneezing, pawing at the nose, reverse sneezing, chronic nasal discharge, facial edema, pruritus and epistaxis. Diagnosis is usually done by rhinoscopic visualization or by examination of the material obtained from nasal flushing. *Capillaria boehmi* lives on the nasal and the frontal sinus mucosa. Clinical signs exhibited by the affected dogs are sneezing, mucopurulent discharge and occasional epistaxis. Diagnosis is made by identification of the parasite eggs on fecal flotation and the adult parasites on rhinoscopy (King *et al* 1990, Nelson and Couto 2014).

Nasal foreign bodies can be inhaled via external nares or through the posterior choanae following gagging or vomiting episodes. Plant materials like twigs, grass awns and thorns are the most common type of foreign bodies. Penetrating foreign bodies can lodge in the nasal cavity and are rare. Clinical signs commonly include an acute onset of paroxysmal sneezing, head shaking, pawing at the nose and acute unilateral serous or purulent nasal discharge or the epistaxis (Rudd and Richardson 1985). Nasal foreign bodies can be diagnosed on basis of acute onset of clinical signs, nasal radiography or rhinoscopy (Sullivan 1987). For diagnosis of nasal foreign bodies, CT scan was found more sensitive and specific than the radiographic studies (Johnson and Wisner 2007).

2.3 Diseases affecting lower respiratory tract in dogs

Most common form of lower airway disease (LAD) reported in dogs is chronic bronchitis. In majority of cases, the cause of LAD remained unproven. The primary

symptom of LAD in case of dogs and cats is chronic cough. Diagnosis is mainly based on the detailed history, physical examination and diagnostic tests used to rule out the other causes of cough and dyspnea such as pneumonia, congestive heart failure and heartworm infestation. More advanced tests, such as bronchoscopy, radioisotope ventilation scans and flow volume loops are available to indicate the extent of the disease process. Glucocorticoids are mainly used as chronic therapy for most dogs and cats having LAD. Bronchodilators are mainly indicated for use in most of cats with symptoms of acute bronchoconstriction, whereas a few number of dogs may respond to bronchodilator administration and indicate an increase in exercise capacity and a decrease in cough frequency. LAD is a progressive disorder in dogs and cats and prognosis is mostly guarded. However, with use of aggressive medical management, most of these animals can live a relatively symptom free lives (Padrid 1992).

Chronic bronchitis (CB) in dogs is defined as chronic inflammatory pulmonary disease resulting in cough and can also lead to exercise intolerance and respiratory distress. Clinical signs can vary from mild to severe, whereas the most severe cases mainly result in respiratory failure (Rozanski 2014). Range of findings can be obtained on auscultation of the lungs which include normal to harsh lung sounds, crackles or the expiratory wheezes (Nelson and Couto 2014). Narrowing of airway lumen, developing due to airway thickening and excessive mucus production and accumulation, result in increased airway resistance in dogs with chronic bronchitis. Bronchial thickening and increased donuts and tram lines are observed on thoracic radiographs (Kumrow and Rozanski 2012). In chronic bronchitis dog, transtracheal wash (TTW) cytology usually reveals a primarily neutrophilic infiltrate along with excessive mucus whereas only small numbers of lymphocytes, goblet cells, eosinophils, ciliated cells and epithelial cells and inconsistent number of alveolar macrophages are observed (Kumrow and Rozanski 2012, Rozanski 2014).

Canine infectious tracheobronchitis (ITB) (kennel cough) is an acute contagious respiratory disease affecting the larynx, trachea, bronchi and occasionally the lower respiratory tract and the parenchyma. Clinical manifestations shown by affected dogs range from very mild to severe and also reflect the localization of infection to the respiratory mucosa (Priestnall *et al* 2014, Cave *et al* 2015). ITB have been described in two clinical forms. The uncomplicated mild form is mainly

characterized by dry hacking cough, but it is often linked with expectoration and gagging. Recovery generally occurs within 5-14 days as disease is self-limiting (primary viral infection of the trachea and the bronchi). Mainly sporadic complicated cases of more severe forms had been reported but outbreaks of more serious disease can also be seen. In severely affected or immune-compromised dogs, secondary bacterial bronchopneumonia can develop and also the acute severe hemorrhagic forms had been explained, which may be associated with one or two single pathogens (Cave *et al* 2015). The principal pathogens causing kennel cough include *Bordetella bronchiseptica*, canine parainfluenza virus (PI) and canine adenovirus type-2 (CAV-2). Although, vaccinated dogs also commonly develop clinical signs of kennel cough, which can arise due to vaccine failure, new strain of the organisms (present in the vaccine) causing infection or involvement of the new pathogens that are not included in the presently available vaccines. Other pathogens causing kennel cough include bacteria that can cause either primary infections or act as secondary "opportunistic" pathogens and mainly include *Pasturella multocida* and *Streptococcus zooepidemicus* (Priestnall *et al* 2014). Viruses involved in the aetiology of kennel cough other than PI and CAV-2, include the canine herpes virus, canine influenza virus and canine respiratory corona virus (Cave *et al* 2015). Priestnall *et al* (2014) also recorded role of canine pneumovirus, pantropic canine corona virus, canine bocavirus and canine hepaci virus and *Mycoplasma cynos* bacteria in the development of canine infectious respiratory disease (CIRD) also known as kennel cough.

Coughing can be differentiated on the basis of body weight as animal will be normal or obese with the respiratory diseases and thin or weight loss with the cardiac diseases (Atkins 2001). Two most common forms of chronic trachea-bronchial diseases in the dogs are tracheobronchial collapse and chronic bronchitis. Tracheal collapse is defined as the progressive, dorsoventral flattening of the tracheal lumen. The highest incidence of tracheal collapse was found in middle-aged, small-breed dogs (Yorkshire terrier, Pomeranian, Toy poodle, Chihuahua and pug) (Sura and Durant 2012) but also has been reported in large breed dogs. Aetiology of tracheal collapse is unknown but considered to be a combination of environmental and genetic factors (Sura and Durant 2012). In affected dogs, on histological examination, cartilaginous rings were found hypocellular, with decreased glycoprotein and glycosaminoglycan and following reduction in water retention (White and Williams

1994). Association of obesity, pollutants, environmental allergens and kennel cough has been seen with the disease progression (Oskouizadeh *et al* 2011).

Bacterial bronchopneumonia occur more commonly in young dogs, whereas chronic bronchitis and bronchiectasis occurs mainly in middle age or older dogs and chronic bronchitis occurs predominantly in obese small breed dogs (Hoover *et al* 1989). Chronic tracheobronchial syndrome was reported in clinically normal eight dogs which had a chronic mild cough for 2 to 13 months. The coughing mainly occurs when the dogs were pulled by their leads or became excited. Three out of 8 dogs had active airway inflammation on bronchoscopic examination and five had varied numbers of inflammatory cells (neutrophils) in the bronchial aspirates. Increased bronchial pattern on radiographs were seen in seven dogs (Corcoran *et al* 1992).

Bronchogenic cyst was reported in a two and half year old male German shepherd dog with intermittent episodes of fever, severe dyspnoea and cyanosis; and there were sudden onset of the clinical signs which disappeared within the eight hours. On histological examination of the structure, it was found that the walls contain traces of the bronchial epithelial cells and on bacteriological examination of the fluid, mucus-forming gram positive bacteria were seen. The definitive diagnosis was a bronchogenic cyst which had developed secondarily to the bronchiectasis (Dahl *et al* 2002).

Bronchiectasis is defined as a permanent and debilitating consequence to the chronic or severe airway injury resulting in progressive and irreversible dilatation of the airways (Chan and Iseman 2016). The highest prevalence of Bronchiectasis is recorded in Cocker spaniels and Miniature poodles and the older dogs of various breeds (Hawkins *et al* 2003). Johnson *et al* (2016) documented bronchoscopy being highly useful in recording bronchiectasis in 92 percent of the affected dogs. Radiography of the dog with bronchiectasis revealed increased pulmonary density along with mixed bronchial, interstitial and alveolar patterns. On cytological examination, mixed cell population of mainly neutrophils and mononuclear cells was observed (Johnson *et al* 2016).

For lungworm larvae examination, faecal samples of 4151 dogs from Denmark, 958 dogs and 231 cats from Germany with clinical signs were examined using the baermann funnel technique between year 2003 and 2007. A total of 3.6

percent of the total Danish and German dogs shed lungworm larvae. In Denmark, *Angiostrongylus vasorum* infection was found to be more prevalent (2.2%) as compared with *Crenosoma vulpis* (1.4%). In Germany, canine faecal samples were found to be more frequently positive for *C. vulpis* than for the *A. vasorum* larvae (2.4% and 1.2%, respectively). Relative high number of lungworm infected dogs and cats indicate that these parasitic diseases should always be considered in the differential diagnosis of the cases of treatment-resistant respiratory/cardiopulmonary distress (Taubert *et al* 2009).

Eosinophilic bronchopneumopathy (EBP) is a characterized by the eosinophilic infiltration of the lung and the bronchial mucosa, as shown by the cytological examination of the bronchoalveolar lavage fluid or the histological examination of bronchial mucosa. However the exact cause of EBP is not known, but a hypersensitivity to the aeroallergens is suspected. The diagnosis mainly depends on the typical history, clinical signs and cytological demonstration of bronchopulmonary eosinophilia or the histopathological examination and the exclusion of other known causes of the lower airway eosinophilia. Majority of the dogs show excellent response to the oral corticosteroid therapy (Clercx and Peeters 2007).

Most commonly seen pattern of pneumonia in clinical cases of small animal in veterinary medicine is bronchopneumonia. Bronchopneumonia can arise in every case as a result of primary disease process or due to injury to the lung, either of two which would result in compromising the lung's innate immunity. Bronchopneumonia can be difficult condition to diagnose and treat as patient can exhibit a wide range of clinical signs ranging from the mild coughing, lethargy, fever, rapid progressive weight loss and ultimately leading to fatal clinical syndrome (Carey 2009). Underlying causes of bronchopneumonia include viral infection, aspiration injury, foreign body inhalation, nosocomial infection and immune dysfunction.

Aspiration pneumonia occurs due to the accidental inhalation of gastric acid or ingesta and remains an important cause of bacterial pneumonia. Risk factors involved in the development of aspiration pneumonia include refractory vomiting, esophageal disease, prolonged anesthesia, seizures and laryngeal dysfunction (Tart *et al* 2010). Aspiration injury occurs either due to inhalation of sterile, acidic gastric contents (resulting from gastric regurgitation or vomiting) or of septic material from the gastric

or oral secretions. Acid inhalation induces irritation and creates an environment in which bacterial colonization can occur and lead to bacterial pneumonia. The severity of the disease depends on the quantity and the nature of the material aspirated as well as the time period between the occurrence of the event and its diagnosis.

Infectious or community acquired pneumonias in dogs commonly occur with viral colonization and infection of the upper respiratory tract (canine respiratory coronavirus, pneumovirus, herpesvirus and parainfluenza virus) (Brownlie *et al* 2013). Mostly these diseases are acute and self-limiting, but these organisms affect the host's immune defences and predispose to infection with bacterial respiratory pathogens (Radhakrishnan *et al* 2007). Many bacteria have been involved in the canine infectious respiratory disease (CIRD), but the main focus has been given toward *Streptococcus*, *Mycoplasma cynos* and *Bordetella bronchiseptica*. CIRD is mostly prevalent in dogs present in overcrowded, stressful environments such as boarding kennels, animal shelters and treatment facilities.

Inhaled foreign bodies mostly carry a mixed bacterial and fungal organism into the lungs and causes focal pneumonias that are mostly initially responsive to antimicrobial therapy but relapse shortly after discontinuation of the therapy. Foreign bodies include grass awns, plant materials or plastic materials and organisms related with grass awn inhalation include *Streptococcus*, *Pasteurella*, *Nocardia*, *Actinomyces* and anaerobic bacteria (Tenwolde *et al* 2010). Mostly foreign material remains at carina or enters the caudodorsal principal bronchi. Characteristic features linked with pulmonary foreign bodies include young, sporting breeds, environmental exposure to the grass awns, focal recurrent radiographic alveolar pattern, history of other cutaneous or visceral foreign bodies and spontaneous pneumothorax or pyothorax. Marked inflammation can be seen in cases of chronic pulmonary foreign bodies which can lead to severe airway remodeling and bronchiectasis that can be seen on radiographs (Workman *et al* 2008).

Ventilator associated pneumonia (VAP) is a very common cause of hospital acquired pneumonia in people, however, there are only few veterinary reports in literature. Oropharynx is colonized by the pathogenic and multidrug resistant bacteria and the endotracheal tube acts as a channel to transmit pathogens into the airways leading to tracheobronchitis and potentially pneumonia. Any animal having a

compromised respiratory tract or any serious systemic disease becomes very prone to development of the infectious airway disease while being hospitalized (Epstein *et al* 2010).

Both immune systems, the innate and the adaptive, protects against development of infectious airway disease and a breakdown in either of the system results in increase of the likelihood of opportunistic infection. Congenital immune deficiencies are found to make an animal specifically sensitive to infectious disease. Young animals are particularly prone to the development of bacterial pneumonia due to their naive immune systems. Moreover, alterations to the innate immune system, such as complement deficiency or primary ciliary dyskinesia (PCD) increase the risk of life-threatening infections greatly. Systemic immunity compromise or any additional alteration to the body's natural defence mechanisms increases the risk for bacterial pneumonia. Medications such as immunosuppressive therapy, chemotherapy or antitussive therapy significantly increase the possibility of, bacterial pneumonia (Crapo *et al* 2000).

Lung lobe torsion (LLT) may be defined as the rotation of a lung lobe along its long axis with twisting of the bronchovascular pedicle at the hilus. A total of 23 dogs were diagnosed with LLT, out of which 10 were large-breed dogs and 13 were small-breed dogs. Seven among the small-breed dogs were Pugs. Age of affected Pugs ranges from 4.5 months to 4 years. Young male Pugs were found to be more predisposed to develop spontaneous LLT. Factors which contribute to the development of LLT in Pugs are unknown (Murphy and Brisson 2006).

Pulmonary neoplasia has also been advocated as a cause of respiratory affections. Six cases of primary neoplasia were diagnosed in the older dogs having mean age 11 years and among which, there were four females and two males. Three tumours on histopathology were adenocarcinomas of bronchial/alveolar origin and one was identified as anaplastic sarcoma. All the primary tumours were found in the caudal lung lobes, of which four were observed on the right side (Brownlie 1990).

Interstitial lung diseases (ILDs) are the diffuse parenchymal lung diseases which form a large heterogenous group of the non-infectious, non-neoplastic disorders categorized by diverse patterns of inflammation and fibrosis (Travis *et al* 2002). ILDs classification in dogs includes three major groups: idiopathic interstitial

pneumonias (IIPs), ILDs secondary to known causes and miscellaneous ILDs (Travis *et al* 2002). Non-specific interstitial pneumonia (NSIP) belonging to IIPs group in humans, is a distinct clinical disorder showing a highly diverse clinical course that may evident as cellular or fibrotic forms (Travis *et al* 2008, Travis *et al* 2013). Generally Idiopathic NSIP has a better prognosis than idiopathic pulmonary fibrosis (IPF), as the patients with the cellular form can recover or stabilize with therapy (Bjoraker *et al* 1998, Travis *et al* 2013).

In humans known cause of ILDs are not clearly defined, but comprise those developing from inhalational, drug, biological agent and radiation exposure along with different collagen vascular diseases and connective tissue disorders. In dogs and cats, ILDs occur as a result of exposure via inhalational routes or secondary to the drugs, radiation and immune-mediated disorders. In ILD classification, finding an identifiable etiological agent is important and its avoidance or removal is the part of therapy. Pneumoconiosis is defined as an inflammatory and fibrotic ILD caused by exposure to environmental factors which include mineral dusts and fibers including silica, asbestos, coal dust and other small particulates (Jp *et al* 2017). Anthracosis (a milder form of pneumoconiosis) occurs from chronic exposure to air pollution or inhalation of coal dust or smoke resulting in accumulation of black dust particles (Mirsadraee 2014).

2.4 Clinical findings in respiratory affections

The diagnosis of respiratory diseases is always challenging as dogs can exhibit a wide range of clinical presentations (Norris *et al* 2001, Priestnall *et al* 2010). Clinical manifestations may include nasal discharge, cough, exercise intolerance, increased respiratory effort or apparent respiratory distress along with systemic signs of illness such as lethargy, anorexia and fever (Centil *et al* 2012).

The major problem in lower respiratory tract infection is the differentiation of infection from colonization or contamination, and isolation of true pathogen (Lode *et al* 1993). In pneumonia or infective exacerbations of chronic bronchitis, the clinical findings and differentiation of patients should provide early definitive diagnosis. Expectorated sputum may be unreliable for diagnosis of pneumonia because of contamination with the oropharyngeal flora.

Expiratory wheezes are considered as the hallmark of chronic bronchitis (Johnson 2000). Thoracic auscultation can be found normal but commonly reveals

coarse, diffuse crackles and tracheal sensitivity is usually seen due to nonspecific airway inflammation.

Nathan and Norris (2002) studied thirty-six cases of hemoptysis in dogs. History and clinical examination revealed that the common signs were coughing, tachypnea and dyspnea. Anaemia was found in 11 out of 36 dogs and the most common pattern evident on radiographs was alveolar and interstitial pattern. The predisposing causes of hemoptysis include bacterial bronchopneumonia (n=7), neoplasia (n=5), trauma (n=5), immune-mediated thrombocytopenia (n=4), heartworm disease (n=4), rodenticide poisoning (n=3), lung-lobe torsion (n=1), left-sided congestive heart failure (n=1), pulmonary hypertension (n=1), and foreign-body pneumonia (n=1).

Normal respiration pattern seen in canines is thoracic or coastal and the abdominal respiration predominates mainly during the painful conditions of the thorax such as pleuritis, etc. (Reece 2004). The frequency of respiration is an excellent indicator of the health status and an increased frequency is usually observed in diseased conditions.

B. dermatitidis organisms were identified in 76 percent of transtracheal aspiration samples from 17 non sedated dogs having pulmonary blastomycosis (McMillan and Taylor 2008). Clinical signs of *B. dermatitidis* infection expressed the inflammatory and the multisystemic nature of the disease. Common signs in affected dogs include anorexia, weight loss and fever. Pulmonary lesions more often associated with respiratory signs, including exercise intolerance, tachypnea, and cough. Lymphadenopathy can be seen in 40 to 60 percent of the affected dogs. Ocular involvement and granulomatous or ulcerative cutaneous lesions can occur in 30 to 50 percent of the affected cases.

The major signs associated with bacterial pneumonia or bronchopulmonary infection are lethargy, decreased exercise tolerance, reduced activity, inappetence, increased respiratory rate and effort, cough and persistent nasal discharge (Ford 2009). All these signs may not be present in all the cases. The physical findings like increased respiratory rate at rest, fever, cough, presence of purulent nasal and ocular discharge in lethargic patients permit further diagnosis of lower airway disease.

The evaluation of the clinical signs, along with the physical examination findings, provides the initial step in guiding the diagnostic workup (Ettinger and

Feldman 2010). Pulse oximetry measurements help to regulate the need for oxygen administration. In dogs and cats, the normal hemoglobin saturation is 95 to 100 percent. Hematological and biochemical analysis are important in differential diagnosis. Leucocytosis with left shift is generally present in animals having moderate to severe airway inflammation, infection, or neoplasia, however, leucopenia is commonly seen in animals with acute bacterial bronchopneumonia or sepsis, whereas eosinophilia is usually present in animals with parasitic airway disease, asthma, bronchitis, or pulmonary infiltration. Hypoalbuminaemia is a common finding seen in cases of pleural effusions and pulmonary infiltration. Culture sensitivity results should be used for immediate diagnosis and treatment. Fecal examination should be done in cases suspected for lung worm infestation.

Hawkins *et al* (2010) conducted a study to identify the demographic and historical factors associated with chronic cough in dogs. A total of 115 dogs having a history of coughing from 2 months were considered and written questionnaires were answered by the owners. Comparison of demographic and historical data was done between the coughing and control dogs. Demographic data and the exposure to environmental tobacco smoke (ETS) were also compared with hospital appointments and adult smoking rates, respectively. Association between the characteristics of cough and diagnosis was observed. Tracheobronchomalacia was diagnosed as the most common cause of chronic cough. Association of the demographic risk factors including older age, smaller body weight and being toy breed were strong ($P < 0.001$). No association was observed between coughing and month ($P = 0.239$) or season ($P = 0.414$) of presentation. Exposure to ETS was not recognized as a risk factor ($P = 0.243$).

The importance of clinical signs, signalment and duration of clinical signs in providing guide for assessment of the underlying condition and prognosis was determined in 229 dogs with dyspnea (Fonfara *et al* 2011). The occurrence rate of 32, 33, 19 and 12 percent was observed for upper airway, lower airway diseases, pleural space, and cardiac diseases. The incidence rate of upper airway and pleural space diseases was found to be more significant in younger dogs whereas lower respiratory tract and cardiac diseases were more commonly seen in older dogs. Non-traumatic acute onset dyspnea is commonly linked with poor prognosis, but stabilization is possible in patients with cardiac disease. Obesity can be an important contributing or aggravating factor in dyspneic dogs.

Ninety clinical cases of dogs with the history of respiratory diseases, were studied over a period of 26 months and were grouped into mild, moderate and severe consisting of 30 animals each (Ayodhya *et al* 2013). The highest prevalence of respiratory diseases was observed in younger age group and the female dogs. Pugs were found to be more prone to respiratory diseases. The highest prevalence of the respiratory diseases in dogs was recorded in the cold season. The common clinical symptoms were dyspnoea, nasal discharge, cough and fever.

Tracheal collapse is a progressive disease of middle-aged and small breed dogs (Tappin 2016). Clinical signs are proportional to the degree of collapse, ranging from mild airway irritation and paroxysmal "goose-honking" coughing to respiratory distress and dyspnea. Diagnosis is usually made on the basis of radiographs, bronchoscopy or fluoroscopy. Majority of dogs respond well to medical management and treatment of any collateral comorbidity. Surgical intervention should be considered in dogs that do not respond or have respiratory compromise.

2.5 Diagnosis of respiratory affections

2.5.1 Haematology

Dogs suffering with a respiratory disease often show unremarkable or non-specific changes in the haematology and biochemistry. The haematological and biochemistry profiles mainly help to uncover any systemic or metabolic diseases that might be affecting the respiratory system (acid-base imbalance, anemia). Some common haematological findings related with respiratory disease include polycythemia from chronic hypoxia, leukocytosis with respiratory infections or eosinophilia with pulmonary infiltrates with eosinophils (PIE) or parasitic lung infections. Basophilia is usually associated with the heartworm infection (Corcoran 2000). In case of hemoptysis or unexplained respiratory distress, the coagulation profile must be performed to rule out warfarin toxicity (Bailiff and Norris 2002). Serology helps in the diagnosis of pulmonary mycotic diseases, particularly coccidioidomycosis or cryptococcosis (Corcoran 2000). Dogs affected with pulmonary blastomycosis exhibit a moderate leukocytosis, hyperglobulinemia and mild hypoalbuminemia (Legendre 2006).

A non- significant difference was seen in the haemoglobin concentration of the dogs that are infected with the respiratory diseases when compared to the normal

values (Piva *et al* 2010, Ayodhya *et al* 2013). Significant increase in the leukocyte counts were observed while a decline was noticed in the lymphocyte and macrophage counts (Maden *et al* 2000, Bolln *et al* 2003, Billen *et al* 2006 and Brendan 2006). The major haematological finding reported in the respiratory diseases was eosinophilia and the leucocytosis (Anusz 2005, Piva *et al* 2010). Differential changes revealed neutrophilic leucocytosis (Amrute *et al* 2009, Ayodhya *et al* 2013) and absolute circulating eosinophilia (Charkrabarthi 2006, Spuzak 2006 and Wray 2006, Ayodhya *et al* 2013). In another study, canine distemper infected dogs having secondary infection showed significant leukocytosis, neutrophilia, lymphopenia and monocytosis whereas dogs without secondary infection revealed significant monocytosis and lymphopenia (Khattab *et al* 2017). Canine distemper inclusion bodies were seen inside the neutrophils and the red blood corpuscle in all infected cases. Toxoplasma tachyzoites can be observed during acute illness in various tissues and body fluids by cytology and may be found in transtracheal or bronchoalveolar washings. Neutrophilic leukocytosis and monocytosis was observed in toxoplasma gondii cases (Khattab *et al* 2017).

Radhakrishnan *et al* (2007) identified etiology, treatment and prognosis for 65 dogs below 1 year of age suffering with community acquired infectious pneumonia. About 57 percent of dogs had an inflammatory leukogram characterized by leukocytosis, neutrophilia and band neutrophilia and rest had unremarkable changes despite of severe bronchopneumonia. *Bordetella bronchiseptica* was isolated from tracheal wash fluid from 32 dogs and from other 33 dogs, there were predominantly gram-negative enteric bacteria. Disease was more severe in dogs with *Bordetella* pneumonia than in dogs with pneumonia caused by other bacterial organisms.

2.5.2 Arterial Blood Gas

An arterial blood gas measurement allows the most definitive assessment of overall pulmonary function as it directly assesses the gas exchange. Most common site used for arterial blood gas measurement in dogs is the femoral artery and the other alternative sites include the dorsal metatarsal, carotid, brachial and the auricular arteries (King and Hendricks 1995). Small-gauge needles on 1 to 3 ml syringes are usually recommended and a small volume of heparin (1000 U/ml) is drawn to coat the needle hub and the barrel. The sample should be analyzed as soon as possible or to be

kept on ice until analysis, to minimize the sources of error (King and Hendricks 1995, Haskins 2004). PaO_2 level in a normal dog at sea level should be greater than 80 mmHg and the conditions leading to decrease in PaO_2 include hypoventilation, decrease in the partial pressure of atmospheric O_2 (high altitude) or with the venous admixture. Most common cause of hypoxemia is venous admixture occurring with venous shunting (lung atelectasis, pneumonia) or physiologic dead space (PTE) (King and Hendricks 1995, Haskins 2004). The alveolar-arterial O_2 gradient is independent of the effect of the ventilation and gives an estimate of the effectiveness of gas transfer. Its normal value in dogs is usually less than 10 to 15 mmHg (King and Hendricks 1995, West 1990).

Dogs with community acquired infectious pneumonia were found hypoxemic and hypercarbic at the time of initial examination and that pulmonary function tended to worsen during the early hospitalization period before improving (Radhakrishnan *et al* 2007). On comparison median venous partial pressure of CO_2 (P_VCO_2) was higher in dogs with pneumonia caused by *B. bronchiseptica* (48.7 mmHg) than in dogs with pneumonia caused by other organisms (41.3 mmHg) whereas median PCV was found to be lower in dogs with pneumonia caused by *B. bronchiseptica* than in dogs with pneumonia caused by other organisms at the time of initial examination.

Kogan *et al* (2008) evaluated clinical, clinico-pathological and radiographic findings in 88 dogs with aspiration pneumonia. Arterial blood gas analyses within 48 hours of the aspiration revealed hypoxemia with mean PaO_2 of 68.5 ± 14.6 in 22 out of 28 (79%) dogs. Partial pressure of carbon dioxide ranged from 20.8 to 52.5 mmHg. Overall, hypercapnia was observed in 2 (7%) dogs and hypocapnia in 14 (50%) dogs. Variable dysfunction in pulmonary gas exchange results in reduction of PaO_2 values. In this study, only 2 dogs were found hypercapnic, showing that the alveolar hypoventilation was not a common problem. Aspiration pneumonia can cause severe lung damage as confirmed by poor oxygen responsiveness to supplemental oxygen, confirmed on arterial blood gas analysis

2.5.3 Radiography

Thoracic radiography plays a crucial role in exploring the potential differential diagnoses of various respiratory diseases. The diagnostic information may be restricted due to the poor radiographic technique, poor cooperation of the patient and

an inherently low diagnostic sensitivity and the specificity. To maximize the chances of lesion detection and to minimize the superimposition of thoracic structures, the three view thoracic radiography is often recommended. The importance of sedation or general anesthesia should be considered as an important tool to help in the proper positioning of the patient (Thrall and Widmer 1998, Saunders and Keith 2004).

Air being radiolucent provides good contrast for thoracic structures (normal and pathologic) that are radiopaque. The heart and the blood vessels which are radiopaque in nature, due to the presence of blood in them which is relatively radiopaque, appears to be superimposed on the radiolucent background of the air. The blood vessels appear as the branching white tubes on the radiographs (Reece 2015).

A set of procedure should be followed for proper assessment of any radiograph and it must be consistent for the reader (Thrall and Widmer 1998, Saunders and Keith 2004). All the bony structures must be checked for the presence of any abnormalities including the lysis, proliferation, osteoporosis or fractures. The diaphragm and the mediastinum must be evaluated for the presence of any anatomic abnormality. The assessment of the position and size of the cardiac silhouette, great vessels and associated structures must be done and also the radiograph must be reviewed for sternal or hilar lymphadenopathy (Saunders and Keith 2004).

The trachea should be evaluated in the cervical and the intrathoracic region for any narrowing, compression, or deviations. Deviation of trachea can be a normal variant or due to improper positioning of the patient. Trachea elevated at the level of carina indicates the presence of mediastinal or cardiac mass (Kneller 1998).

The pulmonary vessels can be seen within the pulmonary parenchyma and the airways are usually visualized between the paired artery and the vein. Bronchial walls are normally not visible but can be visualized as end-on ring structures (doughnuts) or parallel line markings (tramlines) when they become thickened due to inflammation. These findings usually reflect the pathologic change. Interstitial pattern usually appears as the linear densities which gave a hazy appearance to the lung field and masks the visualization of the vasculature. This pattern is very difficult to identify and is very sensitive to obesity or the changes in radiographic technique. An alveolar pattern is obtained when the air in the alveoli is replaced by material having higher density includes exudate, haemorrhage or oedema fluid. It appears as a soft tissue

density in the lung containing air bronchograms and the airways are outlined by infiltrated pulmonary parenchyma. In conditions like pulmonary masses, atelectasis, lung lobe torsion, or pulmonary granuloma, an alveolar pattern without the presence of air bronchograms can occur (Lamb 1998, Saunders and Keith 2004). These patterns indicate the location (bronchioles, interstitium, or alveoli) of the pathologic change but do not provide any definitive diagnosis.

Lang *et al* (1986) evaluated the thoracic radiographs which were taken in right lateral recumbency (RLR), left lateral recumbency (LLR) and ventrodorsal (VD) projections in 80 dogs with known or suspected malignant tumours. Radiographs in each projection were interpreted independently by four radiologists and were classified as positive or negative for one or more lung metastases. The three radiographic projections varied in their sensitivity for the detection of lung metastases. In this study, the RLR view was found to be the most sensitive and the VD view was the least sensitive. This study showed that sensitivity of detection of lung metastases in the dog can be improved by including the RLR view in any diagnostic protocol and by using a minimum of two readers. A three-view protocol must be used if only one reader is available.

Mantis *et al* (1998) studied thoracic radiographs of 23 dogs suffering with chronic bronchitis which were mixed with those taken from 11 dogs (matched by age and bodyweight) without respiratory disease and were interpreted to determine the accuracy of radiography for the diagnosis of chronic bronchitis. The important radiographic signs found commonly in dogs with chronic bronchitis were thickening of the bronchial walls and increased numbers of visible bronchial walls ($P<0.01$). Other signs like bronchial calcification and interstitial pattern appeared with similar frequency in both the groups of dogs. The accuracy of radiographic diagnosis of chronic bronchitis is limited chiefly by their insensitivity for bronchial lesions.

Chest radiography was observed to have a diagnostic accuracy of 47 percent for pleural effusion, 75 percent for alveolar consolidation and 72 percent for alveolar-interstitial syndrome in patients having acute respiratory distress syndrome (Lichtenstein *et al* 2004).

Kogan *et al* (2008) evaluated radiographic findings in 88 dogs suffering with aspiration pneumonia. Aspiration pneumonia was evident at admission in 65 dogs and

developed during hospitalization in 23 dogs. Neutrophilia, hypoalbuminemia, and hypoxemia were frequently detected along with radiographic evidence of infiltrates in the right middle lung lobe. Most common thoracic radiographic pattern observed was an alveolar infiltrates in 65 (74%) dogs and interstitial pattern in 23 (26%) dogs. The right middle lobe was found to be most commonly affected in the 46 dogs with involvement of one lung region and the left cranial-caudal segment was found to be most commonly affected in 28 dogs having involvement of 2 lung regions. Most commonly affected lung region found in all the dogs was the right middle lobe (48%) and the least commonly affected was the accessory lung lobe (3%).

McMillan and Taylor (2008) reviewed thoracic radiographs from the 17 dogs having pulmonary blastomycosis in which transtracheal aspiration was done. Most common radiographic pattern was nodular interstitial followed by miliary interstitial pattern, diffuse interstitial pattern and a focal region of consolidation or a solitary nodule. Cytological examination of transtracheal aspirate yielded a diagnosis in 66 to 100 percent of the affected dogs with different radiographic classifications of pulmonary disease.

Pajas *et al* (2015) studied thirty lateral thoracic radiographs of dogs presented with coughing and assessed tracheal diameter and thoracic inlet ratio (TD: TI), pulmonary patterns present, cardiac silhouette abnormalities and vertebral heart size (VHS). Grouping of data collected was done on the basis of age of the animal and the cephalic index. The TD: TI ratio was normal in all the radiographs irrespective of cephalic index. Mixed pulmonary patterns consisting of alveolar and bronchial forms were observed in dogs with cough. The varied radiographic abnormalities that were found in coughing dogs suggest that a thorough clinical examination of patients should be done to rule out primary cardiac disease and secondary respiratory problems.

2.5.4 Thoracic ultrasound

Thoracic ultrasound is the new tool emerged in the field of thoracic imaging. Thoracic ultrasound is best if used along with the thoracic radiographs so as to localize the pulmonary mass lesions. Structures present within the lungs which are surrounded by the aerated lung are not accessible through thoracic ultrasound. Ultrasonography is most useful in evaluating cardiac or mediastinal masses,

consolidated or collapsed lung lobes, pleural effusion, thoracic wall masses and diaphragmatic hernias. It can also be used to guide needle aspiration or the biopsy instruments into the localized mass lesions for specimen collection (Reichle and Wisner 2000, Saunders and Keith 2004) and in diagnosis of the laryngeal paralysis in dogs (Rudorf *et al* 2001).

Murphy and Brisson (2006) reported that young male pugs were more predisposed to develop spontaneous lung lobe torsion (LLT). A total of 23 dogs (7 pugs and 16 dogs of other breeds) were diagnosed with LLT. Thoracic ultrasonography in 4 pugs revealed a soft tissue mass in the left cranial thorax and pleural effusion in each case. Partial aeration of the soft tissue mass was observed in the 2 pugs with air bronchograms. Thoracic ultrasonography was also done in 4 other small-breed dogs and 5 large-breed dogs. Tissue consolidation in the region of the affected lung lobe and pleural effusion was the consistent findings in all these dogs. Multiple gas bubbles were seen in the consolidated lung lobe in 2 small-breed dogs.

2.5.5 Tracheal wash: microbial flora and cell cytology

Hawkins and De Nicola (1990) evaluated the tracheal wash and bronchoalveolar lavage fluid in 9 dogs with mycotic infections with pulmonary involvement. Blastomycosis causal organisms were identified in tracheal wash fluid of 3 out of 7 dogs and in bronchoalveolar lavage fluid in 5 out of 7 dogs. Only one dog was diagnosed for histoplasmosis and none for coccidioidomycosis. These invasive procedures should be done in the dogs suspected with mycotic infections that involve the lungs and that cannot be diagnosed by less invasive means.

Mayer *et al* (1990) analyzed proteins and respiratory cells in the bronchoalveolar fluid from eighteen healthy Beagle dogs of different ages. Albumin, IgA and IgG was found in BAL fluid. Respiratory cells obtained in BAL were alveolar macrophages, lymphocytes, epithelial cells and neutrophils. It was observed that the age of the dog does not change the content of immunoglobulins or in the differential cell counts of respiratory cells.

Clercx *et al* (2000) evaluated 23 young dogs diagnosed for eosinophilic bronchopneumopathy. Peripheral blood eosinophilia was noted in 14 of 23 dogs. Inflammatory cells in brush cytology or bronchoalveolar lavage (BAL) fluid comprised more than 50 percent eosinophils in 14 of 23 dogs and 20–50 percent

eosinophils in 6 dogs. Eosinophilic infiltration of the bronchial mucosa was observed in biopsies from 19 dogs and was graded as mild (37%), moderate (32%), or severe (32%).

Williams *et al* (2006) studied a 4-month-old, intact male Boxer puppy presented with the signs of dyspnea, nasal discharge, dehydration and coughing. Radiographic findings showed the presence of increased bronchointerstitial pattern throughout the lungs. On direct cytologic examination of transtracheal wash, Wright-Giemsa–stained smears, small basophilic coccoid structures (0.3–0.9 µm in diameter) were seen in low to moderate numbers within neutrophils and adhered to epithelial cells. Culture of the transtracheal wash fluid on MYCOTRIM media (sterol rich media for mycoplasma) for 7 days at 35ºC resulted in growth of a Mycoplasma sp. The cytological findings were septic suppurative inflammation. Presence of Mycoplasma in transtracheal washes helps in recommending the appropriate culture media or immunologic techniques, which could result in an accurate diagnosis of mycoplasmosis.

Bordetella bronchiseptica was isolated from tracheal wash fluid from 32 dogs and gram-negative enteric bacteria organisms from 33 dogs. Disease was more severe in dogs with *Bordetella* pneumonia than in dogs with pneumonia caused by other bacterial organisms (Radhakrishnan *et al* 2007).

McMillan and Taylor (2008) concluded that the definitive diagnosis is based on the cytological demonstration of the organism in the affected tissues. Though, previous studies revealed a low diagnostic yield of *B. dermatitidis* organisms from transtracheal aspiration. In this retrospective study, *B. dermatitidis* organisms were identified in 76 percent of samples when transtracheal aspiration was performed in 17 non- sedated dogs with pulmonary blastomycosis. So whenever blastomycosis is a differential diagnosis in dogs affected with the pulmonary disease, the transtracheal aspiration should be considered as an early diagnostic test.

Ford (2009) stated that the cytology, gram stain and culture of BAL fluid can be very important for therapeutic success in clinical cases. Bacterial pneumonia can be confirmed by the presence of intracellular bacteria in the leukocytes whereas the absence of bacteria does not exclude a diagnosis of bacterial pneumonia.

Benson *et al* (2013) reported a case of a 7-year-old spayed female German wire haired pointer that was presented with history of difficult breathing after being

found seizing in a water-filled drainage ditch while out hunting. Tracheal wash aspirates contained numerous degenerated neutrophils, fewer macrophages, some of which contained basophilic debris, low numbers of extracellular diatoms and a single intracellular short bacterial rod. On necropsy, major finding was a severe granulomatous bronchopneumonia that was mostly due to aspiration of foreign material based on the microscopic presence of plant-like material, birefringent crystalline material, non-cellular debris and occasional fungal structures. On cytologic examination of tracheal aspirate, the presence of diatoms and inflammation in the tracheal wash were interpreted as a likely result of the aspiration of surface water. This was the first reported case of diatoms seen in a cytologic specimen in a non-human mammal with aspiration pneumonia.

Johnson *et al* (2013) stated that the confirmation of the lower respiratory tract infection in dogs is challenging and the organisms can be isolated from the dogs in which no bacteria are detected on cytologic examination.

Khattab *et al* (2017) conducted study on 25 German shepherd dogs grouped into two categories one having 12 apparently healthy dogs of different ages used as control and other having 13 canine distemper infected dogs (8 with secondary infections and 5 without secondary infections) diagnosed by immune-chromatographic assay on ocular and nasal discharges. On cytological examination, transtracheal wash fluids of infected dogs with secondary infections showed significant increase of both total nucleated cell count (TNCC) and urea adjusted TNCC (multiplied by the dilution factor) along with degenerated neutrophils and activated macrophages. In both the groups, the differential nucleated cell count showed significant increase in neutrophils and reactive macrophages as well as significant decrease in lymphocytes, epithelial cells and alveolar macrophages. Biochemical analysis showed that the matrix metalloproteinases, alkaline phosphatase and lactate dehydrogenase were the most sensitive constituent in trans-tracheal wash for detecting inflammation.

2.6 Treatment

Boothe and McKiernan (1992) stated that most successful treatment for various respiratory tract diseases of small animals can be done on the basis of

knowledge and understanding of the normal physiology and the pathophysiology of the respiratory tract diseases.

Olsen (2000) stated that the treatment of the respiratory tract infections poses some unique challenges to the veterinary practitioner. He stated that the treatment targeted at the infecting pathogens is best accomplished with bacterial culture and susceptibility testing. On absence of suitable data, rational antibiotic treatment should be based on familiarity with the historical data and the clinical experience. Ideal drug selection is based on estimated microbial susceptibility, drug distribution in the respiratory tract and safety of the patient. Appropriate dosage regimen and duration of therapy maximizes the chances of a successful resolution of bacterial infections.

Clercx *et al* (2000) evaluated 23 young dogs diagnosed for eosinophilic bronchopneumopathy. Amoxicillin clavulanate (12.5–15 mg/kg q12h orally) was used as the initial choice and was administered for 1–8 weeks. Oral corticotherapy was initiated at a dosage of 1 mg/kg q12h during first week then on alternate days with progressive tapering of the dose; the dosage at maintenance varied between 0.1 and 1.0 mg/kg every other day. All dogs were treated with fenbendazole (50 mg/kg q24h for 3 days).

Datz (2003) stated that the penicillin derivatives achieve good penetration into the pulmonary parenchyma whereas they have relatively poor ability to penetrate the blood-bronchus barrier and therefore, may not be able to reach in therapeutic concentrations in the airways.

Williams *et al* (2006) treated a 4-month-old, intact male Boxer puppy diagnosed for Mycoplasma infection, with enrofloxacin and amoxicillin/clavulanate and observed a full recovery.

Rozanski *et al* (2007) stated that the most effective respiratory therapy depends on availability of a definitive diagnosis and following established recommendations for treatment. Some respiratory diseases can be effectively treated with antibiotics, anti-inflammatory agents or the chemotherapeutic drugs, however chronic inflammatory diseases and those with idiopathic origin remain difficult to manage. In some conditions, disorders are controlled rather than being cured. Recent advances in pulmonary therapeutics include the use of new drugs to treat common

diseases and application of new methods of drug delivery to enhance drug effect and minimize side effects.

Radhakrishnan *et al* (2007) conducted study on treatment of community acquired infectious pneumonia in 65 dogs of below 1 year age. Forty-one out of 65 (63%) dogs received supplemental oxygen when they were hospitalized. Out of these, 40 dogs had oxygen saturation (SO_2) $\leq$ 92 percent. Antimicrobials used during hospitalization include ampicillin, aminoglycosides, ticarcillin-clavulanate, enrofloxacin and cefazolin. In 49 out of the 65 (75%) dogs, nebulization and coupage were used. In 16 dogs, bronchodilators were used out of which 13 dogs were having SO_2 values $\leq$ 92%. Fluid therapy was given IV in 75% dogs. Dogs with pneumonia caused by *B. bronchiseptica* were more likely to receive supplemental oxygen and require higher proportion of bronchodilators than the dogs with pneumonia caused by other organisms. Mostly the *Bordetella* isolates from these dogs were found to be susceptible to a wide range of antimicrobial agents, with the exception that only 9 out of 31 *B. bronchiseptica* isolates were susceptible to trimethoprim-sulfonamide.

Attili *et al* (2012) evaluated the efficacy of enrofloxacin and N-acetylcysteine combination in the treatment of recurrent bronchopneumopathies caused by biofilm producer bacteria, in a total of 30 dogs presented with recurrent respiratory diseases. Bronchoalveolar lavage fluid taken from each animal was submitted for bacteriological and cytological examinations. Twelve dogs were treated with the above mentioned combination orally or by using an aerosol. Three randomly selected dogs were orally treated with Enrofloxacin only and another group of three animals had been randomly chosen to test the treatment with N-acetylcysteine only. One dog was found to be bacteriologically negative while biofilm producer bacteria were identified in 19 (65.5%) out of the 29 bacteriological positive dogs. Biofilm formation and its abundance decreased the bacterial sensitivity towards enrofloxacin ($p<0.01$) and cephalosporin ($p = 0.031$) while significant differences were not seen for other antibiotics. This association proved to be safe and effective in eliminating biofilm producer bacteria from all treated animals.

Rheinwald *et al* (2015) isolated causative bacterial species from BAL fluid samples of 493 dogs with respiratory signs and determined their antibiotic susceptibility. In 35 percent of samples, no bacteria could be cultured. Bacterial

isolates included *Streptococcus* species (31%), Enterobacteriaceae (30%, including *Escherichia coli*), *Staphylococcus* species (19%), *Pasteurella* species (16%) and *Pseudomonas* species (14%) from positive samples. *Bordetella bronchiseptica* as a primary respiratory pathogen was isolated in 8 percent of cases. Enrofloxacin showed the best susceptibility pattern against 86 percent of all isolates and 87 percent of Gram-negative bacteria. Amoxicillin/clavulanic acid yielded the best susceptibility pattern in Gram-positive bacteria (92%).

Lappin *et al* (2017) recommended the use of doxycycline empirically for 7–10 days as the first-line antimicrobial option in dogs suspected with bacterial CIRDC (Canine infectious respiratory disease complex) and with mild pneumonia. Doxycycline was found to have clinical activity against Mycoplasma. Alternate antimicrobials were also recommended as first-line antimicrobials for the treatment of secondary bacterial infections. For *Pasteurella* spp. and *Streptococcus* spp., amoxicillin is found adequate, whereas strains of Staphylococcus spp. are usually susceptible in vitro to amoxicillin–clavulanic acid. Some *B. bronchiseptica* isolates and all Mycoplasma organisms are found to be resistant to amoxicillin–clavulanate. *Streptococcus equi* subspecies *zooepidemicus* strains isolated from dogs are found to susceptible to penicillin, amoxicillin, and ampicillin. In cases of pneumonia along with the existence of sepsis, concurrent parenteral administration of either enrofloxacin or marbofloxacin combined with a drug with Gram-positive and anaerobic spectra (ampicillin or clindamycin) was recommended until bacterial culture and antimicrobial susceptibility testing results are obtained.

CHAPTER – III

MATERIALS AND METHODS

3.1 Place of study

The present study was undertaken at Small Animal Clinics of Guru Angad Dev Veterinary and animal Sciences University, Ludhiana.

3.2 Source of animals

3.2.1 Healthy control group

Ten clinically healthy dogs without any evidence of respiratory disease on clinical examination were included in this group. The animals had no history of clinical illness for 6 months to 1 year.

3.2.2 Diseased group

Forty diseased dogs suffering from various respiratory affections, referred to the Small Animal Clinics of Teaching Veterinary Hospital of the university were included in the diseased group. The major clinical signs in these dogs were chronic cough, dyspnoea, costal or abdominal respiration, hemoptysis, respiratory distress, epistaxis, nasal discharge, sneezing, lethargy and weight loss. The dogs suffering from cardiac diseases with clinical signs of exercise intolerance, weakness, coughing, abdominal swelling, syncope, cyanosis, loss of appetite and weight, were excluded from this group.

3.3 History

History of inappetence or anorexia, any change in management (housing and environment), onset of respiratory signs (coughing, nasal discharge, dyspnoea, fever, epistaxis), duration of present illness, weight loss, exercise intolerance and history of any trauma or accident were recorded.

3.4 Physical examination

Physiological parameters viz. colour of visible mucus membranes, rectal temperature (^{o}F), heart rate (bpm) and respiratory rate (breaths/ min) were recorded. Palpation of larynx and trachea for cough induction was done. Auscultation of the lungs was done for normal and abnormal lung sounds (crackles, wheezes, frictional rubs and fluid sounds).

3.5 Hemato-biochemical parameters

Blood was collected from all the healthy animals as well as diseased (clinical) dogs. After proper restraining of the animal, 2 ml of blood was collected aseptically from cephalic/saphenous vein in EDTA coated vials (Hemo Tube, MB Lab consumables). Five ml of blood was collected in serum collection tubes, which was then centrifuged to harvest the serum. The collected serum was refrigerated at -20°C for biochemical estimations.

a) Haematological parameters

After collection, whole blood was used for determination of Hemoglobin (Hb) and total leukocyte count (TLC) done by Fully Automatic Laser Based Hematology Analyser (ADVIA® 2120 Hematology system, Siemens Healthcare diagnostics Inc., USA). Leishman stained blood smears were prepared and evaluated for Differential Leukocytes Count (DLC) by the method described by Jain (1986).

b) Plasma fibrinogen

Plasma fibrinogen (mg/dl) was estimated from the whole blood sample taken in EDTA vials by heat precipitation method using hand held refractometer (Schalm *et al* 1975).

c) Arterial acid base gas analysis

For acid base gas analysis, 1 ml blood was collected from femoral artery of dogs in heparinized syringe (10 IU heparin/ml blood), maintaining the anaerobic conditions. The blood samples were placed on ice and immediately taken to the laboratory for acid base gas analysis. Blood pH, partial pressure of carbon dioxide ($PaCO_2$), partial pressure of oxygen (PaO_2), bicarbonate (HCO_3^-) and base excess (BE) were estimated at 37^0C using IDEXX VetSTAT Electrolyte and blood gas analyzer, USA, within 1 hour of sample collection to avoid alteration in blood gas tension (Fig. 1).

d) Biochemical parameters

Total serum protein (g/dl) and albumin (g/dl) were estimated by the fully automatic Vitros DT 350 Chemistry system (Ortho Clinical Diagnostics, Johnson & Johnson Company). Globulin levels (g/dl) were calculated by subtracting the albumin from the total serum protein and A: G ratio by dividing albumin values by globulin.

Fig. 2: Collection of transtracheal wash (TTW) in a dog

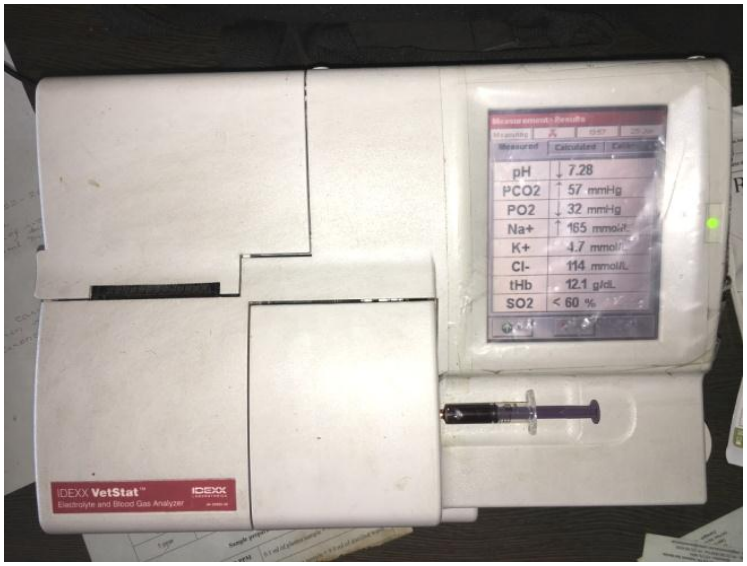

Fig. 1: Vet stat blood gas analyzer

Fig. 3: Transtracheal wash (aspirate)

3.6 Faecal Examination

3.6.1 Faecal sample collection

The fresh faecal sample was collected directly from the rectum of the dog. A total of 50 faecal samples were collected from diseased as well as healthy dogs. The samples were collected in the collection vials and were taken immediately to laboratory.

3.6.2 Floatation concentration technique

1. Two to 5 grams of faeces were weighed and mixed with approximately 10 ml of sugar solution using pestle and mortar.
2. Pour the mixture through a tea strainer into a beaker.
3. This strained solution was poured from beaker into a centrifuge tube.
4. Tube was filled with sugar solution to form a slight positive meniscus and a coverslip was placed on the top of tube.
5. Solution in the tube was left to stand for 30 minutes.
6. Coverslip was removed from tube and placed on the glass slide.
7. Entire area under the cover slip was examined at 10 x and 40 x and used to identify parasites or eggs and to confirm the diagnosis.

3.7 Examination of Nasal Swab

3.7.1 Nasal swab collection

After the proper restraining of the dog, insert the swab parallel to the dog's nose so that it extends to the medial canthus of the eye. This is the maximum depth to which the swab can be inserted into the nasal cavity and varies with the length of the dog's nose.

Hold the stick portion of the swab at the measured length to ensure that the swab has been inserted as far as possible, which maximizes the contact with the respiratory epithelium. To minimize the chances of mucosal damage, quickly but gently twist the swab around its axis as it is advanced into the nasal cavity. A small amount of blood on the swab is usually normal but larger amounts may be seen due to inflammation of the nasal epithelium.

Most of the dogs will have a small amount of blood-tinged discharge and may sneeze blood intermittently following swabbing which is usually self-limiting. Deep

nasal swabs were taken for evaluation of the cell cytology of the nasal cavity of the diseased dogs.

3.7.2 Nasal swab cytology

The nasal swab was rolled gently on the glass slide and a smear was made. The slides were then kept on a hot plate for drying. After drying, the slides were stained with the Leishman stain and were observed under the microscope, first under 10x and then under the 100x (oil immersion lens) for presence of any abnormal cell type.

3.8 Thoracic radiography

a) Radiographic examination

Lateral (mostly right lateral or wherever possible both right lateral and left lateral) and ventrodorsal thoracic radiograph were taken for all healthy and diseased animals and dorsoventral view of nasal cavity was done wherever required. Thoracic radiographs were taken by Small animal X-ray machine (Siemens India, Mumbai) and radiographs were processed using computed radiography system (Kodak, India). These thoracic radiographs were evaluated for different lung patterns (bronchial, interstitial, alveolar, vascular and mixed patterns), opacities, mass or fluid within the chest. The radiographic findings were correlated with the cytologic findings in different respiratory affections.

b) Radiographic interpretation

Radiographic evaluation was done based on radiodensity and visibility of margins of blood vessels (Spasov *et al* 2018).

1. *Alveolar pattern:* An alveolar pattern is obtained when the air present in the alveoli is replaced by material of higher density such as exudate, haemorrhage or oedema fluid. Alveolar pattern is characterized by the presence of air bronchograms and lobe signs. Air bronchogram are seen due to the filling of the alveoli with fluid, a bronchus is clearly visualized in this area while the adjacent vessels become invisible and the lobe sign is the sharp edge formed when a higher density lobe touches a normal lobe. The normal arrangement of artery- bronchus-vein should not be confused with the air bronchograms. In a lateral projection, the artery is always located dorsally to the bronchus and in the VD/DV projection –

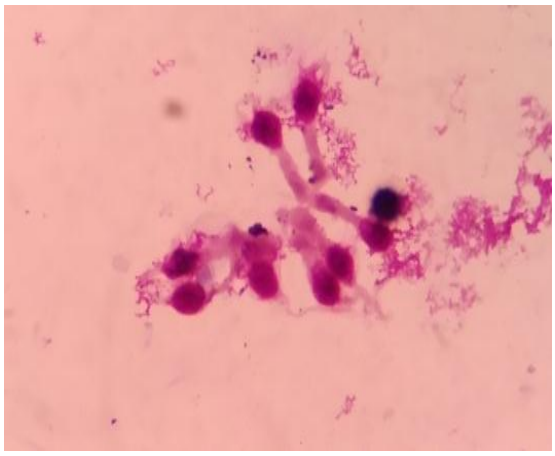

Fig. 5: Ciliated columnar epithelial cells from transtracheal wash in a healthy dog -100x (Leishman staining)

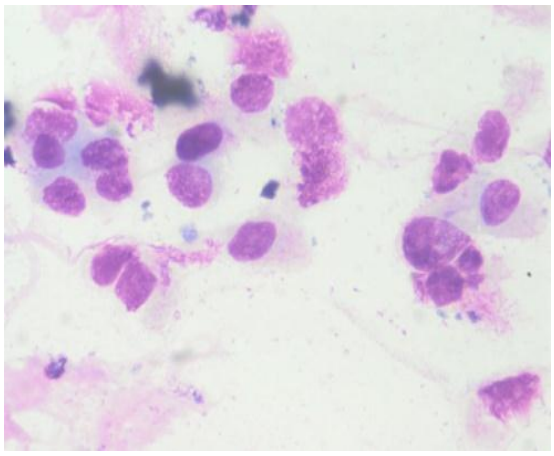

Fig. 6: Sloughed cuboidal epithelial cells in transtracheal wash of a healthy dog-100x. (Leishman staining)

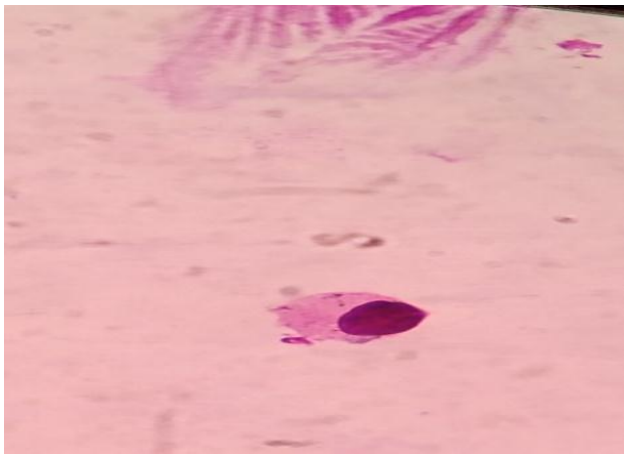

Fig. 7: Normal macrophage and slight mucus in TTW fluid of a healthy dog -100x (Leishman staining)

laterally to the bronchus. These "air bronchogram signs" may be so small as to be called "air alveolograms or bronchiolograms" or so large as to represent segmental bronchi. They are a definitive sign of some type of fluid entering and flooding the air spaces.

2. *Interstitial lung pattern:* An interstitial pattern refers to a lung field or portion of the field that lacks definition of either "air bronchogram sign" as occurs with the alveolar pattern or normal vascular detail. It is of two types: structural (nodular) and non-structural (diffuse). In nodular interstitial pattern an aggregate of cells is observed in the interstitium which are growing and displacing the normal lung tissue. These nodules become visible when they reach 4–5 mm size. It should be considered that the inflammation could also form nodules (under 2 cm) or masses (over 2 cm). A definitive diagnosis cannot be made only based on the radiographic examination. Diffuse interstitial pattern consists of small fibres, military nodules or a combination of both. Normal interstitial structures include alveolar and bronchial walls, the septa between different lobes, as well as the connective tissue supporting the vessels. Each of these structures is radiographically invisible but the presence of fluid, cells or fibrin in the interstitial space makes them denser in a radiography aspect.
3. *Bronchial lung pattern:* The bronchial pattern is obtained when the bronchial wall is infiltrated by cells or fluid or when the peribronchial space is substituted by cells or fluid. The thickening of those structures resulted in enhanced radiographic visualization of the bronchial tree. The most common causes of a bronchial pattern are chronic inflammation, peribronchial oedema, atelectasis, lobe collapse, bronchiectasis, rib fracture, chronic bronchitis, bronchial mineralization, etc. Radiological signs of the bronchial lung pattern are “ring-like shadows” and “tram lines”. Bronchiectasis is an abnormal permanent dilatation of the bronchi. Risk factors include chronic infections, mucociliary disorders, obstruction and aging.
4. *Vascular lung pattern:* A vascular pattern is obtained when there is a larger quantity of blood in the artery or its adjacent vein. This results in a change of the size, form and direction of the vessel which commonly becomes more visible.
5. *Pleural effusions:* Apparent as distinct, horizontal fluid line and a soft tissue opacity that silhouetted the diaphragm present in the ventral thorax caused by pleural fluid.

6. *Mixed lung pattern:* More than one visible lung pattern.

3.9 Ultrasonography

Dogs suffering from the lower respiratory tract affections, with nonspecific radiographic changes were subjected to ultrasonography wherever required. Thoracic ultrasonography was done with the help of portable ultrasound machine (GE Logiq P5) by applying coupling gel, using 2-5 MHz convex transducer. The entire thorax must be examined in a dorsal to ventral direction from the third to twelfth intercoastal space. The findings were correlated with the cytologic findings in different type of conditions. Ultrasonographic interpretation was done as per criteria described by Larson (2009).

3.10 Trans-tracheal wash (TTW)

Trans-tracheal wash was performed by the standard methods outlined by Taylor (2015) in all the dogs which were more than 10 kg body weight (Fig. 2). In the present study, trans-tracheal wash (TTW) was performed using 14 gauge IV cannula and disposable dog catheter (4FG, 50 cm) (SURU International Pvt. Ltd.) Strict asepsis was maintained throughout the procedure. Patient was placed in either sternal recumbency or in a sitting position with the neck extended and the nose elevated towards the ceiling. The ventral neck region including the larynx and the cervical trachea was shaved and aseptically prepared. 1 ml of local anaesthetic drug (2% lidocaine) was infused into the skin and the subcutaneous (SC) tissue. Three 20 ml-syringes were loaded with approximately 6-7 ml of sterile, non-bacteriostatic 0.9 percent saline. The procedure was performed, maintaining utmost asepsis.

The cricothyroid ligament was palpated as a triangular depression distal to the thyroid cartilage. The IV cannula was placed either through the cricothyroid ligament or between the two tracheal rings just distal to the larynx. IV cannula was inserted through the skin and cricothyroid ligament into the tracheal lumen with the bevel facing ventrally. A slight “pop” was felt as the IV cannula passed the ligament. Retching was seen in dogs while using this cricothyroid approach. In few cases, the IV cannula was passed between two tracheal rings on midline, just caudal to the larynx. The cricothyroid ligament approach was found superior as it was easy to locate even in a obese dog. Once the IV cannula is in the tracheal lumen, it was angled down approximately 45^{o} and the needle was removed from the cannula. The

dog catheter was inserted into the tracheal lumen through the cannula. Minimal resistance was noticed when the catheter was advanced properly. In some cases, when resistance was encountered, the catheter was reinserted without any resistance. Coughing reflex was observed every time when cathetar was in the right direction. The catheter was passed down into the tracheal lumen up to the level of 6^{th} intercostal space (approximately at the level of carina). The use of dog tube was advantageous as it was sufficiently long and rigid enough to pass down the trachea easily.

The preloaded syringe was attached to the catheter and the sterile saline was infused into the tracheal lumen. In some cases, an assistant performed coupage during instillation of the fluid so as to promote coughing. The infused saline was aspirated back into the syringe. When excess of air was aspirated, the syringe was detached from the catheter and the excess air was expelled out so as to aspirate as much fluid back as possible. Mostly 10 percent or less of the infused volume was recovered. The procedure was repeated mostly with all the 3 preloaded syringes to recover sufficient amount of aspirate (Fig. 3). After an adequate sample was obtained, the syringe was removed along with the catheter from the trachea in a very smooth motion and a soft, padded bandage laced with betadine was placed over the catheter site for approximately1hour to minimize the formation of SC emphysema (Fig. 2).

3.11 Endotracheal Wash (ETW)

Endotracheal wash was performed by the standard method described by Taylor (2015). ETW sampling was done in smaller patients (cats and dogs <10kg) due to their smaller tracheal lumen diameter which prevents the placement of IV cannula between the tracheal rings. ETW can be considered in the larger dogs that are very ferocious or resistant to restraint and in which doing a transtracheal wash would result in clinician or patient injury. However in the present study, ETW was done only in the brachycephalic breeds. In ETW, higher risk to the patient was involved, as the general anesthesia was required and also the anesthesia prevents the cough reflex. So, the recovered fluid was found as the more representative sample of the large airways and also there were more chances of oropharyngeal contamination.

The patient was preoxygenated for 5-10 minutes before the anesthesia. During this period, three 20 ml- syringes were preloaded with approximately 5 ml of sterile, non- bacteriostatic 0.9 percent saline. As the risk of bronchospasm was involved

during the procedure, so bronchodilators were given in dogs which are suspected with inflammatory airway disease. Terbutaline was given @ 1.25-5mg/kg/day PO q12 hours before the procedure and also final dose being given 2-4 hours before the anesthetic induction. In most of the patients induction was done with the injectable anesthetics such as propofol, due to the inability to provide oxygen and the inhalant anesthesia during the procedure. These short-acting anesthetics allow quick recovery after the procedure.

Similar to TTW, aseptic technique was followed for an endotracheal lavage. The patient was placed in sternal or lateral recumbency with the affected side down. A sterile ET tube was carefully passed through the arytenoids cartilages into the trachea, avoiding the contamination with the oropharyngeal secretions. The anesthesia machine tubing was never attached to the ET tube till the wash had been performed.

As soon as the patient was intubated, baby feeding tube (5 FG) was put through the ET tube while maintaining sterility. A preloaded syringe was then attached to the catheter and sterile saline was infused through the catheter as described in TTW. Then infused saline was aspirated back into the syringe.

After a total of 1.5 to 3 ml of turbid fluid was obtained in the trans-tracheal wash (TTW) or ETW, the fluid was transferred from the syringe into the sterile tube collector and was properly labelled which was then carried to the laboratory as soon as possible on ice packs. Separate samples were collected for culturing and cytological examination.

3.11.1 Cytology of transtracheal wash (TTW)

Two ml aliquots of TTW fluid was obtained from the animal and was transferred from the syringe into EDTA vials for cytological analysis. Samples were first measured for volume, although they were not filtered. The samples were centrifuged (1000 rpm, 5 minutes) and smears were prepared from the sediment and stained with Leishman's stain. Two hundred cells from each sample were counted for differential cell counts (Finke 2013).

3.11.2 Staining

Rapid staining was done using Gram's stain and the smears were observed for the presence of bacteria. However, Lactophenol Cotton Blue Staining was also done on the same samples for the presence of fungal spores.

3.11.3 Bacteriological culture of TTW

At least 2 ml aliquot of TTW was transferred into sterile containers for bacteriology and was kept on ice packs till processed. All the samples were processed within 1-2 hours of collection. In few cases, samples were centrifuged at 3000 rpm for 5 min and the supernatant was discarded but in others, sample was directly used for culture. The sediment was cultured on 5 per cent defibrinated sheep blood agar and was incubated overnight at 37°C in aerobic conditions and for fungal isolation, the sediment was cultured on sabouraud's dextrose agar (SDA) and was incubated for one week at 37◦C. All isolates were characterized using the growth, staining and biochemical characteristics (Quinn *et al* 1994). The bacterial isolates were purified by picking single colony and subculturing it on fresh blood agar plates. On the basis of the colonial morphology, gram staining, further streaking on differential and specific media, catalase and oxidase tests, bacterial isolates were identified up to the species level.

a) Characterization of isolates

For the isolation of the etiological agent, tracheal fluids were cultured on Blood agar, Brain heart infusion (BHI) agar and Sabouraud Dextrose agar media. The Gram-positive cocci were subjected to the catalase test to differentiate between Staphylococci and Streptococci. *Staphylococcus* produces medium sized round, smooth white coloured colonies on Blood agar. On gram staining, violet coloured cocci in irregular clusters or shape of bunch of grapes were found which were positive for the catalase test and negative for the oxidase test. Round black colored colonies were observed on Baird-Parker Media which is a selective and diagnostic media for *Staphylococcus aureus.*

Klebsiella and *E. coli* were differentiated on the basis of growth on Eosin methylene blue agar (EMB) and MacConkey's lactose agar (MLA) media. Gram negative rods with a greenish metallic sheen on the EMB agar are characteristic of *E. coli* that produces large, pale colonies on MLA agar. Non-hemolytic, mucoid colonies on blood agar which is further isolated on MLA and appeared as pink colour circular, mucoid and smooth lactose fermenting colonies, are the characteristic for *Klebsiella pneumoniae*. On gram staining, *Klebsiella pneumoniae* were seen as pink coloured rod-shaped bacteria.

b) Antibiogram of bacterial isolates

The antibiotic sensitivity test (AST) of the isolates was performed on Nutrient agar or Mueller-Hinton agar (Hi-Media, India) by Kirby-Bauer disc diffusion method. The isolates were tested for sensitivity against 9 antibiotics (HiMedia, India); amikacin, amoxyclav, ampicillin, ceftriaxone-tazobactum, ciprofloxacin, cloxacillin enrofloxacin, gentamicin and tetracycline. The results were read after 24 hours of incubation at 37°C on the basis of the size of zone of inhibition. The interpretation of the results as sensitive or resistant to antibacterials was done as per the manufacturer's instructions.

3.12 Necropsy

Necropsy was conducted in two animals suffering from severe respiratory distress which died during treatment. Macroscopic and microscopic post mortem findings were recorded to confirm the diagnosis. Tissue samples were collected in 10 per cent buffered formalin for histopathology. Samples were processed routinely and stained with hematoxylin and eosin and examined with an optical microscope.

3.13 Treatment

Treatment was initiated in every dog affected with respiratory infection with amoxicillin-clavulanic acid/ amoxicillin-sulbactum @15 mg/kg PO or IM 12h for 3 days followed by antimicrobial therapy based on antibiotic sensitivity test results of the TTW samples along with Terbutaline @1.25-5mg/dog PO 12 h up to 2 weeks. Fluoro-quinolone (enrofloxacin) or aminoglycosides (gentamicin) was added depending upon the clinical condition of the patient. The supportive therapy with oxygen and fluids was done as and when needed. The hematology and radiography was repeated after two weeks in clinically recovered dogs. However, the treatment was extended up to 3 to 4 weeks in convalescing dogs.

3.14 Statistical analysis

Means and standard errors for each evaluated variable of healthy and diseased groups were calculated using the descriptive statistical procedures. Statistical analyses were performed using the SAS software. Mann-Whitney Test was used for comparison of median values between the groups. Wilcoxon Signed Rank Test was used to compare median values between pre and post treatment groups.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Clinically healthy dogs without any respiratory sign

Clinically healthy dogs had no history of any clinical illness for 6 months to 1 year. All the animals were having normal appetite and performance. Breathing was normal, quiet and apparently effortless. Mucous membrane was pink and moist. Vital parameters viz. heart rate, rectal temperature and respiratory rate were within normal range. On auscultation of the lungs, no abnormal sounds were heard. It has been mentioned that normal lung sounds are produced by the high velocity turbulent airflow in the trachea and bronchi and heard through a stethoscope. In bronchioles, low velocity laminar flow produces no sound (Reece 2015). The mean age of healthy animals was 3.8±0.52 years. The mean body weight was 24.68±2.36 Kg. The mean rectal temperature was 102.12±0.20°F which was in normal range. The mean heart rate and respiration were 99.2±5.23 bpm and 27.6±2.29 breaths per minute, respectively.

4.1.1 Hemato-biochemistry

Blood represents an important medium for assessment of the health status of the animals. Through the hematological and biochemical analyses of the blood, the physiological and the pathological conditions of the animals can be assessed (Khan *et al* 2011). The mean hemoglobin concentration was 13.00±0.78 g/dl which was within the reference range (12.0-18.0 g/dl) described by Rizzi *et al* (2011). Mean packed cell volume was 39.76±2.13 percent with levels varying from 31.5 to 49.9 percent in healthy control animals without evidence of any respiratory sign. Mean levels of erythrocytic count (TEC) in dogs was $6.03\pm0.32 \times 10^6$ /µl. These findings fall within the reference range of PCV (37-55 %) and TEC ($5.5\text{-}8.5 \times 10^6$ /µl) as documented by Rizzi *et al* (2011).

The mean total leukocyte count in healthy controls was 11376.00±1372 cells/µl which was similar to levels (6000-17000 cells/µl) reported by Rizzi *et al* (2011). Mean absolute neutrophil count was 7724±1114 cells/µl whereas mean

platelet count was 228.80±39.9×10^3/µl which was similar to the reference range of 3000-11500 cells/µl and 200-500 ×10^3/µl, respectively reported by Rizzi *et al* (2011).

Acute phase response is the innate nonspecific immune reaction of the body. Acute phase proteins (such as fibrinogen) production is mainly triggered by different stimuli including trauma, stress, infection, inflammation and neoplasia (Pradeep 2014). Mean plasma fibrinogen value in healthy dogs in the present study was 3.2±0.3 g/L which lies in the normal reference range (1.3-4.8 g/L) documented by Jolivet *et al* (2017). Mean total protein and albumin levels in healthy dogs were 6.38±0.08 g/dl and 2.77±0.11 g/dl, respectively. Mean albumin to globulin ratio in healthy group was 0.78±0.05 which within the levels for healthy dogs established by Klaassen (1999) and Krimer (2011).

4.1.2 Blood gas analysis

Acid-base and electrolyte assessment is vital to monitor health, specify diagnosis and therapy of disease and perform nutritional trials (Bouda *et al* 2009). Blood gas analysis gives us an estimate of animal's oxygenation and acid-base status (Kules *et al* 2015). Thus, acid base status provides significant data mainly associated with the respiratory, endocrine, digestive and urinary systems (Bouda and Jagos 1991).

The mean arterial blood pH of healthy dogs was 7.44±0.01 in our study, which falls in the normal pH range (7.37-7.44) measured by Kules *et al* (2015) in 40 healthy dogs. Mean anion gap in our animals was 16.67±0.70 mmol/L that were comparable with the levels described by Kules *et al* (2015) (5.49-19.67 mmol/L) in their study. The mean partial pressure of carbon dioxide ($PaCO_2$) was 27.17±1.25 mmHg whereas the mean bicarbonate, base excess, partial pressure of oxygen and oxygen saturation were 17.17±0.34 mmol/L, -3.85±0.42 mmol/L, 87.17±1.74 mmHg and 93.00±1.06 percent, respectively. Mean sodium, potassium and chloride levels were 135.17±3.71 mmol/L, 3.41±0.12 mmol/L and 105.17±2.94 mmol/L, respectively, as per study undertaken by Kules *et al* (2015).

4.1.3 Transtracheal wash cytology

Normal transtracheal wash (TTW) include cells that are easily washed away from the proximal mucosal surface and comprise respiratory epithelial cells (ciliated

and nonciliated columnar to cuboidal epithelial cells), macrophages, neutrophils, lymphocytes, eosinophils and mucus (Burkhard *et al* 2001). Cytologic interpretation should include estimated cellularity, differential cell counts and morphologic description of the cells encountered (Creevy 2009).

Mean cell number (cells per HPF) in the tracheal wash smears of the healthy control animals was 8.35±2.68 cells. Burkhard *et al* (2001) reported that the tracheal wash from healthy dogs usually had low cellularity. Tracheal wash smears in healthy animals in our study comprised 49.57±5.28 percent pulmonary alveolar macrophages (Fig.7) and 11.43±1.00 percent neutrophils (Fig. 4). Dunn (2010) in his study also recorded alveolar macrophages as the predominant cell in tracheal wash of clinically normal animals whereas neutrophils constituted only 5-8 percent of the total nucleated cell count (Dunn 2010, Rozanski 2014).

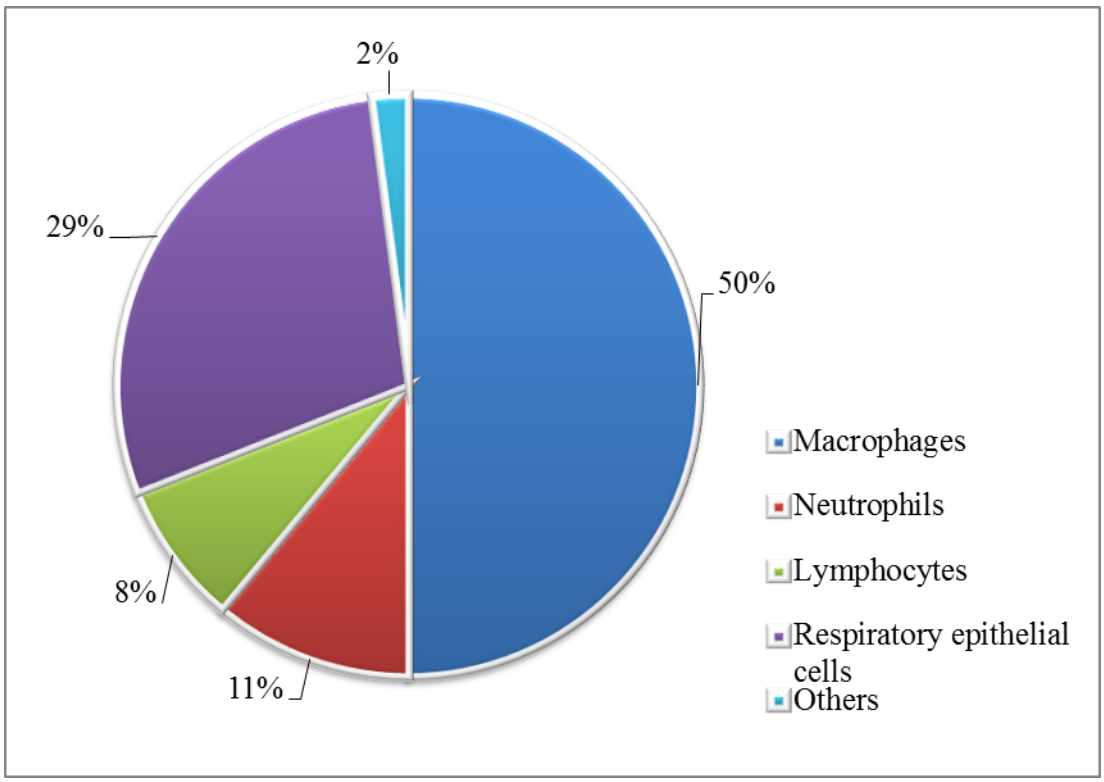

Fig. 4: Transtracheal wash (TTW) cytology of healthy animal

Overall 29.14±7.13 percent respiratory epithelial cells were observed in tracheal wash fluid from healthy dogs in the present study (Fig.4). Trachea and bronchi are lined by the ciliated columnar epithelial cells (Fig.5) and bronchioles by ciliated and non-ciliated cuboidal epithelial cells (Fig. 6). Epithelial cells may be

present as single cell or clusters in tracheal wash (Dunn 2010). TTW sample primarily represents the central large airways, hence it has a larger population of ciliated columnar epithelial cells (McCullough and Brinson 1999).

Nearly 7.71±2.25 percent lymphocytes, 0.14±0.14 percent eosinophils and 2±0.79 percent other cells (mast cells, plasma cells, basophils etc.) were observed in tracheal aspirates in our study. Dunn (2010) proposed that the tracheal aspirates should have 5 – 14 percent lymphocytes, <5 percent eosinophils and rarely mast cells (<2%).

4.1.4 Culture of TTW

Transtracheal wash (TTW) was collected from seven healthy dogs and it was cultured on Blood agar, Brain heart infusion agar and Sabouraud dextrose agar which were incubated for 18 -24 hours at 37 ºC. Culture reports were negative for all TTW samples.

4.1.5 Radiography of Healthy dogs

Thoracic radiography plays a vital role in investigation of the different respiratory diseases (Miller 2007). Thoracic radiographs were found normal in all the healthy dogs in the present study. Cardiac diseases can be differentiated from respiratory diseases using radiography especially in coughing dogs (Spier 2011).

4.2 Identification of different respiratory affections in dogs

4.2.1 Upper respiratory tract affections in dogs

4.2.1.1 Nasal squamous cell carcinoma (n=3)

Dogs rarely have tumors of nasal planum. Most of the nasal tumors in dogs are malignant. Squamous cell carcinoma (SCC) was seen as the most common tumour of nasal cavity. However, cutaneous lymphoma, fibrosarcoma, melanoma, haemangioma, mast cell tumour, fibroma, and eosinophillic granulomas have also been recorded (Withrow 1996).

Three dogs (2 German shepherd and 1 Non-descript) were presented to the small animal clinics of Teaching Veterinary hospital of the university with history of epistaxis/bleeding (Fig.8) and sneezing. Bilateral epistaxis was seen in 2 and unilateral in 1 along with sneezing in 1 dog. Other clinical observations include

tachypnea in 1, fever in 1, lethargy in 2, decreased appetite in 1 and occasional non productive cough in 1 animal. Mean age of the affected dogs in our study was 4.17±1.96 years. McEntee (2004) and Tasker *et al* (1999) identified the occurrence of sinonasal tumors in 33% of middle to old aged dogs. All the clinical signs in dogs having squamous cell carcinoma were found similar to signs reported by Lascelles *et al* (2000). In one study, medium to large breed dogs were seen more prone to SCC (Lana and Withrow 2001) whereas in other report, dolichocephalic breeds were found to be on higher risk as compared to brachycephalic dogs (Wilson and Dungworth 2002, Rief *et al* 1998). Lana and Withrow (2001) also recorded the slight predilection for male dogs. The said justification is not valid for the present study as the number of dogs is just 3 comprising 2 males and one female dog. No metastasis was noticed in lung tissue in any dog though harsh lung sounds were heard during auscultation in two dogs whereas the third dog revealed normal lung sounds. The mean respiratory rate was found to be 34.67±7.06 breaths/min (range 24-48). The mean rectal temperature (102.33±0.88 °F) was in normal range similar to that of control group. The mean heart rate was higher than the healthy control animals (110.67±14.67 vs 99.2±5.23 beats/min, respectively) (Table 1).

Table 1: Comparison of age and physiological parameters (mean ± SE) between healthy and squamous cell carcinoma dogs

Parameters	Healthy (n=10)	Squamous cell carcinoma (n=3)
Age (years)	3.8±0.52 (1.5-7)	4.17±1.96 (1.5-8)
Body weight (Kg)	24.68±2.36 (13.7-35)	26±6.24 (17-38)
RT (ºF)	102.12±0.20 (101.4-103.2)	102.33±0.88 (101-104)
HR (bpm)	99.2±5.23 (76-136)	110.67±14.67 (96-140)
RR (breaths/min)	27.6±2.29 (18-40)	34.67±7.06 (24-48)

Values in parenthesis depicts range

Table 2: Comparison of hemato-biochemical parameters (mean ± SE) between healthy and squamous cell carcinoma dogs

Parameters	Healthy (n=10)	Squamous cell carcinoma (n=3)
Hb (g/dl)	13±0.78 (9.3-16.5)	11±1.83 (8.6-14.6)
TEC (count/µl × 10^6)	6.03±0.32 (4.5-7.47)	5.29±1.11 (3.27-7.11)
TLC (count/µl)	11376±1372 (6790-19100)	20996.7±9386.7 (9000-39500)
PCV (%)	39.76±2.13 (31.5- 49.9)	33.27±5.72 (26.2-44.6)
Platelets (count/µl×10^3)	228.8±39.9 (119-542)	218.67±16.60 (196-251)
Absolute neutrophils (count/µl)	7724±1114 (2717-12926)	17723.6±7964.74 (6660-33180)
Absolute lymphocytes (count/µl)	3243±790 (1161-9168)	2386.47±889.62 (869.4-3950)
Absolute eosinophillia (count/µl)	409±120 (0-901.8)	886.6±746.42 (0-2370)
Total protein (g/dl)	6.38±0.08 (5.9-6.8)	6.67±0.24 (6.2-7)
Albumin (g/dl)	2.77±0.11 (2.4-3.4)	2.9±0.06 (2.8-3)
Globulin (g/dl)	3.61±0.10 (3.1-4.1)	3.77± 0.26 (3.3-4.2)
A : G ratio	0.78±0.05 (0.58-1.10)	0.78±0.06 (0.67-0.88)
Fibrinogen (g/dl)	0.32±0.03 (0.2-0.4)	0.47±0.07 (0.4-0.6)

Values in parenthesis depicts range

a) Hemato-biochemical parameters

Mean hemoglobin concentration in affected dogs was 11±1.83 g/dl which was lower as compared to the healthy control group (13±0.78 g/dl). The total leukocyte count in these dogs was 20996.7±9386.7 cells/µl which was significantly higher than

the healthy control group (11376±1372 cells/µl) (Table 2). A marked inflammatory response of the nasal mucosa followed by secondary bacterial infection could be the cause of leucocytosis which was corroborated by increase number of polymorphonuclear cells in the cytological examination. Similarly, the absolute neutrophils count was significantly higher (17723.6±7964.74 cells/µl) when compared to the healthy control group (7724±1114 cells/µl) in dogs with squamous cell carcinoma (Table 2).

Serum report showed mean albumin:globulin ratio of 0.78±0.06. Mean plasma fibrinogen level in diseased dogs (0.47±0.07 g/dl) was higher as compared to the healthy group (0.32±0.03 g/dl) (Table 2). Pradeep (2014) recorded the increase in fibrinogen level in inflammation, neoplasia, stress, infection and trauma.

b) Blood gas analysis

The mean partial pressure of oxygen and oxygen saturation of SCC group (88.5±1.22 mmHg and 94.5±1.22 %, respectively) was near normal when compared with the healthy group (Table 3).

c) Nasal Swab cytology

Acute or chronic nasal discharge whether unilateral or bilateral is commonly portrayed as a feature of upper respiratory disease and can be serous, suppurative, mucoid, to serosanguineous depending upon the underlying cause. However, it can be present with infectious, inflammatory or neoplastic disorders involving the lower airways. Epistaxis is mostly seen with trauma, fungal infections and neoplasia (Brown 2010). Cytological examination of nasal swab is the simplest and non-invasive technique and has been recommended as a preliminary test in all nasal diseases (De Lorenzi 2006).

In our study, clusters of pleomorphic epithelial cells with abundant cytoplasm and centrally placed nuclei were observed, indicative of squamous cell carcinoma (Fig. 10). Squamous cell carcinoma is mainly characterized by the presence of cells with angular borders, rich in homogenous, glassy cytoplasm and centrally placed nuclei. Neoplastic cells depicted a varied range in maturation, ranging from immature small, cuboidal, nucleated, epithelial cells with deeply basophilic cytoplasm to mature anucleated, fully keratinized cells containing abundant, pale, basophilic cytoplasm, as described by Kleiter and Malarkey (2004).

Table 3: Comparison of blood gas analysis (mean ± SE) in healthy and squamous cell carcinoma dogs

Parameters	Healthy group (n=6)	Squamous cell carcinoma (n=3)
pH	7.44±0.01 (7.38-7.48)	7.47±0 (7.47-7.47)
$PaCO_2$ (mmHg)	27.17±1.25 (23-32)	27±1.63 (25-29)
HCO_3 (mmol/L)	17.17±0.34 (15.6-17.9)	18.25±0.94 (17.1-19.4)
AnGap (mmol/L)	16.67±0.70 (14.4-18.7)	17.45±2.82 (14-20.9)
tCO_2 (mmol/L)	18±0.36 (16.3-18.8)	19.1±0.98 (17.9-20.3)
BE (mmol/L)	-3.85±0.43 (-5.5- -2.5)	-2.9±0.41 (-3.4- -2.4)
$stHCO_3$ (mmol/L)	20.68±0.30 (19.7-21.5)	21.65±0.53 (21-22.3)
st pH	7.34±0.01 (7.32-7.35)	7.36±0.01 (7.34-7.37)
cH+ (nmol/L)	35.98±1.32 (33.1-41.9)	33.7±0.16 (33.5-33.9)
PaO_2 (mmHg)	87.17±1.74 (81-92)	88.5±1.22 (87-90)
tHb (g/dL)	12.57±0.67 (11-15.3)	10.35±1.10 (9-11.7)
SO_2 (%)	93±1.06 (89-97)	94.5±1.22 (93-96)
Na+ (mmol/L)	135.17±3.71 (119-142)	140±7.35 (131-149)
K+ (mmol/L)	3.41±0.12 (3-3.78)	3.45±0.04 (3.4-3.5)
Cl- (mmol/L)	105.17±2.94 (92-111)	103±1.63 (101-105)

Values in parenthesis depicts range

Fig. 8: Epistaxis in dogs with nasal squamous cell carcinoma

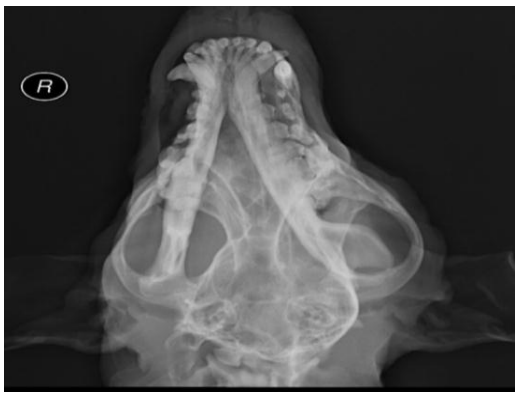

Fig. 9: A dorsoventral radiograph of nasal cavity showing soft tissue density in the nasal cavity and maxillary sinus in a dog with squamous cell carcinoma

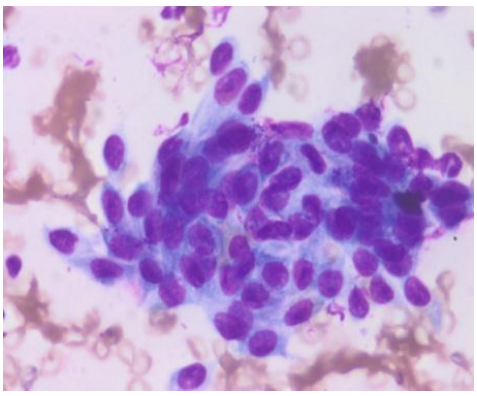

Fig. 10: Cluster of pleomorphic epithelial cells in nasal swab smear cytology suggestive of squamous cell carcinoma-100x (Leishman staining)

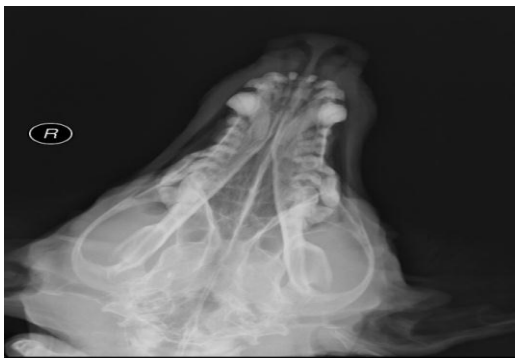

Fig. 11: A dorsoventral radiograph of nasal cavity showing normal nasal passage in a dog with allergic rhinitis.

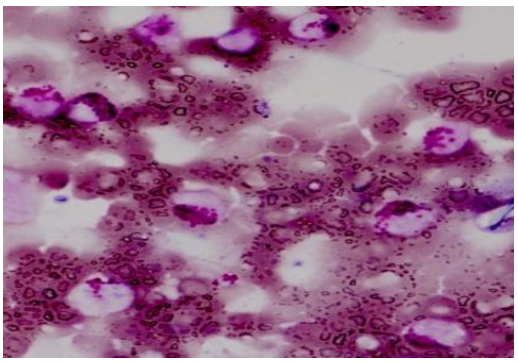

Fig. 12: Large number of eosinophils in nasal swab smear cytology in dog with a allergic rhinitis-100x (Leishman staining)

d) Radiography

Radiograph of nasal cavity (DV view) was normal in two dogs and increased soft tissue density in nasal cavity and maxillary sinus was observed in one dog (Fig. 9). Nasal radiographs are usually beneficial in advanced cases as the changes recorded in early cases are minimal (Ruslander 2011). Thoracic radiographs were found normal in our study at the time of diagnosis similarly as described by Lana and Withrow (2001). Olgilvie and Moore (2006) reported the presence of soft-tissue densities within the nasal cavity along with bony destruction and proliferation as the consistent radiographic sign with the sinonasal tumors.

4.2.1.2 Allergic rhinitis

In dogs and cats, allergic rhinitis has not been well documented. Airborne allergens producing hypersensitivity response within the nasal cavity and sinuses is expressed as allergic rhinitis (Nelson and Couto 2014). Seasonal cause of allergic rhinitis is mainly the pollen production and annually due to house dusts and molds. Sudden rhinitis can be caused by lodging of foreign objects in the nasal cavity or due to inhalation of smoke or irritant gases (Kuehn 2016).

A 10 year old female German shepherd was presented to the small animal clinics with history of intermittent epistaxis and sneezing from 3 months. Dog was found to be alert and active with normal appetite. Allergic rhinitis was found to be a cause of intermittent epistaxis in dogs in a previous study (McDougal 1977). Sneezing and serous or mucopurulent nasal discharge in allergic rhinitic dogs was recorded by Nelson and Couto (2014). After taking detailed history from the owner it was found that new furniture was brought to the house, which could be a source of allergy due to being recently varnished. Nelson and Couto (2014) stated that the worsening of signs can occur in definite seasons, due to cigarette smoke or after the introduction of brand new kitty litter or new perfumes, cleaning agents and new furniture or fabric for the house. The respiratory rate (52 breaths/min) of the diseased dog was found to be higher than the healthy control group (27.6±2.29 breaths/min). The heart rate and rectal temperature were 88 beats/min and 102.2 °F, respectively, which were found to be in the normal range as in the control dogs (Table 4).

Table 4: Comparison of age and physiological parameters (mean ± SE) between healthy dogs and allergic rhinitic dog

Parameters	Healthy (n=10)	Allergic rhinitis (n=1)
Age (years)	3.8±0.52 (1.5-7)	10
Body weight (Kg)	24.68±2.36 (13.7-35)	35
RT (ºF)	102.12±0.20 (101.4-103.2)	102.2
HR (bpm)	99.2±5.23 (76-136)	88
RR (breaths/min)	27.6±2.29 (18-40)	52

Values in parenthesis depicts range

a) Hemato-biochemical parameters

The hemoglobin concentration in the diseased dog was 12.9 g/dl. The total leukocyte and neutrophil counts in allergic rhinitic dog (15250 and 10370 cells/µl, respectively) were higher as compared to the healthy control group (11376±1372 and 7724±1114 cells/µl, respectively). Absolute eosinophilia was observed in this dog (2440 cells/µl) when compared with the healthy control group (409±120 cells/µl). Total protein was found higher in diseased dog (8.2 g/dl) in comparison to the healthy animals 6.38±0.08 g/dl (range 5.9-6.8) (Table 5). Globulin concentration was higher (6 g/dl) whereas albumin was found to be on lower side in diseased dog (2.2 g/dl). Albumin to globulin ratio in diseased dog (0.37) was lower than the healthy group (0.78±0.05) (Table 5). The fibrinogen concentration was higher in diseased dog (0.6 g/dl) as compared to the healthy group (0.32±0.03 g/dl). Solter *et al* (1991) recorded significant changes in the acute phase proteins even in the absence of changes in total or differential WBC count in a retrospective assessment conducted in dog suffering with inflammatory conditions. Patel and Nagpal (2014) did not find eosinophilia in most of the human patients suffering from allergic rhinitis.

Table 5: Comparison of hemato-biochemical parameters (mean ± SE) between healthy and allergic rhinitis group

Parameters	Healthy (n=10)	Allergic rhinitis (n=1)
Hb (g/dl)	13±0.78 (9.3-16.5)	12.9
TEC (count/µl × 10^6)	6.03±0.32 (4.5-7.47)	6.87
TLC (count/µl)	11376±1372 (6790-19100)	15250
PCV (%)	39.76±2.13 (31.5- 49.9)	41.3
Platelets (count/µl×10^3)	228.8±39.9 (119-542)	285
Absolute neutrophils (count/µl)	7724±1114 (2717-12926)	10370
Absolute lymphocytes (count/µl)	3243±790 (1161-9168)	3965
Absolute eosinophillia (count/µl)	409±120 (0-901.8)	2440
Total protein (g/dl)	6.38±0.08 (5.9-6.8)	8.2
Albumin (g/dl)	2.77±0.11 (2.4-3.4)	2.2
Globulin (g/dl)	3.61±0.10 (3.1-4.1)	6
A : G ratio	0.78±0.05 (0.58-1.10)	0.37
Fibrinogen (g/dl)	0.32±0.03 (0.2-0.4)	0.6

Values in parenthesis depicts range

b) Blood gas analysis

The partial pressure of oxygen (PaO_2) in diseased dog (71 mmHg) was less as compared to the healthy control group (87.17±1.74 mm Hg) whereas the saturation of oxygen in diseased dog was found to be within normal levels (93%). No significant difference was seen in other parameters (Table 6).

Table 6: Comparison of blood gas analysis (mean ± SE) in healthy and allergic rhinitis group

Parameters	Healthy group (n=6)	Allergic rhinitis (n=1)
pH	7.44±0.01 (7.38-7.48)	7.47
$PaCO_2$ (mmHg)	27.17±1.25 (23-32)	28
HCO_3 (mmol/L)	17.17±0.34 (15.6-17.9)	19.1
AnGap (mmol/L)	16.67±0.70 (14.4-18.7)	21.4
tCO_2 (mmol/L)	18±0.36 (16.3-18.8)	20
BE (mmol/L)	-3.85±0.43 (-5.5- -2.5)	-1.8
$stHCO_3$ (mmol/L)	20.68±0.30 (19.7-21.5)	22.5
st pH	7.34±0.01 (7.32-7.35)	7.38
cH+ (nmol/L)	35.98±1.32 (33.1-41.9)	33.8
PaO_2 (mmHg)	87.17±1.74 (81-92)	71
tHb (g/dL)	12.57±0.67 (11-15.3)	12.9
SO_2 (%)	93±1.06 (89-97)	93
Na+ (mmol/L)	135.17±3.71 (119-142)	148
K+ (mmol/L)	3.41±0.12 (3-3.78)	4.3
Cl- (mmol/L)	105.17±2.94 (92-111)	112

Values in parenthesis depicts range

c) Nasal Swab cytology

On cytological examination of nasal swab, scattered neutrophils along with increased number of eosinophils was observed (Fig. 12). Nelson and Couto (2014) observed eosinophilic inflammation in nasal biopsy in allergic rhinitic dogs. Increased number of eosinophils was observed in nasal smears of 70% of the cases (Binder et al 1984). A good correlation has been observed between nasal smear eosinophila and clinical score severity in human allergic rhinitis patients (Patel and Nagpal 2014) suggesting it, a valuable test for diagnosis of allergic rhinitis.

d) Radiography

Nasal cavity radiograph (DV view) was found normal in the rhinitic dog (Fig. 11). Many diseases can lead to non-destructive non-neoplastic changes which are non-specific. A normal nasal radiograph doesn't rule out nasal disease. Even with chronic foreign bodies, localized inflammatory changes may be seen, although nasal radiographs are generally normal (McConnell 2008).

4.2.1 Lower respiratory tract affections in dogs

4.2.1.1 Bacterial bronchopneumonia (n=7)

Bacterial Bronchopneumonia/bacterial pneumonia (BP) is one of the most commonly occurring type of pneumonia. It occurs as a result of primary disease process or due to injury to the lung, either of these that would result in compromising lung's innate immunity (Amrute *et al* 2009). This immunodeficiency will let the resident bacteria of the respiratory system to proliferate and cause infection. Bronchopneumonia is a challenging condition to diagnose due to the wide range of clinical signs exhibited by the patient (Carey 2009).

Lungs can be infected by a wide variety of the bacteria. Common bacterial isolates which can proliferate under the favourable conditions, are mostly gram negative aerobes and include *Bordetella bronchiseptica*, *Pasturella*, *Klebsiella*, *Proteus* spp. and *E.coli*. Gram positive organisms *Staphylococcus* and *Streptococcus* can also be isolated. The exact role of *Mycoplasma* spp in pneumonia in dogs is not known (Corcoran *et al* 1999). Predisposing factors leading to bacterial pneumonia include aspiration of ingested food material or gastric contents accidently or due to megaoesophagus and cleft palate, or other conditions causing aspiration pneumonia. Comparatively low clearance of debris in chronic respiratory diseases such as chronic bronchitis, ciliary dyskinesia and bronchiectasis could be another factor. Inhalation/

migration of foreign substances; immunosuppression caused from drugs, malnutrition, stress, endocrinopathies; systemic viral infections (canine distemper and canine influenza); and rarely neoplasia, fungal or parasitic infection could also cause bronchopneumonia due to interference in the clearance of debris from the respiratory tract (Nelson and Couto 2014).

Seven dogs were presented to small animal clinics of Teaching Veterinary hospital of the university with history of cough, bilateral nasal discharge, exercise intolerance and respiratory distress. Severe bilateral nasal discharge was there in 5 dogs (mucopurulent=3, serous=2) with no nasal discharge in 2 dogs along with productive cough (marked=4, moderate=3). Other clinical observations include dyspnea in 3, tachypnea in 3, fever in 1, lethargy in 3, weight loss in 2, dehydration in 4, exercise intolerance in 5, decreased appetite in 3 and positive inducible cough reflex in 6 dogs. Mean age of dogs affected with bacterial bronchopneumonia was (4.8±1.02 years). All the clinical signs in dogs suffering with bacterial bronchopneumonia were similar to the respiratory signs documented by Corcoran (2004).

Abnormal lung sounds were heard during auscultation in all the dogs with apparent crackles in 2, wheezes in 2 and harsh sounds in 3 animals. The mean respiratory rate was significantly higher than the healthy control animals (70.14±10.04 vs. 27.6±2.29 breaths/min, respectively; $P<0.05$). The mean rectal temperature was 102.57±0.54 °F. The mean heart rate was significantly higher than the healthy control group (115.86±6.77 vs 99.2±5.23 beats/min, respectively; $P<0.05$) (Table 7). Wide range of clinical manifestations were shown by dogs suffering with bronchopneumonia, ranging from mild coughing, lethargy, fever, rapid progressive weight loss and ultimately leading to fatal clinical syndrome (Carey 2009). Involvement of pulmonary parenchyma was found to be the reason for more severe signs in bronchopneumonia in dogs. However, some animals may exhibit no or few of the signs (Bailiff and Norris 2002) which normally range from fever, anorexia, lethargy, weakness, dehydration, weight loss, serous to mucopurulent nasal discharge, moist or productive cough, tachypnea, tachycardia, crackles, wheezes, harsh lung sounds and cyanosis (Kogan *et al* 2008, Tart *et al* 2010). Absence of fever in 6 dogs in the present study could be due to the fact that dogs were presented after 1 to 8 months of sickness. Fecal floatation was performed in all dogs suffering with bacterial bronchopneumonia to rule out parasitic infection and was found negative for presence of any parasitic eggs or larvae.

Table 7: Comparison of age and physiological parameters (mean ± SE) between healthy and bacterial bronchopneumonia

Parameters	Healthy (n=10)	Bacterial Bronchopneumonia (n=7)
Age (years)	3.8±0.52 (4.00, 1.5-7)	4.8±1.02 (5, 1-9)
Body weight (Kg)	24.68±2.36 (27.20, 13.7-35)	24.36±5.87 (22, 5.5-45)
RT (°F)	102.12±0.20 (102, 101.4-103.2)	102.57±0.54 (102.8, 100-104.8)
HR (bpm)	99.2±5.23 (98^{b}, 76-136)	115.86±6.77 (120^{a}, 85-140)
RR (breaths/min)	27.6±2.29 (26^{b}, 18-40)	70.1±10.0 (64^{a}, 34-105)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at $P<0.05$

a) Hemato-biochemical parameters

Mean hemoglobin concentration in bacterial bronchopneumonia dogs was 11.96±1.10 g/dl. The total leukocyte count in these animals was 14844±2325 cells/µl which was higher than the healthy control group (11376±1372 cells/µl). Absolute neutrophil count (12270±1898 cells/µl) was significantly higher in dogs suffering from bacterial bronchopneumonia than the healthy control group (7724±1114 cells/µl; $P<0.05$). Relative neutrophilia was evident in 4 whereas absolute neutrophilia was seen in 2 dogs along with mild to moderate left shift in all dogs. The specific neutophilic leucocytosis was observed in only one dog with TLC levels 26,620 cells/µl. Nelson and Couto (2014) described neutrophilic leukocytosis with a left shift or neutropenia with a degenerative left shift or moderate to severe toxic changes in neutrophils, in hematological findings of dogs with bacterial pneumonia (Peeters *et al* 2000). But there is also possibility of finding a normal or stress leukogram (Sykes 2013, Nelson and Couto 2014).

Biochemical levels revealed a non-significant decrease in albumin level in dogs suffering with bacterial pneumonia (2.27±0.29 g/dl) as compared to healthy control group (2.77±0.11 g/dl). Ettinger and Feldman (2010) stated that the increased pulmonary and systemic capillary permeability leads to hypoalbuminaemia. Eckersall and Bell (2010) recorded that the albumin concentration decreases during the

inflammatory response and it is the only negative acute phase protein that is found useful in veterinary pathology. Similarly, Pradeep (2014) also recommended albumin as a significant biomarker for bacterial infections. Mean plasma fibrinogen level in bacterial pneumonia animals (0.88±0.11 g/dl) was significantly higher as compared to the healthy control group (0.32±0.03 g/dl; P<0.05) (Table 8).

Table 8: Comparison of hemato-biochemical parameters (mean ± SE) between healthy and bacterial bronchopneumonia dogs

Parameters	Healthy (n=10)	Bacterial Bronchopneumonia (n=7)
Hb (g/dl)	13±0.78 (13.55, 9.3-16.5)	11.96±1.10 (13.8, 7.4-14.5)
TEC (count/µl ×10^6)	6.03±0.32 (6.24^a, 4.5-7.47)	4.68±0.55 (4.80^b, 2.47-6.87)
TLC (count/µl)	11376±1372 (10030, 6790-19100)	14844±2325 (13870, 6770-26620)
PCV (%)	39.76±2.13 (40.85, 31.5- 49.9)	36.17±3.28 (41.4, 22.4-43.5)
Platelets (count/µl×10^3)	228.8±39.9 (193.5, 119-542)	255.7±58.50 (190, 51-462)
Absolute neutrophils (count/µl)	7724±1114 (6502^b, 2717-12926)	12270±1898 (11096^a, 5687-21828)
Absolute lymphocytes (count/µl)	3243±790 (2219, 1161-9168)	1977±373 (1939, 640-3727)
Absolute eosinophilia (count/µl)	409±120 (467, 0-901.8)	597±362 (0, 0-2560)
Total protein (g/dl)	6.38±0.08 (6.45, 5.9-6.8)	6.01±0.46 (6.2, 3.9-7.6)
Albumin (g/dl)	2.77±0.11 (2.7, 2.4-3.4)	2.27±0.29 (2.4, 1.3-3.2)
Globulin (g/dl)	3.61±0.10 (3.6, 3.1-4.1)	3.74±0.37 (3.5, 2.6-5.2)
A : G ratio	0.78±0.05 (0.77, 0.58-1.10)	0.64±0.09 (0.63, 0.26-0.94)
Fibrinogen (g/dl)	0.32±0.03 (0.4^b, 0.2-0.4)	0.88±0.11 (0.9^a, 0.4-1.2)

Values in parenthesis depicts range

Values having different superscript in the same row differ significantly at P<0.05

Zapryanova *et al* (2013) reported that the serum fibrinogen level was more reliable than albumin due to persistency of its elevated level up to 21 days of post inoculation with *Staphylococci*.

b) Blood gas analysis

The partial pressure of oxygen (PaO_2) in the bronchopneumonia dogs was found significantly lower than the healthy group (55.25±6.80 vs 87.17±1.74 mmHg, respectively; $P<0.05$). Similarly the oxygen saturation was also slightly lower in diseased dogs than the healthy group (88±1.00 and 93±1.06 mmHg, respectively) (Table 9). Corcoran (2004) observed the degree of ventilation-perfusion mismatch present in the bacterial bronchopneumonia cases through the blood gas analysis. Hypoxemia was recorded in more than 75 percent of dogs with aspiration pneumonia (Kogan *et al* 2008, Tart *et al* 2010) with PaO_2 levels between 69 to 77 mmHg (Peeter *et al* 2000).

Table 9: Comparison of blood gas parameters (mean ± SE) between healthy and bacterial bronchopneumonia in dogs

Parameters	Healthy group (n=6)	Bacterial Bronchopneumonia (n=6)
pH	7.44±0.01 (7.44, 7.38-7.48)	7.40±0.02 (7.39, 7.37-7.44)
$PaCO_2$ (mmHg)	27.17±1.25 (27.5, 23-32)	32.75±2.25 (33, 27-38)
HCO_3 (mmol/L)	17.17±0.34 (17.3, 15.6-17.9)	19.15±0.94 (18.5, 17.8-21.8)
AnGap (mmol/L)	16.67±0.70 (16.3, 14.4-18.7)	20.95±1.83 (21.95, 16-23.9)
tCO_2 (mmol/L)	18±0.36 (18.2^b, 16.3-18.8)	19.8±1.07 (18.8^a, 18.6-23)
BE (mmol/L)	-3.85±0.43 (-3.7, -5.5- -2.5)	-4.38±0.99 (-4.85, -6.1- -1.7)
stHCO_3 (mmol/L)	20.68±0.30 (21, 19.7-21.5)	21.02±0.81 (20.6, 19.7-23.2)
st pH	7.34±0.01 (7.34, 7.32-7.35)	7.33±0.02 (7.32, 7.31-7.39)

Parameters	Healthy group (n=6)	Bacterial Bronchopneumonia (n=6)
cH+ (nmol/L)	35.98±1.32 (35.65, 33.1-41.9)	40.05±1.49 (40.80, 36.1-42.5)
PaO_2 (mmHg)	87.17±1.74 (86.5[a], 81-92)	55.25±6.80 (61[b], 35-64)
tHb (g/dL)	12.57±0.67 (12.15, 11-15.3)	10.7±1.09 (11.6, 7.5-12.1)
SO_2 (%)	93±1.06 (93, 89-97)	88±1.53 (89, 85-90)
Na+ (mmol/L)	135.17±3.71 (139, 119-142)	146.25±4.21 (149, 134-153)
K+ (mmol/L)	3.41±0.12 (3.45, 3-3.78)	4.32±0.74 (3.85, 3.2-6.4)
Cl- (mmol/L)	105.17±2.94 (107.5, 92-111)	110.75±2.66 (112.5, 103-115)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at $P<0.05$

c) Radiography

Thoracic radiographic findings of dogs suffering with bacterial broncho-pneumonia showed interstitial pattern (mild in 1 and severe in 5) along with alveolar pattern (mild in 3, moderate in 2 and severe in 1) (Fig. 13, 14, 15). In 5 dogs, mixed pattern (interstitial and alveolar) (Fig. 14), in 1 only alveolar and in another one only interstitial pattern was observed. Right middle lung lobe congestion was observed in 2 dogs (Fig. 13). Pulmonary edema was observed in cranial and caudal lobes in 4 dogs and perihilar edema in 2 dogs (Fig. 15). Bacterial pneumonia is mainly diagnosed on the basis of clinical signs (cough, tachypnea and hypoxemia), radiographic changes showing alveolar infiltrates and the positive culture reports (Dear 2014, Kogan *et al* 2008 and Radhakrishnan *et al* 2007). Similar radiographic findings of interstitial and alveolar patterns were witnessed by Kogan *et al* (2008) and Tart *et al* (2010). Nelson and Couto (2014) related the only interstitial pattern in dogs with brochopmeumonia to mild or early disease or in those dogs where the infection is of hematogenous origin.

d) Transtracheal wash (TTW) cytology

TTW cytology revealed markedly increased cellularity with majority of degenerated and non degenerated neutrophils along with few macrophages and occasional sloughed epithelial cells indicative of long standing chronic inflammation (Fig. 16). The presence of degenerated neutrophils in BAL cytology was also considered as a sign of bacterial infection in another study (Hawkins *et al* 1995). Nelson and Couto (2014) also proposed septic neutrophilic inflammation in dogs with bacterial pneumonia along with growth of organisms on bacterial culture.

Mean cell number (cells/HPF) in bacterial pneumonia group (192.5±24.2 cells/HPF) was significantly higher than the healthy control group (8.35±2.68 cells/HPF; $P<0.05$). Mean macrophage count of bacterial pneumonia group (12.8±1.66 %) was significantly lower than the healthy control group (49.57±5.28 %; $P<0.05$) whereas the mean neutrophil count (73±2.52 %) was significantly higher than the healthy group (11.43±1.00 %; $P<0.05$). Mean lymphocyte count (2.4±0.24 %; $P<0.05$) was significantly lower than the healthy control group (7.71±2.25 %) and mean epithelial cell count of the bacterial pneumonia group (9.4±1.86 %) was comparatively lower than the healthy group (29.14±7.13 %) (Table 10).

Table 10: Comparison of tracheal wash cytology (mean ± SE) between healthy and bacterial bronchopneumonia

Parameters	Healthy group (n=7)	Bacterial Bronchopneumonia (n=5)
Macrophages (%)	49.57±5.28 (51[a], 28-66)	12.8±1.66 (12[b], 8-17)
Neutrophils (%)	11.43±1.00 (11[b], 8-15)	73±2.98 (74[a], 63-81)
Lymphocytes (%)	7.71±2.25 (7[a], 0-18)	2.4±0.24 (2[b], 2-3)
Eosinophils (%)	0.14±0.14 (0, 0-1)	1±1 (0, 0-5)
Respiratory epithelial cells (%)	29.14±7.13 (32, 9-60)	9.4±2.20 (8, 5-16)
Others (%)	2±0.79 (2, 0-5)	1.4±0.24 (1, 1-2)
Mean cell number (cells/HPF)	8.35±2.68 (5.8[b], 1.85-21.4)	192.5±24.2 (226.1[a], 132.2-239.7)

Values in parenthesis depicts range

Values having different superscript in the same row differ significantly at $P<0.05$

Viitanen *et al* (2014) on cytological analysis of TTW fluid in 5 dogs suffering with bacterial pneumonia recorded median value of neutrophils (67.5%), eosinophils (1.4%), lymphocytes (3.0%), macrophages (12.5%) and respiratory epithelial cells (0.7%). Similar counts were seen in the present study with median value of neutrophils 74 percent, macrophages 12 and respiratory epithelial cells 8 percent. On histopathological examination of lung tissue, fibrosis of interstitial tissue and hyperplasia of interstitium was observed in a dog with bacterial bronchopneumonia (Fig. 17, 18) similarly as observed by Viitanen *et al* (2014).

e) Culture of Transtracheal wash (TTW)

For the isolation of the etiological agent, tracheal fluids were cultured on Blood agar, Brain heart infusion (BHI) agar and Sabouraud Dextrose agar media. *Staphylococcus aureus* was identified in three and *E.coli* in two dogs in this group. On Blood agar, medium sized round, smooth white coloured colonies of *Staphylococcus* were seen. On gram staining, violet coloured cocci in irregular clusters or shape of bunch of grapes (Fig. 22) were found which were positive for the catalase test (Fig. 24) and negative for the oxidase test. Round black colored colonies were observed on Baird-Parker Media (Fig. 20) which is a selective and diagnostic media for *Staphylococcus aureus.* Biochemical tests were done to confirm the organism (Fig. 23). *Escherichia coli* organisms were isolated on the MacConkey agar and appeared as the pink coloured lactose fermenting colonies. Greenish metallic sheen type of colonies of *E coli* were observed on Eosin methylene blue (EMB) agar (Fig. 19) , a selective and differential media for coliforms, which yield large pink coloured rods on gram staining (Fig. 21) and were positive for catalase and negative for oxidase test. The organisms (including *Pasturella*, *Klebsiella*, *Proteus* spp., *E.coli*, *Staphylococcus* and *Streptococcus*) usually present in the respiratory system which starts proliferating under suitable conditions (Corcoran 2004).

f) Antibiotic sensitivity tests

Organisms obtained from tracheal wash culture were subjected for antibiotic sensitivity tests (Fig. 25, 26). *Staphylococcus aureus* obtained in 3/5 dogs was found sensitive to gentamicin (n=2), enrofloxacin (n=2), ceftriaxone-tazobactum (n=2), cloxacillin (n=2) followed by amikacin (n=3)and ciprofloxacin (n=3) and resistant to ampicillin (n=3) and amoxicillin and clavulanic acid combination (n=2). *E.coli* was isolated in 2 cases and was found sensitive to amikacin in 1 case only and was found

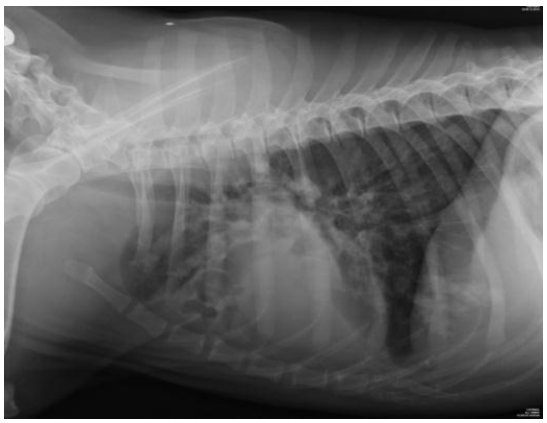

Fig. 13: A lateral thoracic radiograph showing severe alveolar pattern in cranial and right middle lung lobe. Mild interstitial pattern in the caudal lung lobe, marked congestion of right middle lung lobe and soft tissue density at perihilar region in a dog with bacterial bronchopneumonia

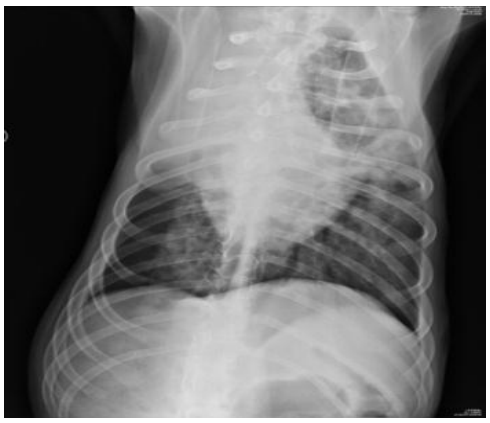

Fig. 14: A ventrodorsal thoracic radiograph showing increased opacity. Alveolar and interstitial pattern was more conspicuous in cranial and middle lobe as compared with caudal lobe.

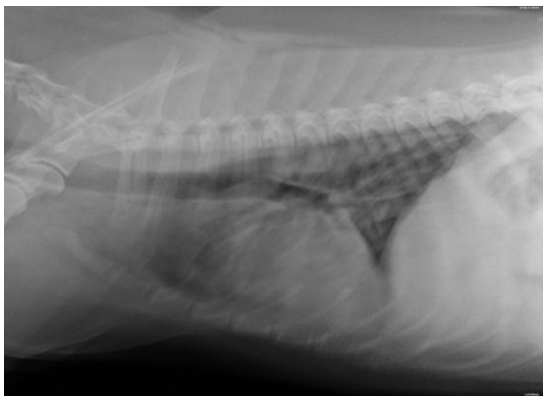

Fig. 15: A lateral thoracic radiograph showing severe interstitial and moderate alveolar pattern. Perhilar edema and lungs edema in cranial and caudal lobe in another dog with bacterial bronchopneumonia

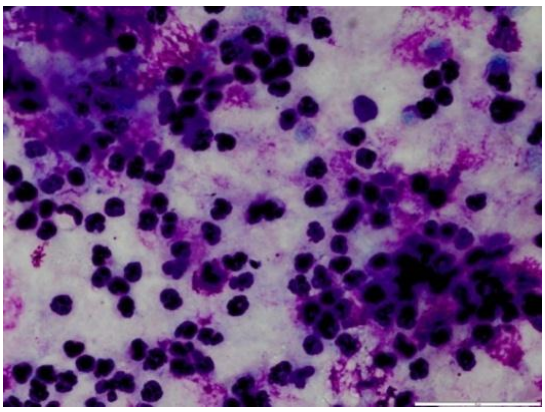

Fig. 16: Purulent exudate showing degenerative and non degenerative neutrophils in TTW of a dog with bacterial bronchopneumonia-100x. (Leishman staining)

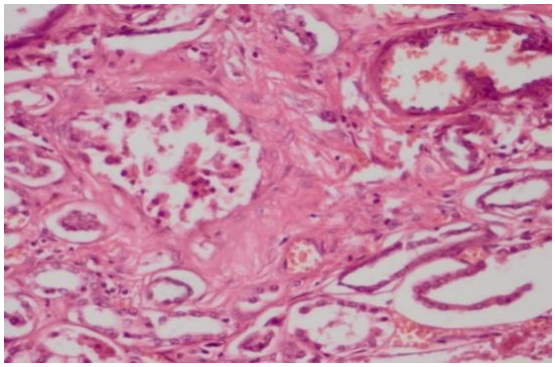

Fig. 17: Histopathology of lung tissue showing fibrosis of interstitial tissue in a dog with bacterial bronchopneumonia-40x (Hematoxylin and Eosin staining)

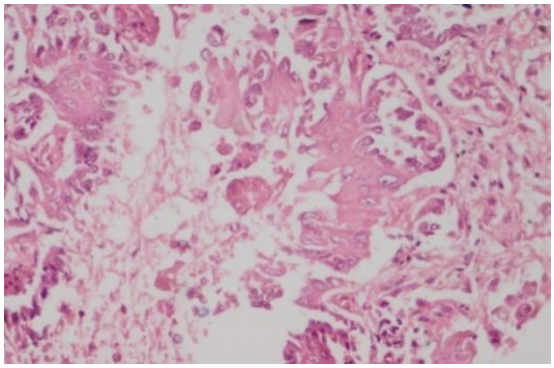

Fig. 18: Histopathology of lung tissue showing hyperplasia of interstitium in a dog with bacterial bronchopneumonia-40x (Hematoxylin and Eosin staining)

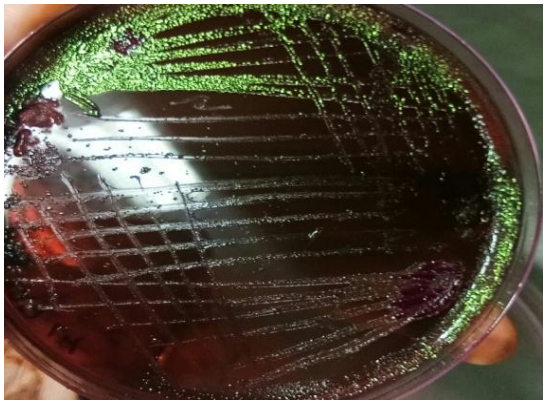

Fig. 19: EMB agar showing typical small round convex colonies of E.coli giving greenish metallic sheen in dog with bacterial bronchopneumonia

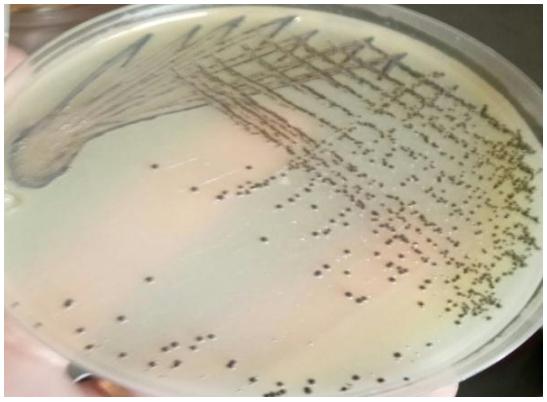

Fig. 20: Baird Parker agar showing typical round black colonies of *Staphylococcus aureus* in dog with bacterial bronchopneumonia

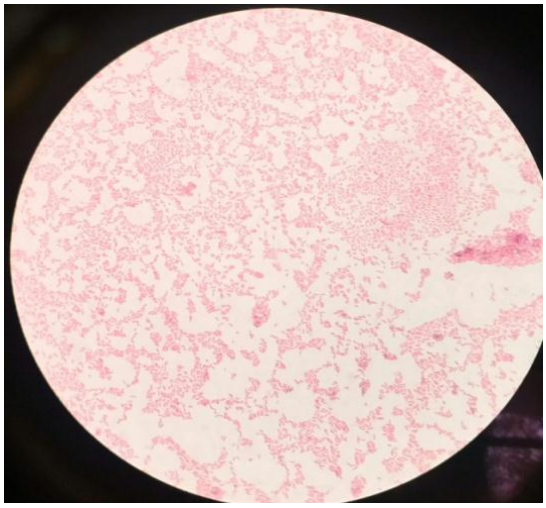

Fig. 21: *Escherichia coli* organisms appeared as pink colour rods on gram staining in a dog with bacterial bronchopneumonia

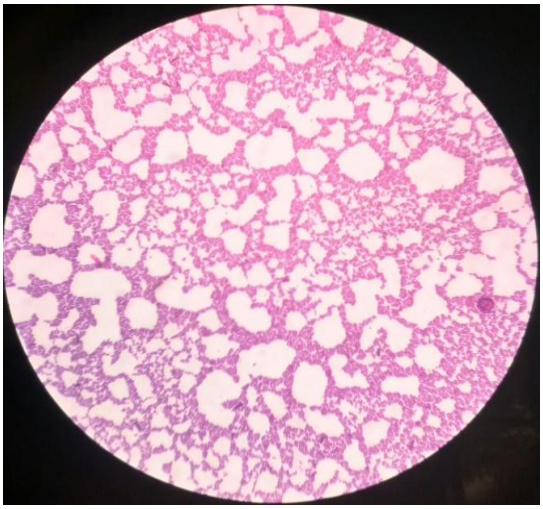

Fig. 22: *Staphylococcus aureus* organisms appeared as violet cocci present in bunches on gram staining in a dog with bacterial bronchopneumonia

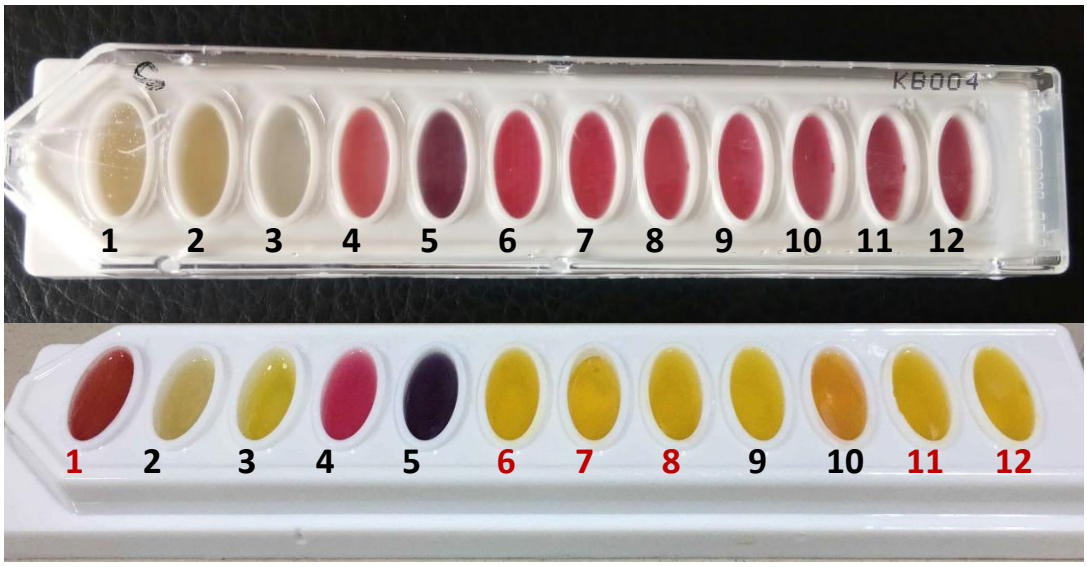

Fig. 23: Biochemical KB004: HiStaph™ identification kit showing positive results (1-Voges Proskauer's, 6-Mannitol, 7-Sucrose, 8-lactose, 11-Trehalose and 12-Maltose) specific for *S. aureus* organisms

Fig. 24: *E. coli* and *Staphylococcus aureus* showing positive catalase test.

Fig. 25: Antibiotic sensitivity test by disk diffusion method

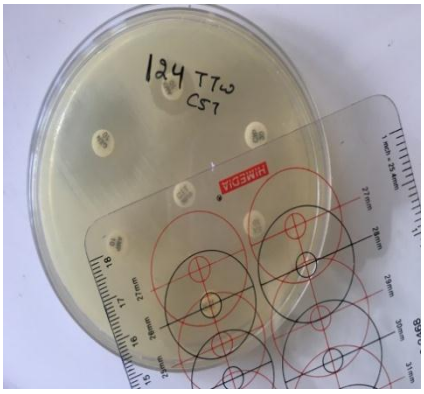

Fig. 26: Zone of inhibition measurement using a ruler

resistant to gentamicin, enrofloxacin, amoxicillin/chavulanic acid, ampicillin, ceftriaxone-tazobactum, ciprofloxacin and cloxacillin in both the cases.

4.2.1.2 Chronic bronchitis (n=11)

Chronic bronchitis (CB) is the most frequently occurring chronic respiratory impairment in the dogs (Carey 2011). It is a chronic inflammatory pulmonary disease (proliferation of neutrophils and excessive mucus production), leading to narrowing and obstruction of the airways, resulting in cough, exercise intolerance and respiratory distress (Brownlie 1990, Padrid 1992, Nelson and Couto 2014, Rozanski 2014). It has been concluded that the chronic bronchitis is a result of long standing inflammatory process initiated by the infection, allergy, inhaled toxins or irritants. This ongoing inflammation cycle starts with mucosal damage leading to mucus hypersecretion and airway obstruction and thus impaired mucociliary clearance. The inflammatory mediators amplifies the response to irritants and the organisms (Kumrow and Rozanski 2012). Long term inflammation of the airways causes histological changes like epithelial hyperplasia, fibrosis, glandular hypertrophy and inflammatory infiltrates (Thurlbeck 1988). Pneumonia is an important differential for cough, although dogs affected with pneumonia shows more systemic signs (fever and lethargy), have a short duration of clinical signs and more commonly exihibit an alveolar infiltrate on thoracic radiographs (Rozanski 2014).

Eleven dogs were presented to the small animal clinics with complaints of coughing, respiratory distress and exercise intolerance similar to the signs documented by Rozanski (2014). Loud harsh non productive cough was observed in 6 dogs with induced cough reflex positive in 7, exercise intolerance in 5 and dyspnea in 3 animals. Other clinical observations include nasal discharge (bilateral serous in 3, mucoid in 1), decreased appetite in 1, lethargy in 1 and weight loss in 1 dog. Similar signs were reported by McKiernan (2000).

The mean respiration rate in CB dogs (48.54±6.35 breaths/min) was significantly higher than the healthy group (27.6±2.29 breaths/min; $P<0.05$) (Table 11). The mean heart rate was 105.27±8.13 bpm and the mean rectal temperature was 101.89±0.34 ^{0}F. On auscultation of the lungs, abnormal lung sounds (harsh in 7, crackles in 1 and wheezes in 1) were heard in 9 affected dogs and normal lung sounds in 2 dogs. Rozanski (2014) concluded that a wide variety of findings could be

expected in chronic bronchitis ranging from normal to harsh lung sounds, crackles or expiratory wheezes. The mean duration of cough in dogs suffering with chronic bronchitis was 34±11.92 days. Chronic bronchitis is defined as the cough that has been occurring continuously for duration of 2 months, without confirmation of presence of other underlying diseases that can cause cough (McKiernan 2000).

Table 11: Comparison of age and physiological parameters (mean ± SE) between healthy and chronic bronchitis dogs

Parameters	Healthy (n=10)	Chronic bronchitis (n=11)
Age (years)	3.8±0.52 (4.00, 1.5-7)	3.64±0.93 (2.5, 0.8-9)
Body weight (Kg)	24.68±2.36 (27.20^{a}, 13.7-35)	16.77±2.45 (16^{b}, 6.8-35)
RT (ºF)	102.12±0.20 (102, 101.4-103.2)	101.89±0.34 (101.80, 100.4-103.6)
HR (bpm)	99.2±5.23 (98, 76-136)	105.27±8.13 (96, 72-172)
RR (breaths/min)	27.6±2.29 (26^{b}, 18-40)	48.55±6.35 (36^{a}, 28-84)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at $P<0.05$

a) Hemato-biochemical parameters

The mean hemoglobin concentration and total erythrocyte count were 12.11±0.74 g/dl and 5.12±0.34 count/µl × 10^{6}, respectively. Mean total leucocyte count was13985±1236 cells/µl while mean haematocrit was 37.03±2.16 percent (Table 12). Mean neutrophil count was significantly higher (11564±1378 cells/µl) than healthy group (7724±1114 cells/µl; $P<0.05$). Padrid *et al* (1990) found hematocrit within the normal limits in 17dogs (ranging 41-50), but was 33% in one dog only. Leukocytosis or eosinophilia was not found in any of the dog (Padrid *et al* 1990). Routine clinical pathology (including complete blood count and serum chemistry) is not usually diagnostic for chronic bronchitis but helps in identifying the other coexisting disorders that can influence the future treatment or the diagnostic procedures (McKiernan 2000). Chemistry of blood revealed normal serum protein, albumin and globulin concentrations. However, mean fibrinogen level was

significantly higher in the affected dogs (0.62±0.03 g/dl; P<0.05) (Table 12). It is proposed that plasma fibrinogen could be considered as a potent biomarker of respiratory diseases. A significant relationship was established between plasma fibrinogen and pulmonary dysfunction in Japanese human population (Shibata *et al* 2013).

Table 12: Comparison of hemato-biochemical parameters (mean ± SE) between healthy and chronic bronchitis dogs

Parameters	Healthy (n=10)	Chronic bronchitis (n=11)
Hb (g/dl)	13±0.78 (13.55, 9.3-16.5)	12.11±0.74 (11.4, 8.4-15.1)
TEC (count/µl × 10^6)	6.03±0.32 (6.24, 4.5-7.47)	5.12±0.34 (4.97, 3.37-7.31)
TLC (count/µl)	11376±1372 (10030, 6790-19100)	13985±1236 (14130, 8680-20900)
PCV (%)	39.76±2.13 (40.85, 31.5- 49.9)	37.03±2.16 (35.60, 27.5-45.6)
Platelets (count/µl× 10^3)	228.8±39.9 (193.5, 119-542)	274.5±34.8 (305, 129-532)
Absolute neutrophils (count/µl)	7724±1114 (6502^b, 2717-12926)	11564±1378 (10928^a, 6423-20064)
Absolute lymphocytes (count/µl)	3243±790 (2219, 1161-9168)	1812±276 (1903, 772-3579)
Absolute eosinophilia (count/µl)	409±120 (467, 0-901.8)	608±326 (188, 0-3391)
Total protein (g/dl)	6.38±0.08 (6.45, 5.9-6.8)	6.42±0.34 (6.4, 4.8-8.8)
Albumin (g/dl)	2.77±0.11 (2.7, 2.4-3.4)	2.66±0.12 (2.7, 2-3.3)
Globulin (g/dl)	3.61±0.10 (3.6, 3.1-4.1)	3.75±0.28 (3.7, 2.8-6)
A : G ratio	0.78±0.05 (0.77, 0.58-1.10)	0.74±0.05 (0.77, 0.47-0.93)
Fibrinogen (g/dl)	0.32±0.03 (0.4^b, 0.2-0.4)	0.62±0.03 (0.6^a, 0.4-0.8)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at P<0.05

b) Blood gas analysis

The mean partial pressure of oxygen in dogs affected with chronic bronchitis was significantly lower than the healthy control animals (62.2±10.8 vs 87.17±1.74 mmHg, respectively; P<0.05) (Table 13). Mean oxygen saturation in affected dogs was 87.75±4.89 percent (ranging 74-97 %). Mild hypoxemia (PaO2<80 mmHg) was documented by Kumrow and Rozanski (2012). In a study of 18 dogs conducted by Padrid *et al* (1990) mean partial pressure of oxygen in arterial blood (PaO_2) was found to be 83±11 mmHg (ranging from 62 to 110mmHg). In 8 of 18 dogs, resting PaO_2 was less than 80 mmHg (Padrid *et al* 1990). In our study mean partial pressure of CO_2 in arterial blood of diseased dogs was observed as 34.8±5.62 mmHg. Partial pressure of CO_2 in arterial blood ($PaCO_2$) was reported between 30 and 41 mmHg, with a mean value of 36±2.6mmHg (Padrid *et al* 1990).

Table 13: Comparison of blood gas parameters (mean ± SE) between Healthy and chronic bronchitis dogs

Parameters	Healthy group (n=6)	Chronic bronchitis (n=6)
pH	7.44±0.01 (7.44, 7.38-7.48)	7.40±0.03 (7.40, 7.29-7.48)
$PaCO_2$ (mmHg)	27.17±1.25 (27.5, 23-32)	34.8±5.62 (30, 27-57)
HCO_3 (mmol/L)	17.17±0.34 (17.3^b, 15.6-17.9)	20.28±1.17 (19.3^a, 18.4-24.9)
AnGap (mmol/L)	16.67±0.70 (16.3, 14.4-18.7)	22.1±3.20 (23, 11.4-30.9)
tCO_2 (mmol/L)	18±0.36 (18.2^b, 16.3-18.8)	21.08±1.45 (20.2^a, 18.6-26.7)
BE (mmol/L)	-3.85±0.43 (-3.7, -5.5- -2.5)	-2.8±0.58 (-2.7, -4.1- -0.9)
$stHCO_3$ (mmol/L)	20.68±0.30 (21, 19.7-21.5)	22.1±0.44 (22.2, 20.9-23.2)
st pH	7.34±0.01 (7.34, 7.32-7.35)	7.36±0.01 (7.37, 7.31-7.39)
cH+ (nmol/L)	35.98±1.32 (35.65, 33.1-41.9)	39.4±3.44 (36.10, 32.9-52.5)

Parameters	Healthy group (n=6)	Chronic bronchitis (n=6)
PaO_2 (mmHg)	87.17±1.74 (86.5[a], 81-92)	62.2±10.8 (67[b], 33-90)
tHb (g/dl)	12.57±0.67 (12.15, 11-15.3)	13.04±0.48 (13, 12-14.4)
SO_2 (%)	93±1.06 (93, 89-97)	87.75±4.89 (90, 74-97)
Na+ (mmol/L)	135.17±3.71 (139, 119-142)	149±6.04 (151, 127-164)
K+ (mmol/L)	3.41±0.12 (3.45, 3-3.78)	3.44±0.33 (3.3, 2.7-4.7)
Cl- (mmol/L)	105.17±2.94 (107.5, 92-111)	109.4±2.60 (111, 100-114)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at $P<0.05$

c) Radiography

Chest radiographs play a vital role in the diagnosis of chronic bronchitis (McKiernan 2000). Chest radiographs are found to be the most useful test if diagnostic testing is limited for an individual patient (Mantis 1998, McKiernan 2000 and Rozanski 2014). Lateral and VD view radiographs of chest are taken in all the dogs. Both views showed mild to moderate bronchial pattern in cranial and caudal lobe along with bronchial thickening and multiple donuts in all the dogs suffering with chronic bronchitis (Fig. 27, 28). Non-structural (diffused) interstitial pattern was observed in 6 dogs (mild in 1, moderate in 4 and severe in 1) on chest radiography (Fig. 29). Radiographic findings observed by McKiernan (2000) in the dogs suffering with chronic bronchitis was increased bronchial markings (doughnuts and tram lines) and a mild diffuse increased interstitial density. The degree of structural changes seen in lungs may not correspond with the functional abnormalities shown by dog clinically suffering with chronic bronchitis (McKiernan 2000).

d) Transtracheal wash (TTW) cytology

Transtracheal wash (TTW) was collected in 8 out of 11 dogs. Increased cellularity with moderate increase in neutrophils along with presence of mucus (Fig.

30) and sloughed hyperplastic epithelial cells (Fig. 31) was observed in all the dogs suffering with chronic bronchitis (Fig. 32, 33). Mean cell number (cells/HPF) of tracheal wash/aspirate in affected dogs was significantly higher than the control group (69±18.6 vs 8.35±2.68, respectively; P<0.05). With respect to differential cell count, mean macrophage count in affected dogs was significantly lower than the healthy control animals (30.62±4.84 vs 49.57±5.28 %, respectively; P<0.05) whereas neutrophils count increased significantly in the dogs suffering with chronic bronchitis as compared to the healthy control group (48.5±6.48 vs 11.43±1.00, respectively; P<0.05) (Table 14). Mean lymphocyte and eosinophil count of the diseased dogs (8.62±4.81 and 1.25±1.11 %, respectively) was higher as compared to healthy ones (7.71±2.25 and 0.14±0.14 %, respectively). Mean respiratory epithelial cells were significantly lower in affected dogs as compared to the healthy group (9.25±2.23 vs 29.14±7.13, respectively; P<0.05) (Table 14).

Table 14: Comparison of tracheal wash cytology (mean ± SE) between healthy and chronic bronchitis dogs

Parameters	Healthy group (n=7)	Chronic bronchitis (n=8)
Macrophages (%)	49.57±5.28 (51^{a}, 28-66)	30.63±4.84 (33.50^{b}, 13-53)
Neutrophils (%)	11.43±1.00 (11^{b}, 8-15)	48.5±6.48 (47^{a}, 26-80)
Lymphocytes (%)	7.71±2.25 (7, 0-18)	8.62±4.81 (3, 0-41)
Eosinophils (%)	0.14±0.14 (0, 0-1)	1.25±1.11 (0, 0-9)
Respiratory epithelial cells (%)	29.14±7.13 (32^{a}, 9-60)	9.25±2.23 (8^{b}, 3-16)
Others (%)	2±0.79 (2, 0-5)	1.75±0.49 (2, 0-4)
Mean cell number (cells/HPF)	8.35±2.68 (5.8^{b}, 1.85-21.4)	69±18.6 (64.8^{a}, 12.3-148.2)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at P<0.05

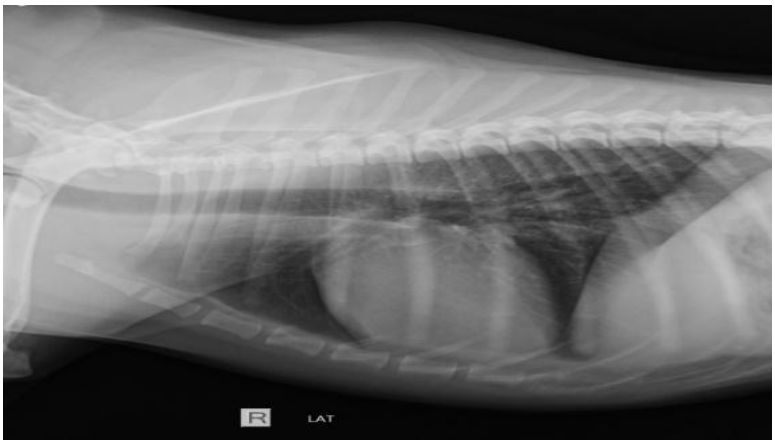

Fig. 27: Lateral thoracic radiograph showing moderate bronchial pattern in cranial and caudal lung lobe along with bronchial wall thickening. Increased opacity of cranial and caudal lung lobe in a dog with chronic bronchitis

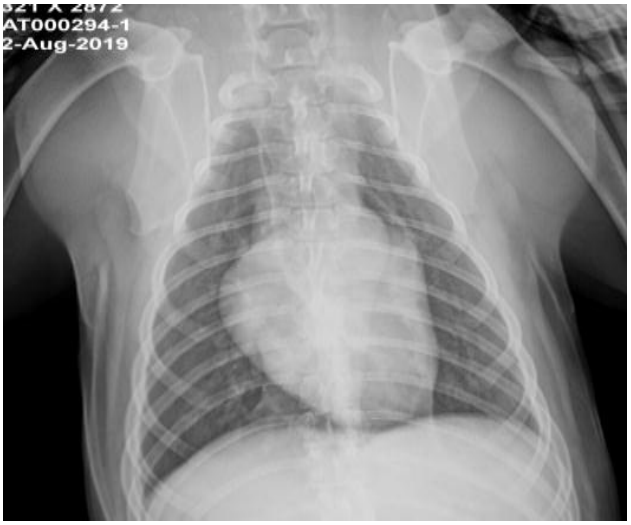

Fig. 28: Ventrodorsal thoracic radiograph showing multiple donuts on both sides of lungs. Increased opacity seen on both sides in a dog with chronic bronchitis

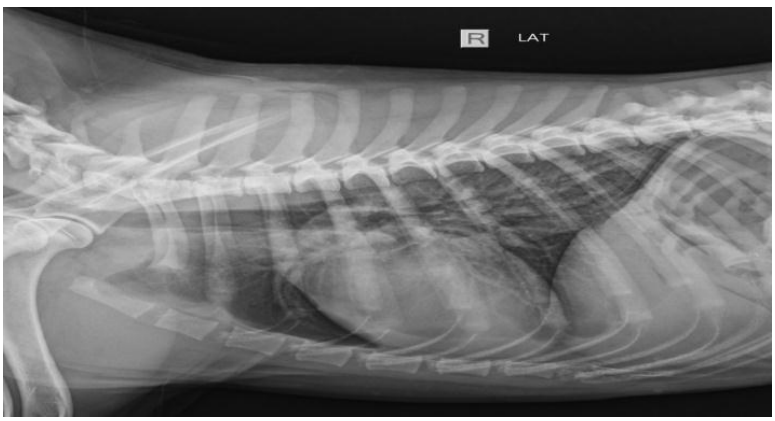

Fig. 29: Lateral radiograph showing mild interstitial pattern in caudal dorsal lung lobe. Peribronchial infiltration in right middle and caudal lung lobe indicating mild bronchial pattern (donuts) in a dog with chronic bronchitis

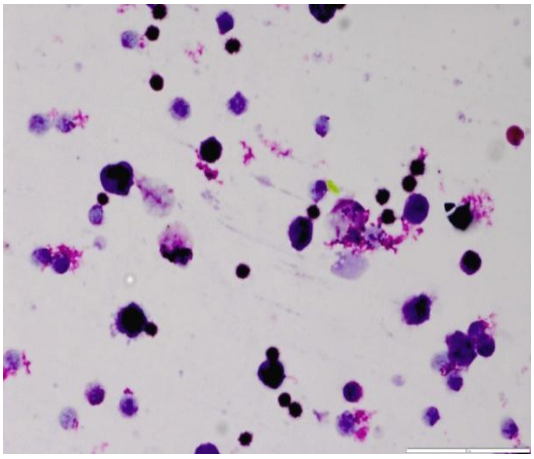

Fig. 30: Few neutrophils and epithelial cells in TTW indicating mild inflammation in chronic bronchitis dog-100x (Leishman staining)

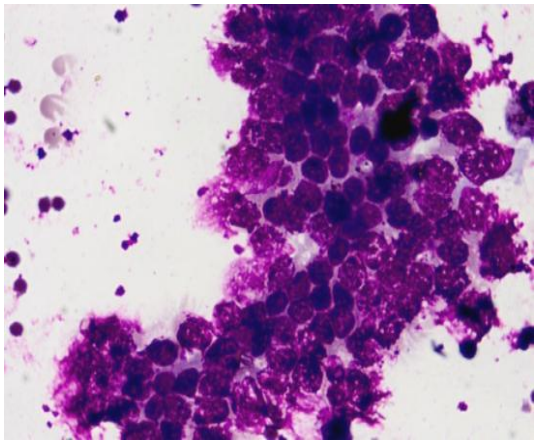

Fig. 31: Hyperplasia of epithelial cells in TTW of dog with chronic bronchitis-100x (Leishman staining)

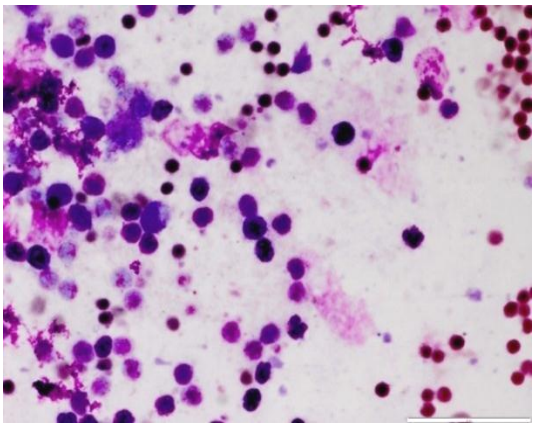

Fig. 32: Many epithelial cells along with neutrophils and RBCs in TTW of a dog with bronchitis-100x (Leishman staining)

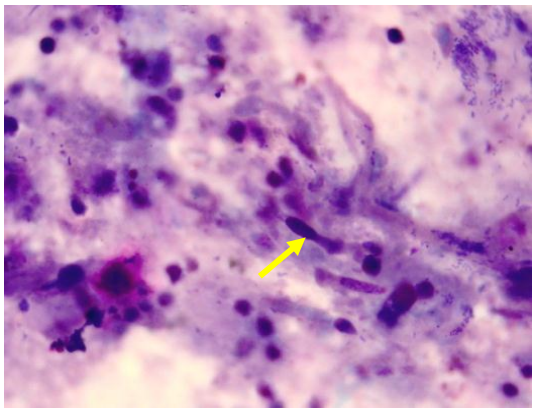

Fig. 33: Fibroblast cell (yellow arrow) along with macrophages and neutrophils in TTW of a dog with bronchitis -100x (Leishman staining)

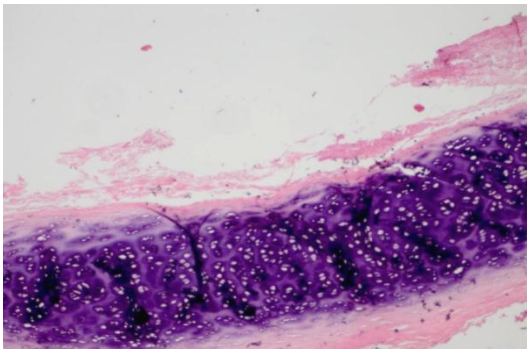

Fig. 34: Histopathology of tracheal tissue showing sloughing of tracheal epithelium in chronic bronchitis-10x (Hematoxylin and Eosin staining)

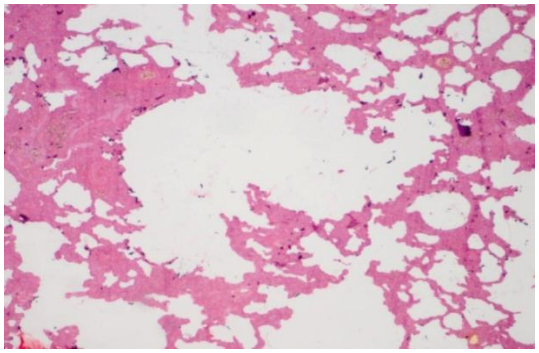

Fig. 35: Histopathology of lung tissue showing lung emphysema and thickening of interlobular septa in chronic bronchitis -10x (Hematoxylin and Eosin staining)

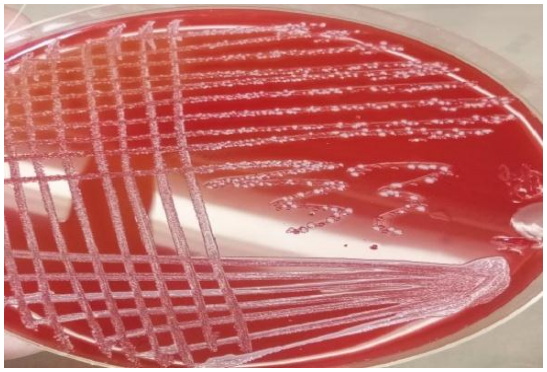

Fig. 36: *Staphylococcus aureus* on Blood Agar showing round smooth convex colonies with hemolysis in a dog with chronic bronchitis

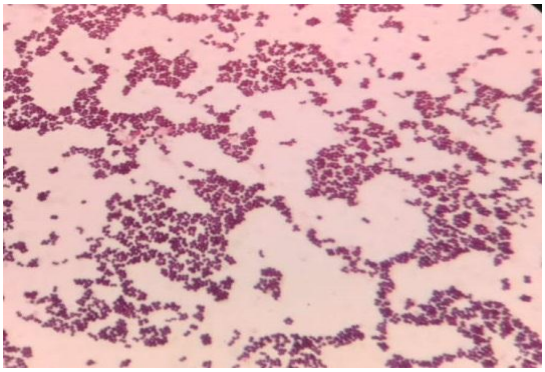

Fig. 37: *Staphylococcus aureus* showing violet colored cocci present in bunches on gram staining in a dog with chronic bronchitis

Fig. 38: *Klebsiella pneumonia* showing non-hemolytic, mucoid colonies on Blood agar in a dog with chronic bronchitis

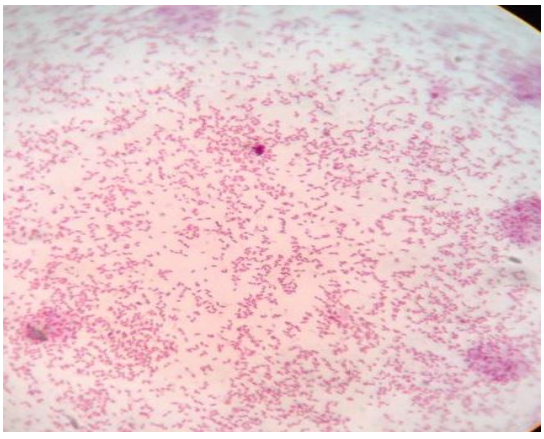

Fig. 39: *Klebsiella pneumoniae* showing gram negative, rod-shaped bacteria on gram staining in a dog with chronic bronchitis

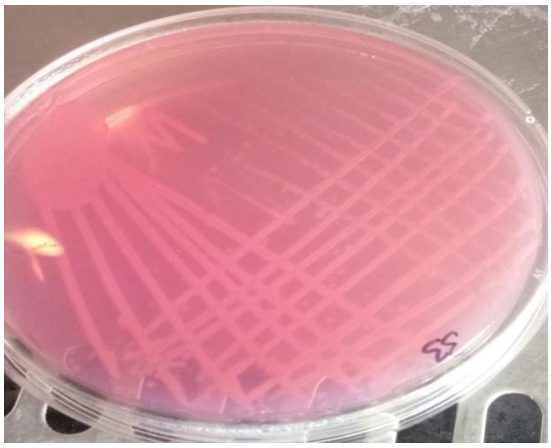

Fig. 40: *E.coli* on MLA giving round convex pink colonies in a dog with chronic bronchitis

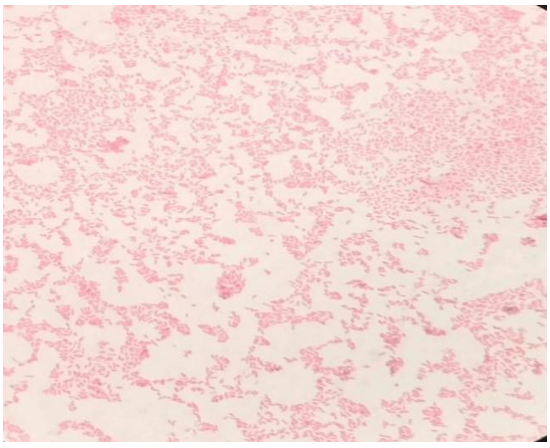

Fig. 41: *E.coli* showing as pink colored cocobacilli on gram staining in a dog with chronic bronchitis

Cytological examination of the airway samples of the dogs with canine chronic bronchitis typically shows predominantly neutrophilic infiltrate with excessive mucus. Small number of lymphocytes, goblet cells, eosinophils, ciliated cells and epithelial cells and variable number of alveolar macrophages were also seen in majority of the cases (Padrid *et al* 1990, Kumrow and Rozanski 2012). In bronchial washing of 2 out of 18 dogs, large numbers of eosinophils were observed in the study conducted by Padrid *et al* (1990). In the present study, histopathology of tracheal and lung tissue in a dog with chronic bronchitis showed sloughing of tracheal epithelium (Fig. 34) and lung emphysema along with thickening of interlobular septa (Fig. 35), respectively, similar to documented by Maxie (2015).

e) Culture of TTW

Transtracheal wash (TTW) collected from eight dogs was cultured on Blood agar, Brain heart infusion and Sabouraud dextrose agar and incubated for 18-24 hours at 37 ^{0}C. Bacterial culture was found negative in 4 dogs and other 4 positive bacterial culture was observed with *Staphylococcus aureus* in 2, *E.coli* in 1 and *Klebsiella* sp. in 1 animal. *Staphylococcus aureus* showed round smooth convex colonies with hemolysis on blood Agar (Fig. 36) which was further isolated on the Baird Parker media (specific media) showing round black colonies. On gram staining, purple coloured cocci present in grape like clusters were observed which are catalase positive and oxidase negative (Fig. 37). Isolation of *Escherichia coli* organisms was done on MacConkey agar and appeared as pink colour circular, moist and smooth lactose fermenting colonies (Fig. 40). *E coli* forms greenish metallic sheen type of colonies on Eosine methylene blue (EMB) agar which yields pink coloured rods on grams staining (Fig. 41). *Klebsiella pneumoniae* shows non-hemolytic, mucoid colonies on blood agar (Fig. 38) which is further isolated on MacConkey agar and appeared as pink colour circular, moist and smooth lactose fermenting colonies. On gram staining, pink coloured rod-shaped bacteria was seen (Fig. 39). The results were in accordance with as observed by Padrid *et al* (1990).

f) Antibiotic sensitivity tests

Staphylococcus aureus (n=2), *E.coli* (n=1) and *Klebsiella sp.* (n=1) obtained from tracheal wash culture was subjected for antibiotic sensitivity tests. *Staphylococcus aureus* was found to be sensitive for ciprofloxacin (n=2), cloxacillin (n=1), enrofloxacin (n=1), gentamicin (n=1), amikacin (n=1) and resistant for

ampicillin, ceftriaxone-tazobactum and amoxicillin/chavulanic acid in both the cases. *E.coli* was found sensitive for gentamicin, ciprofloxacin, amikacin, tetracycline and resistant to enrofloxacin, ampicillin, ceftriaxone-tazobactum and amoxicillin/ chavulanic acid. *Klebsiella pneumoniae* was found sensitive for gentamicin, ampicillin, ciprofloxacin, amikacin, amoxicillin/chavulanic acid and resistant to enrofloxacin and ceftriaxone-tazobactum.

4.2.1.3 Lung tumors/ nodular interstitial lung pattern (n=6)

Primary lung tumors are rare in dogs as compared to humans, but the pulmonary metastasis from other organs is very common (Withrow 1996). Primary lung carcinomas are generally described by the topographical location, which includes bronchiolar, bronchiolo-alveolar or alveolar carcinoma that is synonymously used in human pathology (Liebow 1960, Donaldson *et al* 1978, Carter and Egglestone 1980). In dogs, primary lung tumors are mainly epithelial in origin (Peters *et al* 2001). Based on the predominant histologic pattern, the most common primary lung tumor in dogs and cats is adenocarcinoma whereas less common are squamous cell carcinoma and anaplastic carcinomas (Ogilvie *et al* 1989, Withrow 1996, McNiel *et al* 1997, Fossum 2013). The bronchioloalveolar carcinoma (BAC) is originated from the peripheral airway which includes bronchiolar or alveolar regions (Makino *et al* 2008) and mostly occurs as a solitary sub-pleural mass in dogs (Dungworth *et al* 1999). Though, infrequently BAC can occur in a diffuse form in human beings. Previously described as adenomatosis (Carter and Egglestone 1980), diffuse BAC has been also documented in dogs and in infectious pulmonary carcinomatosis in sheep (Dungworth *et al* 1999). Moreover, it is unique in character as it can involve large areas of pulmonary parenchyma or the entire lung. But it does not alter the lung morphology even after spreading throughout the airways because it does not produce large neoplastic masses. However, neoplastic alveoli and small airways mostly replace the normal alveolar architecture of the pulmonary parenchyma (Liebow 1960, Donaldson *et al* 1978, Carter and Egglestone 1980). For these reasons, diffuse BAC is usually misdiagnosed as they imitate diffused lung diseases (Ludington *et al* 1972, Sestini *et al* 1985).

On the other hand, canine histiocytic tumors denote a group of neoplasms having common morphological features, but differ in cellular origin and biologic behaviour (Dervisis *et al* 2017). On the basis of cellular origin of the tumour and the

clinical signs of the disease, three forms of the disease had been documented which includes disseminated histiocytic sarcoma (DHS), localized HS (LHS) and hemophagocytic HS (HHS) (Fulmer and Mauldin 2007, Schultz *et al* 2007 and Skorupski *et al* 2007). Canine histiocytic sarcoma (HS), either localized or diffuse forms can lead to extensive metastasis ultimately results in grave outcome. Primary metastasis sites for localised HS include periarticular locations (PAHS), subcutaneous tissues and lungs and rarely ocular and central nervous system (Affolter and Moore 2002, Naranjo *et al* 2007, Klahn 2011). Moreover, histiocytic sarcoma is an aggressive and abnormal proliferation of dendritic cells carrying a grave prognosis (Skorupski *et al* 2007). Secondly, both localized and disseminated histiocytic sarcomas have indistinguishable histopathological characters and present as poorly differentiated masses of pleomorphic, single, big round cells, or compactly packed bunch of plump spindle cells (Fulmer and Mauldin 2007).

Pulmonary abscesses are confined areas of pus or necrotic debris present in the lung parenchyma (Seo *et al* 2013). In human pathology, these usually come under lung infections like lung gangrene and the necrotizing pneumonia which are primarily characterized by multiple abscesses (Yazbeck *et al* 2014).

Six dogs were presented to small animal clinics with a complaint of coughing (non-productive), respiratory distress and exercise intolerance. Undiagnosed nodular interstitial cases in the present study showed clinical observations which include dyspnea, tachypnea, weight loss, lethargy, fever, decreased appetite and hemoptysis. Mehlhaff *et al* (1984), Ogilvie *et al* (1989) and McNiel *et al* (1997) recorded nonproductive cough, as the most common presenting sign in dogs (occurring in 52% to 58% of dogs) suffering with primary lung tumor. In addition, dogs with primary lung tumor may show tachypnea, wheezing, hemoptysis and exercise intolerance (Ogilvie *et al* 1989, McNiel *et al* 1997). In the present study, the clinical signs observed in the dog with histiocytic sarcoma are non productive cough, weight loss, dyspnea, exercise intolerance and lameness. Dervisis *et al* (2017) also observed lethargy, lameness, anorexia, weight loss, swelling and dyspnea, in 168 dogs suffering with histiocytic sarcoma. In our study clinical observations of the dog suffering with bronchioloalveolar carcinoma include non productive cough, lethargy, tachypnea, exercise intolerance and decreased appetite. Regardless of the fact that majority of patients are presented with the apparent clinical signs associated with primary lung

tumors, still 30% of the all dogs will have no signs at the time of diagnosis (Ogilvie *et al* 1989, McNiel *et al* 1997 and Fossum 2013).

In our study the dog diagnosed with pulmonary abscessation showed non productive cough, bilateral serous nasal discharge, weight loss, dyspnea, dehydration and exercise intolerance. Though no clinical report has been documented in dogs, the earlier clinical signs of pulmonary abscess in human patients resembles that of pneumonia which include fever, cough (firstly non productive, then productive), dyspnea, weight loss and fatigue, chest pain and occasionally anemia (Yen *et al* 2004, Chan *et al* 2005).

Respiration rate was higher in the diseased dogs. Increase in rectal temperature was recorded in dogs with pulmonary neoplasia (n=2) (Table 15). Bertazzolo *et al* (2002) recorded moderate hyperthermia in a dog diagnosed with diffuse bronchiolo-alveolar carcinoma. Harsh lung sounds were present in all the dogs, with apparent crackles in 1 dog. Nelson and Couto (2014) stated that lung sounds can be normal, increased or decreased in pulmonary neoplasia dogs and in fewer cases, crackles and wheezes can also be heard on auscultation.

Table 15: Comparison of age and physiological parameters (mean ± SE) between healthy and lung tumor/undiagnosed nodular interstitial dogs

Parameters	Healthy (n=10)	Histiocytic sarcoma (n=1)	Bronchioloalveolar carcinoma (n=1)	Undiagnosed Nodular interstitial (n=3)	Pulmonary abscessation (n=1)
Age (years)	3.8±0.62 (1.5-7)	3	8	7.5±2.76 (4.5-13)	4
Body weight (Kg)	24.68±2.82 (13.7-35)	22.4	32	23.33±7.43 (14-38)	20.6
RT (ºF)	102.12±0.24 (101.4-103.2)	103.4	104	102.73±0.75 (101.4-104)	102.6
HR (bpm)	99.2±6.24 (76-136)	104	92	113.33±19.66 (88-152)	156
RR (breaths/min)	27.6±2.73 (18-40)	80	60	72.67±20.36 (40-110)	56

Values in parenthesis depicts range

a) Hemato-biochemical parameters

Mean hemoglobin concentration in the dogs suffering with pulmonary neoplasia (9.25±0.25 g/dl) was relatively lesser as compared to the healthy control group (13±0.78 g/dl), whereas it was normal in nodular interstitial group and higher value (16.8 g/dl) is seen in the dog having pulmonary abscessation. Higher hemoglobin concentration in pulmonary abscessation could be due to long duration inappetence, dehydration and weight loss in that animal. Nelson and Couto (2014) recorded the presence of hypovolemia and anemia along with respiratory compromise in the pulmonary neoplasia dogs due to rapid blood loss. Mean total leukocyte count in nodular interstitial pneumonia cases and pulmonary abscessation was 19113.33± 3258.52 and 16600 cells/µl, respectively, which was appreciably higher than the healthy control group. Mean erythrocytic count in pulmonary neoplasia dogs (3.08± 0.08 cells/µl $\times 10^6$) was relatively lower than the healthy control group (6.03±0.32 count/µl $\times 10^6$).

Mean serum total protein and globulin concentrations in the dog suffering with pulmonary abscessation were 7.5 and 4.9 g/dl, respectively which were appreciably higher than the healthy control group (Table 16). Positive acute phase proteins (APPs) (e.g. fibrinogen) level increases during the inflammatory response of body whereas the negative APPs (e.g. albumin) level decreases (Viitanen *et al* 2014). Mean plasma fibrinogen concentration in the lung tumor/undiagnosed nodular interstitial group was relatively higher than the healthy control group animals (Table 16). Jiang *et al* (2014) recorded that the elevated plasma fibrinogen levels are associated with the tumor progression and poor prognosis in the human patients diagnosed with lung cancer.

b) Blood gas analysis

Mean partial pressure of oxygen in the affected dogs was appreciably lesser than the healthy control group. Oxygen saturation value in histiocytic sarcoma and bronchioloalveolar carcinoma dog was 68 and 60 percent, respectively which was lesser than the healthy group 93± 1.06 percent (Table 17). Tumor infiltration in the lungs can lead to hindrance in the oxygenation, causing increased respiratory effort

and exercise intolerance (Nelson and Couto 2014). Similar interference could be the cause of low partial pressure of oxygen in pulmonary abscessation and undiagnosed nodular interstitial pneumonia.

Table 16: Comparison of hemato-biochemical parameters (mean ± SE) between healthy and lung tumor/undiagnosed nodular interstitial dogs

Parameters	Healthy (n=10)	Histiocytic sarcoma (n=1)	Bronchioloalveolar carcinoma (n=1)	Undiagnosed Nodular interstitial (n=3)	Pulmonary abscessation (n=1)
Hb (g/dl)	13±0.78 (9.3-16.5)	9.5	9	12±1.71 (10-15.4)	16.8
TEC (count/µl × 10^6)	6.03±0.32 (4.5-7.47)	3.17	3	5.00±1.09 (3.33-7.04)	5.6
TLC (count/µl)	11376±1372 (6790-19100)	9160	8100	19113.33±3258.52 (15400-25600)	16600
PCV (%)	39.76±2.13 (31.5- 49.9)	30	27	36.63±5.44 (30-47.4)	50.8
Platelets (count/µl× 10^3)	228.8±39.9 (119-542)	135	254	233.67±78.05 (106-375)	189
Absolute neutrophils (count/µl)	7724±1114 (2717-12926)	7328	7614	17249.2±2677.84 (13860-22528)	14608
Absolute lymphocytes (count/µl)	3243±790 (1161-9168)	1282.4	486	1505.47±406.30 (980.4-2304)	1992
Absolute eosinophillia (count/µl)	409±120 (0-901.8)	549.6	0	358.67±223.41 (0-768)	0
Total protein (g/dl)	6.38±0.08 (5.9-6.8)	5.5	5.1	5.93±0.97 (4-7)	7.5
Albumin (g/dl)	2.77±0.11 (2.4-3.4)	2.9	1.6	2.2±0.40 (1.5-2.9)	2.6
Globulin (g/dl)	3.61±0.10 (3.1-4.1)	2.6	3.5	3.73±0.63 (2.5-4.6)	4.9
A : G ratio	0.78±0.05 (0.58-1.10)	1.12	0.46	0.60±0.10 (0.48-0.70)	0.53
Fibrinogen (g/dl)	0.32±0.03 (0.2-0.4)	0.8	0.6	0.73±0.07 (0.6-0.8)	0.6

Values in parenthesis depicts range

Table 17: Comparison of blood gas parameters (mean ± SE) between Healthy and lung tumor/undiagnosed nodular interstitial dogs

Parameters	Healthy group (n=6)	Histiocytic sarcoma (n=1)	Bronchioloalveolar carcinoma (n=1)	Undiagnosed Nodular interstitial (n=2)	Pulmonary abscessation (n=1)
pH	7.44±0.01 (7.38-7.48)	7.39	7.3	7.44±0.04 (7.41-7.48)	7.37
$PaCO_2$ (mmHg)	27.17±1.25 (23-32)	30	40	25±2.01 (23-27)	46
HCO_3 (mmol/L)	17.17±0.34 (15.6-17.9)	16.9	18.2	16.1±2.41 (13.7-18.5)	24.4
AnGap (mmol/L)	16.67±0.70 (14.4-18.7)	15.1	13.2	18.7±1.40 (17.3-20.1)	23.2
tCO_2 (mmol/L)	18±0.36 (16.3-18.8)	17.8	19.5	16.85±2.46 (14.4-19.3)	25.8
BE (mmol/L)	-3.85±0.43 (-5.5- -2.5)	-5.9	-7.3	-5.35±2.56 (-7.9- -2.8)	-0.9
$stHCO_3$ (mmol/L)	20.68±0.30 (19.7-21.5)	19.5	18.9	19.55±2.26 (17.3-21.8)	24.2
st pH	7.34±0.01 (7.32-7.35)	7.31	7.30	7.31±0.05 (7.26-7.36)	7.41
cH+ (nmol/L)	35.98±1.32 (33.1-41.9)	40.5	50.5	36±2.61 (33.4-38.6)	42.9
PaO_2 (mmHg)	87.17±1.74 (81-92)	38	38	62.5±1.50 (61-64)	64
tHb (g/dL)	12.57±0.67 (11-15.3)	10.3	8.3	9.95±0.05 (9.9-10)	13.3
SO_2 (%)	93±1.06 (89-97)	68	60	89±0 (89-89)	88
Na+ (mmol/L)	135.17±3.71 (119-142)	130	121	143.5±3.51 (140-147)	150
K+ (mmol/L)	3.41±0.12 (3-3.78)	2.5	3.6	4.65±0.05 (4.6-4.7)	3.8
Cl- (mmol/L)	105.17±2.94 (92-111)	100	93	108±2.01 (106-110)	104

Values in parenthesis depicts range

c) Radiography

Chest radiography (Lateral and VD view) was performed in all the diseased dogs. Severe nodular interstitial pattern was observed in all lung lobes in 3 dogs (Fig. 42). In the dog suffering from histiocytic sarcoma, moderate alveolar and bronchial pattern was observed on lateral and VD view, along with air bulla (pneumobulla) seen in left caudal lung lobe (Fig. 47). One collapsed lung lobe was also seen with increased opacity of the parenchyma on both sides (Fig. 48). In dog with diffuse bronchioloalveolar carcinoma, mild to moderate bronchial pattern, moderate alveolar pattern in caudal lung lobe and severe interstitial pattern along with increased radiopacity around perihilar region and edema around secondary bronchus was observed (Fig. 53, 54). In dog with pulmonary abscessation, severe nodular interstitial pattern in all lung lobes was observed (Fig. 43, 44). Ogilvie *et al* (1989), McNiel *et al* (1997) documented radiography as the best aid for early diagnosis of pulmonary neoplasia. Bertazzolo *et al* (2002) observed an increased interstitial reticular pattern in all lung lobes along with diffuse increase in radiopacity in the dog suffering with bronchioloalveolar carcinoma. The characteristic radiographic pattern observed in pulmonary neoplasia is mostly a solitary nodule (Suter and Lord 1984). However, other patterns can be there which include multiple nodules, homogenous lobar consolidation, diffused reticulonodular or mixed alveolar interstitial patterns (Suter and Lord 1984, Barr *et al* 1986 and Nelson and Couto 2014). Nelson and Couto (2014) also observed the presence of distinct cavitation in pulmonary neoplasia cases. Common causes of nodular pattern on radiographs include mycotic infections, tumors and abscesses (Nelson and Couto 2014, Spasov *et al* 2018). Thoracic ultrasonography of dog with histiocytic sarcoma revealed cavitory lesion (1.24 x 1.17cm) on left caudal lung lobe (Fig. 52).

d) Transtracheal wash (TTW) cytology

Nelson and Couto (2014) explained the need of assessment of the lung aspirates, tracheal wash fluid, bronchoalveolar lavage fluid or lung biopsy specimens for establishing a diagnosis in cases of pulmonary neoplasia. In the present study, mean cell number in TTW from dog with pulmonary abscessation (90.25 cells/HPF) was significantly higher than the healthy group (8.35±2.68 cells/HPF) (Table 18). Mean neutrophils in dogs suffering with lung tumors/ undiagnosed nodular interstitial pneumonia cases was found to be appreciably higher than the healthy control group.

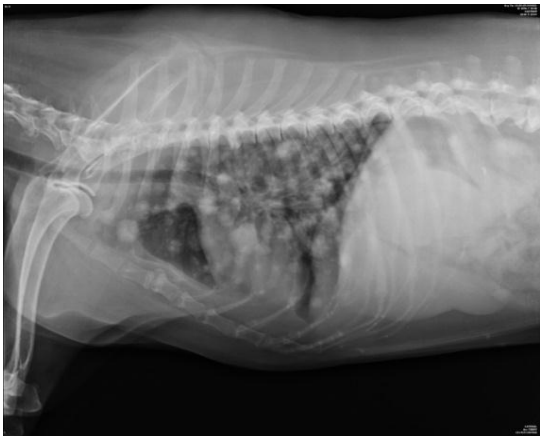

Fig. 42: Lateral thoracic radiograph of dog with undiagnosed nodular interstitial pattern showing severe nodular interstitial pattern in caudal, cranial and middle lung lobes

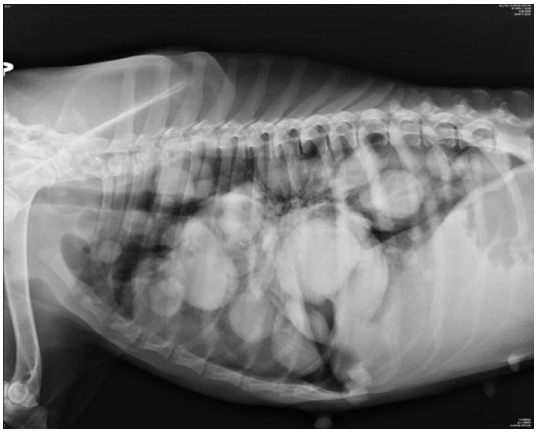

Fig. 43: Lateral thoracic radiograph of dog with pulmonary abscessation showing severe nodular interstitial pattern in all lung lobes. Cardiac silhouette not clearly visible

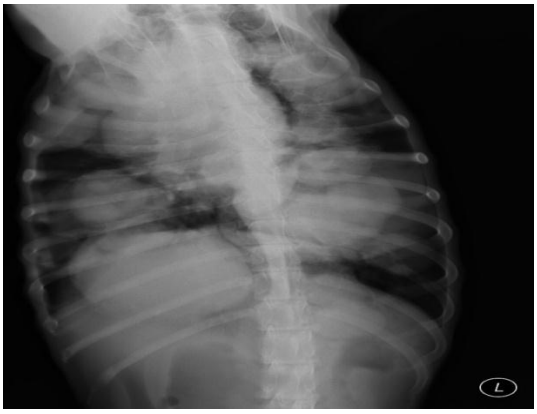

Fig. 44: Ventrodorsal thoracic radiograph of a dog with pulmonary abscessation showing severe nodular interstitial pattern in all lung lobes. Cardiac silhouette not clearly visible

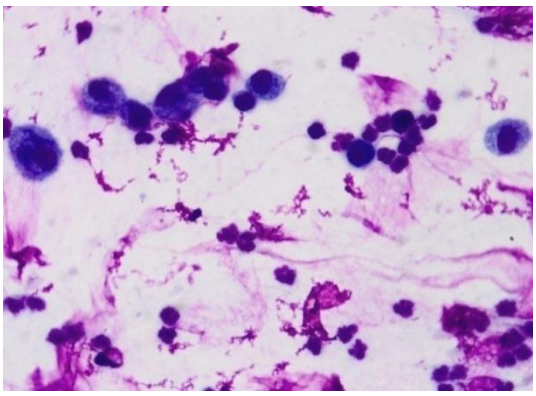

Fig. 45: TTW of dog with pulmonary abscessation showing increased macrophages and neutrophils indicating chronic active inflammation-100x (Leishman staining)

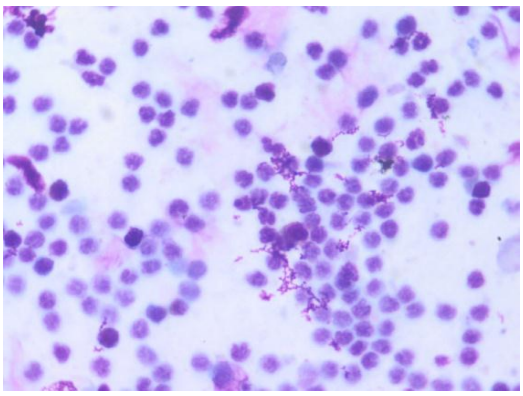

Fig. 46: Large number of neutrophils and few macrophages were seen in the fine needle aspirate taken from 7th and 8th ICS in a dog with pulmonary abscessation -100x Leishman staining)

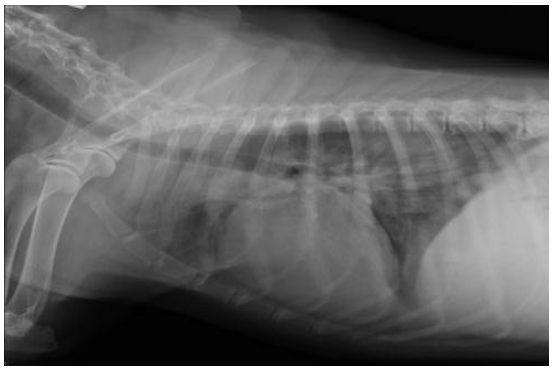

Fig. 53: Lateral radiograph showing mild to moderate bronchial pattern and severe interstitial pattern, radiopacity increased around perihilar region in a dog with bronchioloalveolar carcinoma.

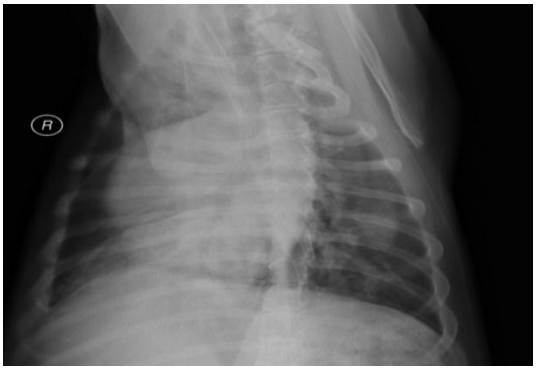

Fig. 54: Ventrodorsal radiograph showing severe interstitial pattern and moderate alveolar pattern in caudal lobe, mild bronchial and edema around secondary bronchus, increased opacity in cranial lung lobe in a dog with bronchioloalveolar carcinoma.

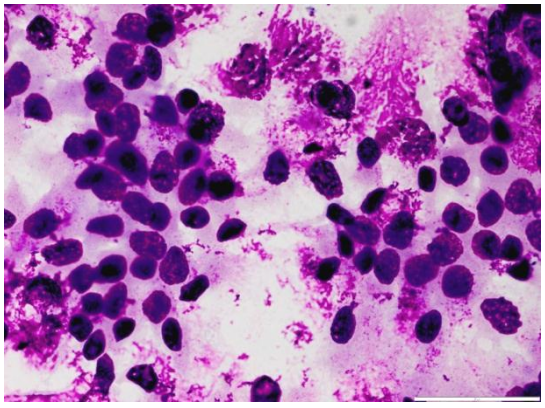

Fig. 55: Cluster of slightly pleomorphic epithelial cells in TTW, suggestive of bronchioloalveolar carcinoma-100x. (Leishman staining)

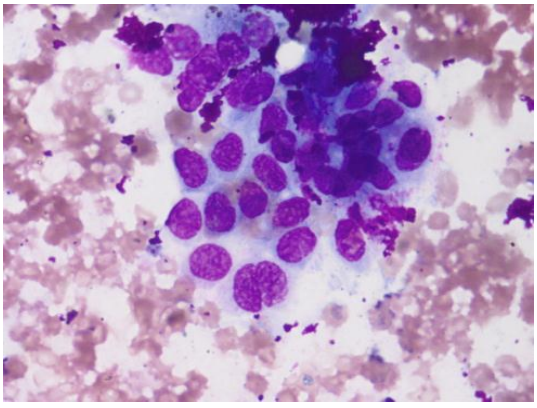

Fig. 56: Cluster of cells showing prominent nucleoli indicating liver metastasis in a dog with bronchioloalveolar carcinoma -100x. (Leishman staining)

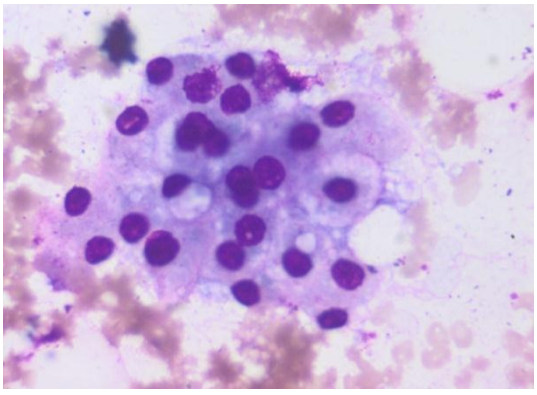

Fig. 57: Cluster of normally appearing hepatocytes showing hyperplasia in a dog with bronchioloalveolar carcinoma-100x. (Leishman staining)

TTW cytology of histiocytic sarcoma showed 5 percent other cells (which include tumor and goblet cells) (Fig. 49) and bronchioloalveolar carcinoma TTW cytology showed 15 percent other cells (which include bunch of pleomorphic epithelial cells) (Table 18). On TTW cytology examination of one of the affected dog suffering from bronchioloalveolar carcinoma, clusters of pleomorphic epithelial cells were observed (Fig. 55). These variable sized cells were having single, round to oval, central or paracentral nucleus, with prominent nucleoli along with some variation in the nucleus to cytoplasm ratio. Liver FNAC in this dog revealed clusters of normally appearing hepatocytes showing hyperplasia, suggestive of liver metastasis (Fig. 56, 57). Bertazzolo *et al* (2002) observed tridimensional clusters of pleomorphic epithelial cells showing anisocytosis, anisokaryosis, prominent nucleoli and varied amount of slightly basophilic cytoplasm with occasional vacuolation, on the cytological examination of transthoracic needle aspiration biopsy in diffused bronchioloalveolar carcinoma.

Table 18: Comparison of tracheal wash cytology (mean ± SE) between healthy and lung tumors/undiagnosed nodular interstitial pattern dogs

Parameters	Healthy group (n=7)	Histiocytic sarcoma (n=1)	Bronchiolo-alveolar carcinoma (n=1)	Nodular interstitial (n=1)	Pulmonary abscessation (n=1)
Macrophages (%)	49.57±5.28 (28-66)	42	34	29	13
Neutrophils (%)	11.43±1.00 (8-15)	40	41	59	80
Lymphocytes (%)	7.71±2.25 (0-18)	9	5	3	1
Eosinophils (%)	0.14±0.14 (0-1)	0	0	0	0
Respiratory epithelial cells (%)	29.14±7.13 (9-60)	4	5	9	6
Others (%)	2±0.79 (0-5)	5	15	0	0
Mean cell number (cells/HPF)	8.35±2.68 (1.85-21.4)	34.1	15.25	28.35	90.25

Values in parenthesis depicts range

Transthoracic fine needle aspiration from the large abscesses was performed in the dog with pulmonary abscessation, with 22 gauge needle through the left seventh and eighth intercostal space which demonstrated large number of neutrophils along with few macrophages suggestive of predominant suppurative inflammation (Fig. 46). Similar results were seen by Nguyen (1989) on fine needle aspirate cytology of lung abscesses. TTW cytology of dog with pulmonary abscessation revealed increased macrophages and neutrophils suggestive of chronic active inflammation (Fig. 45).

In histiocytic sarcoma case, pleomorphic large cells with round to oval eccentric placed nuclei, resembling macrophages were observed in the fine needle aspirate taken from the cavitory lesion of the diseased dog, suggestive of histiocytic sarcoma (Fig. 50). Lymph node aspiration showed reactive hyperplasia of the lymphocytes, indicating metastasis of the tumor (Fig. 51). Coomer and Liptak (2008) on cytologic examination of samples aspirated from histiocytomas observed clusters of round or oval cells having variable amount of pale basophilic cytoplasm without vacuolation and round to ovoid, eccentrically placed nuclei containing finely granular chromatin and non-noticeable nucleoli. Cannon *et al* (2015) reported metastasis of HS to the local lymph nodes in all the affected dogs taken in the study.

e) Culture of TTW

Bacterial culture was found to be negative in 2 dogs and positive in other 2 dogs out of 4 in which TTW aspiration was done. In one dog suffering with pulmonary abscessation, *E.coli* was observed (big, circular and moist colonies on the blood agar). *Escherichia coli* organisms were further isolated on MacConkey agar and appeared as pink colored, circular, moist and smooth lactose fermenting colonies. On gram staining pink colored rods was observed which are catalase positive and oxidase negative and green metallic sheen was observed on the EMB agar. In human patients suffering with lung abscesses, anaerobic bacteria which are mainly isolated aregram-negative (*Bacteroides fragilis, Fusobacterium capsulatum and necrophorum*) and gram-positive (*Peptostreptococcus*), whereas aerobic bacteria predominantly isolated includes *Staphylococcus aureus* (including MRSA), *Streptococcus pyogenes* and *pneumonia, Klebsiella pneumonia, Pseudomonas aeruginosa, Haemophilus influenza* (type B), *Acinetobacter spp, Escherichia coli* and *Legionela* (Liu *et al* 2010, Pande *et al* 2012).

In other one case having nodular interstitial pattern, *Staphylococcus aureus* was observed on blood agar showing round smooth convex colonies with hemolysis which

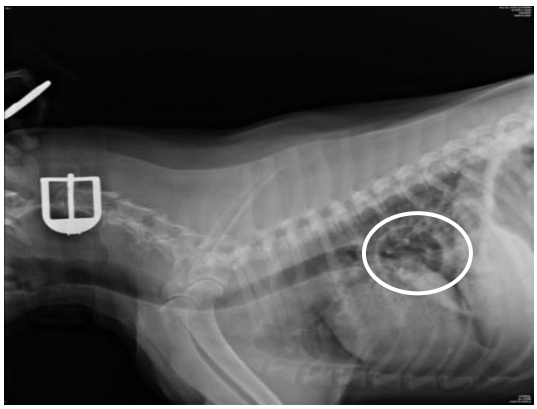

Fig.47: Lateral thoracic radiograph showing moderate alveolar pattern and moderate bronchial pattern. Air bullae (white circle) seen in left caudal lung lobe. One collapsed lung lobe and increased opacity was also seen in dog with histiocytic sarcoma

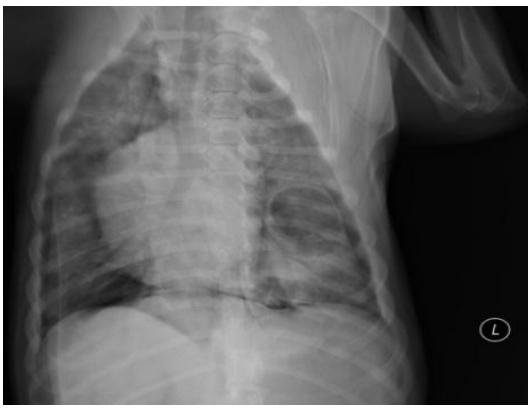

Fig. 48: Ventrodorsal thoracic radiograph showing increased opacity on both sides, moderate alveolar and bronchial pattern and pneumobulla seen in a dog with histiocytic sarcoma

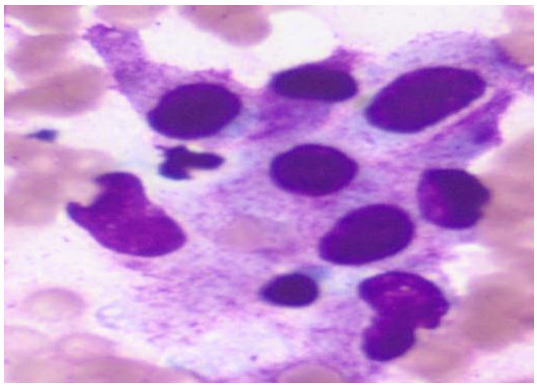

Fig. 49: Pleomorphic cells resembling macrophages in TTW of dog with histiocytic sarcoma along with rbc's-100x. (Leishman staining)

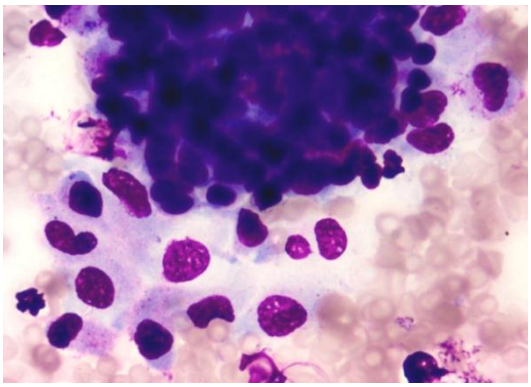

Fig. 50: Pleomorphic large cells resembling macrophages were observed in aspirate taken from cavitory lesion suggestive of histiocytic sarcoma-100x (Leishman staining)

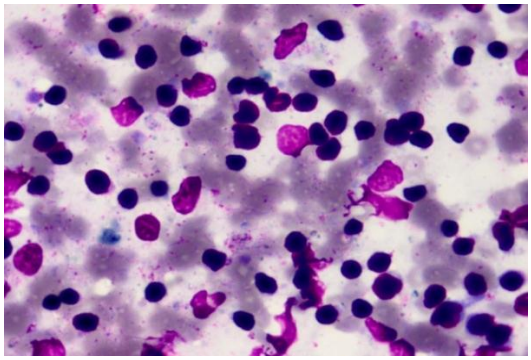

Fig. 51: Lymph node aspirate showing reactive hyperplasia of lymphocytes in a dog with histiocytic sarcoma-100x (Leishman staining)

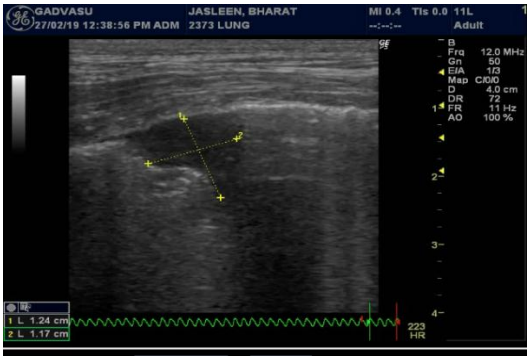

Fig. 52: Thoracic ultrasonograph of dog with histiocytic sarcoma showing cavitory lesion (1.24 x 1.17 cm) on left caudal lung lobe

was further isolated on Baird Parker media showing round black colonies. On gram staining, purple cocci were present in bunches which are catalase positive and oxidase negative.

4.2.1.4 Interstitial lung diseases (ILDs) (n=12)

Interstitial lung diseases (ILDs) are diffuse parenchymal lung diseases constituting the diverse group of non-infectious, non-neoplastic respiratory tract disorders having overlapping clinicopathologic and radiographic features (Travis *et al* 2002). ILDs are mainly classified into three major groups in dogs and cats i.e. idiopathic interstitial pneumonias (IIPs), ILDs secondary to known causes and the miscellaneous ILDs (Travis *et al* 2002).

In this present study, 2 cases of anthracosis (ILDs of known cause), 1 case of eosinophillic bronchopneumopathy (miscellaneous ILDs) and 9 cases of non-specific interstitial pneumonia (NSIP) (idiopathic interstitial pneumonia) were recorded. Mirsadraee (2014) reported the accumulation of the black dust particles from chronic exposure to air pollution or inhalation of coal dust or smoke, being the leading cause resulting in anthracosis (a milder form of pneumoconiosis). Eosinophilic infiltration of lungs and bronchial mucosa is defined as eosinophilic bronchopneumopathy (EBP) (Clercx *et al* 2000).

Twelve dogs were presented to the small animal clinics of Teaching Veterinary hospital with history of coughing and exercise intolerance. Coughing was seen in 10 dogs (productive in 2 and non productive in 8) and exercise intolerance in 6 dogs. Other clinical observations include tachypnea in 3, dyspnea in 3, fever in 7, lethargy in 1, decreased appetite in 2, sneezing in 1 and positive inducible cough reflex in 11 dogs. All clinical signs present in our study were similar to those described by Jones *et al* (2000), Norris *et al* (2002) and Cohn *et al* (2004). However, they also observed that respiratory clinical signs can be absent in some cases. Mean age of the affected dogs was 3.46±0.59 years. Reinero and Cohn (2007) reported that mostly middle to older aged dogs and cats are found to be affected with ILDs with equal tendency among both the genders. Mean rectal temperature was significantly higher in the diseased dogs in comparison to the healthy control group. Mean respiration rate of the NSIP dogs was found to be significantly higher as compared to the healthy control dogs ($P<0.05$) (Table 19).

Table 19: Comparison of age and physiological parameters (mean ± SE) between healthy and interstitial lung diseased dogs

Parameters	Healthy (n=10)	NSIP (n=9)	Anthracosis (n=2)	EBP (n=1)
Age (years)	3.8±0.52 (4.00, 1.5-7)	2.94±0.62 (2.5, 0.75-7)	5±2.00 (3-7)	5
Body weight (Kg)	24.68±2.36 (27.20, 13.7-35)	30.3±3.03 (32, 13-39)	29.35±6.67 (22.7-36)	20
RT(ºF)	102.12±0.20 (102^{b}, 101.4-103.2)	103.58±0.28 (103.6^{a}, 102-105)	102.5±1.30 (101.2-103.8)	104.8
HR (bpm)	99.2±5.23 (98, 76-136)	108±6.99 (104, 80-140)	110±10.03 (100-120)	92
RR (breaths/min)	27.6±2.29 (26^{b}, 18-40)	46.22±6.54 (50^{a}, 14-76)	52±4.01 (48-56)	56

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at $P<0.05$

Abnormal lung sounds were observed on auscultation of the lungs (harsh in 6, wheezes in 1 and crackles in 2 dogs) and normal lung sounds were found in 3 dogs. Typical feature of ILDs is the presence of harsh lung sounds in absence of an alveolar radiographic pattern as recorded by Reinero and Cohn (2007). Faecal floatation was performed in all diseased cases to rule out parasitic infection and was found negative for presence of any parasitic eggs or larvae.

a) Hemato-biochemical parameters

Mean hemoglobin concentration of diseased dogs was found to be in the normal range. Mean total leukocyte count in the non-specific interstitial pneumonia and the eosinophilic bronchopneumopathy group were found to be (14313±3089 and 15520cells/µl, respectively) which were higher than the healthy control group. Complete blood count was done to rule out underlying causes of disease. No significant difference was observed in blood picture of diseased dogs as compared to the healthy dogs. Mean fibrinogen concentration in diseased dog was found significantly higher as compared to healthy dogs ($P<0.05$) (Table 20). It is proposed that plasma fibrinogen could be considered as a potent biomarker of respiratory diseases. A significant relationship was established between plasma fibrinogen and pulmonary dysfunction in Japanese human population (Shibata *et al* 2013).

Table 20: Comparison of hemato-biochemical parameters (mean ± SE) between healthy and interstitial lung diseased dogs

Parameters	Healthy (n=10)	Non-specific interstitial pneumonia (n=9)	Anthracosis (n=2)	Eosinophilic bronchopneumopathy (n=1)
Hb (g/dl)	13±0.78 (13.55, 9.3-16.5)	13.16±0.44 (13.5, 10.1-14.5)	13.35±1.15 (12.2-14.5)	14.2
TEC (count/µl × 10^6)	6.03±0.32 (6.24^a, 4.5-7.47)	4.78±0.20 (4.63^b, 4.17-6.22)	5.43±0.60 (4.83-6.03)	4.73
TLC (count/µl)	11376±1372 (10030, 6790-19100)	14313±3089 (13000, 5800-35310)	8075±2331.95 (5750-10400)	15520
PCV (%)	39.76±2.13 (40.85, 31.5-49.9)	39.61±1.30 (40.50, 30.7-43.5)	41.25±2.26 (39-43.5)	42.6
Platelets (count/µl × 10^3)	228.8±39.9 (193.5, 119-542)	216.11±18.77 (206, 95-287)	262±41.12 (221-303)	62
Absolute neutrophils (count/µl)	7724±1114 (6502, 2717-12926)	11254±2710 (8422, 5330-31073)	6391.5±2142.89 (4255-8528)	11640
Absolute lymphocytes (count/µl)	3243±790 (2219, 1161-9168)	2677±630 (2189, 348-5530)	1338.5±535.09 (805-1872)	1552
Absolute eosinophillia (count/µl)	409±120 (467, 0-901.8)	362±203 (0, 0-1788)	345±346.03 (0-690)	2328
Total protein (g/dl)	6.38±0.08 (6.45, 5.9-6.8)	6.39±0.34 (6.2, 5-8.3)	7±0.10 (6.9-7.1)	5.5
Albumin (g/dl)	2.77±0.11 (2.7, 2.4-3.4)	2.87±0.12 (2.8, 2.2-3.3)	2.55±0.15 (2.4-2.7)	2.7
Globulin (g/dl)	3.61±0.10 (3.6, 3.1-4.1)	3.52±0.32 (3.6, 2.2-5.5)	4.45±0.05 (4.4-4.5)	2.8
A : G ratio	0.78±0.05 (0.77, 0.58-1.10)	0.87±0.09 (0.84, 0.51-1.27)	0.57±0.04 (0.53-0.61)	0.96
Fibrinogen (g/dl)	0.32±0.03 (0.4^b, 0.2-0.4)	0.69±0.06 (0.8^a, 0.4-0.9)	0.8±0.20 (0.6-1)	0.8

Values in parenthesis depicts range

Values having different superscript in the same row differ significantly at $P<0.05$

b) Blood gas analysis

Mean partial pressure of oxygen in dogs with non-specific interstitial pneumonia and eosinophilic bronchopneumopathy was found to be significantly lower than the healthy control group (Table 21).

Table 21: Comparison of blood gas parameters (mean ± SE) between healthy and interstitial lung diseased dogs

Parameters	Healthy group (n=6)	Non-specific interstitial pneumonia (n=7)	Anthracosis (n=1)	EBP (n=1)
pH	7.44±0.01 (7.44, 7.38-7.48)	7.45±0.02 (7.47, 7.34-7.53)	7.48	7.44
$PaCO_2$ (mmHg)	27.17±1.25 (27.5, 23-32)	30.86±3.25 (30, 23-49)	30	27
HCO_3 (mmol/L)	17.17±0.34 (17.3^b, 15.6-17.9)	19.5±0.90 (18.6^a, 17.1-24.3)	20.6	17.1
AnGap (mmol/L)	16.67±0.70 (16.3^b, 14.4-18.7)	21.83±1.44 (23.3^a, 16-25.6)	25.3	20.9
tCO_2 (mmol/L)	18±0.36 (18.2^b, 16.3-18.8)	20.43±0.98 (19.6^a, 17.9-25.7)	21.5	18
BE (mmol/L)	-3.85±0.43 (-3.7^b, -5.5- -2.5)	-2.21±0.48 (-2.2^a, -4.2- -0.6)	-0.6	-4.2
stHCO3 (mmol/L)	20.68±0.30 (21^b, 19.7-21.5)	22.33±0.41 (22.3^a, 21-23.7)	23.7	20.5
st pH	7.34±0.01 (7.34^b, 7.32-7.35)	7.37±0.01 (7.37^a, 7.34-7.40)	7.40	7.33
cH+ (nmol/L)	35.98±1.32 (35.65, 33.1-41.9)	35.54±1.97 (33.50, 29.7-45.7)	33.2	36.3
PaO_2 (mmHg)	87.17±1.74 (86.5^a, 81-92)	71.14±8.21 (84, 33-91)	84	62
tHb (g/dL)	12.57±0.67 (12.15, 11-15.3)	11.97±0.51 (12.2, 9.8-13.5)	12.7	12
SO_2 (%)	93±1.06 (93, 89-97)	92.33±1.76 (94, 86-96)	96	89
Na+ (mmol/L)	135.17±3.71 (139, 119-142)	141.57±2.49 (139, 134-149)	147	150
K+ (mmol/L)	3.41±0.12 (3.45, 3-3.78)	3.36±0.24 (3.2, 2.4-4.4)	3.8	3.2
Cl- (mmol/L)	105.17±2.94 (107.5, 92-111)	105.86±1.49 (105, 100-111)	105	115

Values in parenthesis depicts range

Values having different superscript in the same row differ significantly at $P<0.05$

Mean saturation of oxygen in diseased group was found to be in normal range. Hypoxemia and the degree of pulmonary dysfunction can be independently measured by arterial blood gas analysis (Reinero and Cohn 2007).

c) Radiography

Thoracic radiography (lateral and VD view) was done in all the diseased dogs. In our study, interstitial pattern (moderate in 8 and severe in 4 dogs) and bronchial pattern (mild in 1 and moderate in 2 dogs) was observed on chest radiography (Fig. 60, 63). Reinero and Cohn (2007) stated that in majority of ILDs cases, interstitial pattern was observed, however, an alveolar or bronchointerstitial pattern can also occur. In EBP dog, moderate bronchointerstitial pattern was recorded along with increased radiopacity of cranial and caudal lobe (Fig. 58). This finding is in accordance with the Clercx *et al* (2000) who reported moderate to severe bronchointerstitial pattern in 68 percent of dogs and it was the most common radiographic pattern in their study.

d) Transtracheal wash (TTW) cytology

Mean cell number in diseased dogs especially NSIP dogs was found to be significantly higher than the healthy control dogs ($P<0.05$). In NSIP dogs increased number of non degenerated neutrophils along with mucus was observed on TTW cytology (Fig. 64, 65). Mean neutrophil count in NSIP ($P<0.05$) and anthracosis dogs was found to be significantly higher than the healthy control dogs. TTW cytology examination of the anthracosis dog in the present study, revealed increased number of neutrophils and macrophages with engulfed blackish pigment and lymphoid cells, suggesting long standing chronic active inflammation (Fig. 61, 62). In our study, severe eosinophilia (63%) with mean cell count of 10.1 cells/HPF was observed in tracheal wash of eosinophilic bronchpneumopathic dog (Table 22). Clercx *et al* (2000) observed the increased number of eosinophils (20 to 50%) in EBP dogs. In other study, Kumrow and Rozanski (2012) also stated that if marked eosinophilia was observed, EBP should be considered rather than chronic bronchitis.

Table 22: Comparison of tracheal wash cytology (mean ± SE) between healthy and interstitial lung disease dogs

Parameters	Healthy group (n=7)	Non-specific interstitial pneumonia (n=5)	Anthracosis (n=2)	EBP (n=1)
Macrophages (%)	49.57±5.28 (51[a], 28-66)	26±6.36 (31[b], 8-43)	15.5±3.51 (12-19)	9
Neutrophils (%)	11.43±1.00 (11[b], 8-15)	59.6±6.79 (62[a], 36-78)	47±5.01 (42-52)	18
Lymphocytes (%)	7.71±2.25 (7, 0-18)	4.8±2.13 (4, 1-13)	16.5±11.53 (5-28)	0
Eosinophils (%)	0.14±0.14 (0, 0-1)	0±0 (0, 0-0)	0.50.±0.50 (0-1)	63
Respiratory epithelial cells (%)	29.14±7.13 (32[a], 9-60)	8.8±2.44 (8[b], 2-17)	17.5±14.54 (3-32)	10
Others (%)	2±0.79 (2, 0-5)	0.8±0.37 (1, 0-2)	3±1.00 (2-4)	0
Mean cell number (cells/HPF)	8.35±2.68 (5.8, 1.85-21.4)	44.2±18 (34.1, 6.7-99.8)	40.05±14.09 (26-54.1)	10.1

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at $P<0.05$

e) Culture of TTW

Tracheal wash fluids were cultured on Blood agar, Brain heart infusion agar and Sabouraud dextrose agar for isolating the etiological agent. Culture was found to be negative in 6 out of the 8 dogs. *Staphylococcus aureus* was identified in two dogs in this group. On Blood agar, it showed medium sized round, smooth white colour colonies. On gram staining, violet colour cocci in irregular clusters or shape of bunch of grapes were found which were catalase positive and oxidase negative.

f) Antibiotic sensitivity tests

Staphylococcus spp. obtained from tracheal wash culture in two cases was subjected for antibiotic sensitivity tests. *Staphylococcus aureus* was found to be sensitive for ciprofloxacin, cloxacillin, ampicillin, enrofloxacin, ceftriaxone followed by gentamicin, amoxicillin and amikacin.

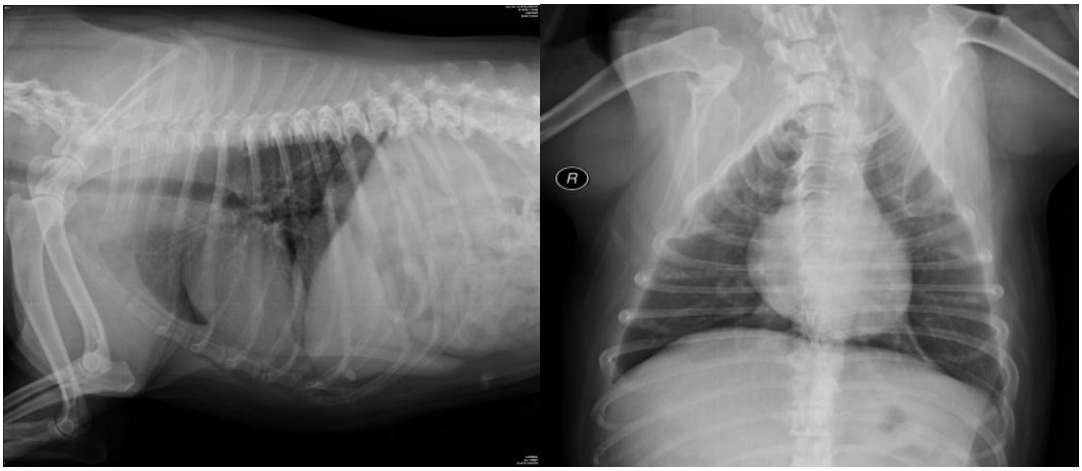

Fig. 58: Lateral and ventrodorsal thoracic radiograph showing moderate broncho-interstitial pattern, increased opacity of cranial and caudal lobe in dog with eosinophilic bronchopneumopathy (EBP)

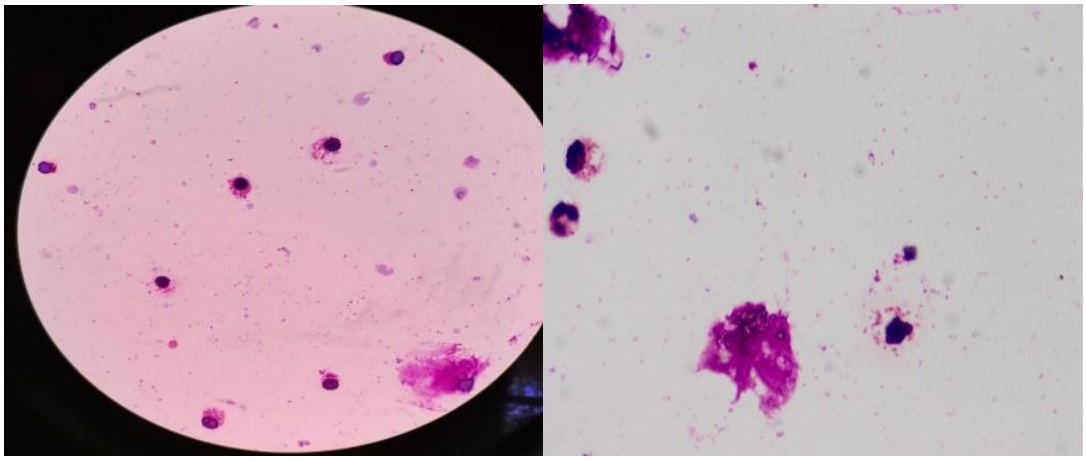

Fig. 59: Severe eosinophilia in TTW of EBP dog-100x. (Leishman staining)

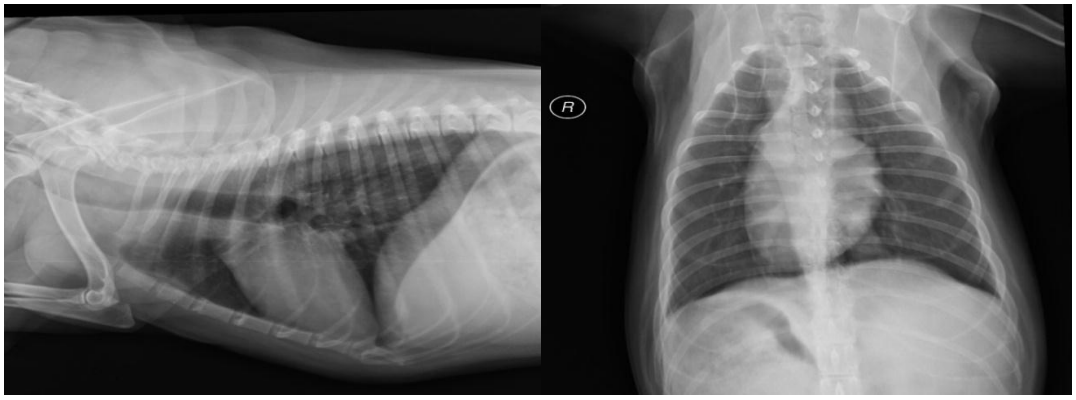

Fig. 60: Lateral and ventrodorsal radiograph in a dog with anthracosis showing moderate interstitial pattern. Mild bronchial pattern and increased opacity was also evident

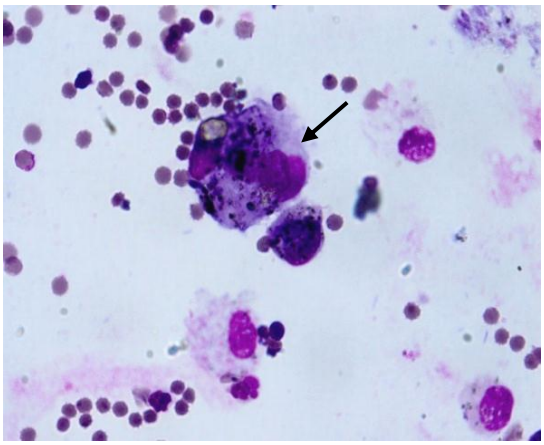

Fig. 61: Giant macrophage (arrow) with engulfed blackish pigment in TTW of a dog with anthracosis-100x. (Leishman staining)

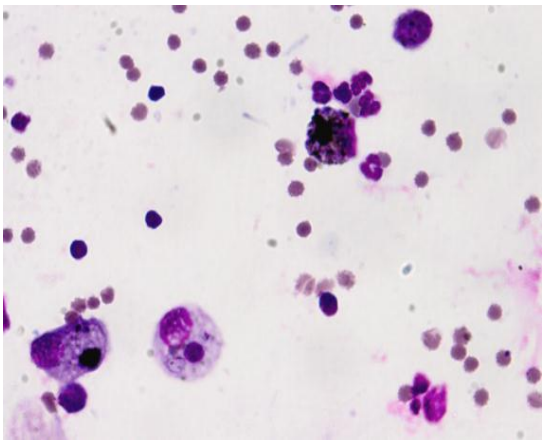

Fig. 62: Macrophage with engulfed blackish pigment in TTW of a dog with anthracosis-100x. (Leishman staining)

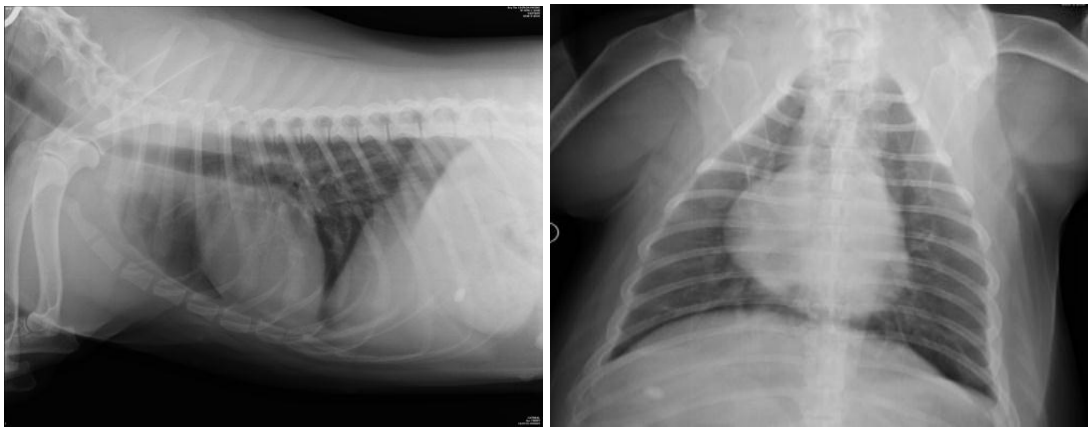

Fig. 63: Lateral and ventrodorsal thoracic radiograph showing moderate interstitial pattern and bilateral increased radiopacity in a dog with NSIP (non-specific interstitial pneumonia)

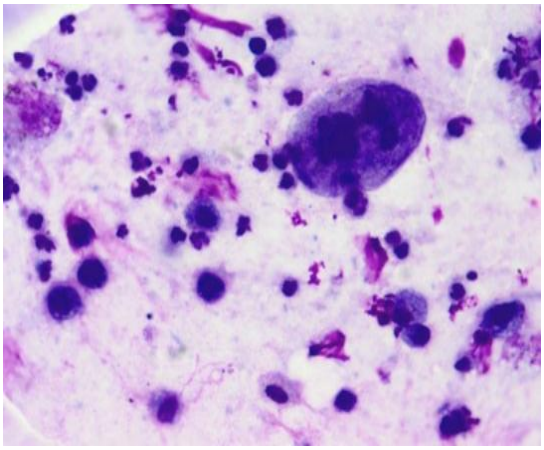

Fig. 64: Giant cell, epithelial cells and neutrophils in TTW of a dog with ILDs. Leishman stain 100x

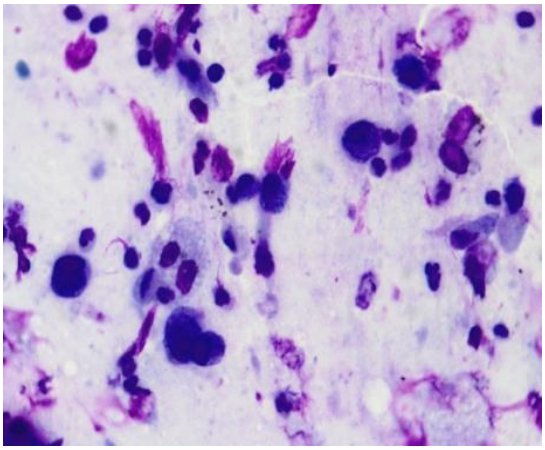

Fig. 65: Macrophages, epithelial cells and many neutrophils seen in TTW of dog with ILDs. Leishman stain 100x

4.2.3 Healthy vs Dogs affected with respiratory affections

Numerous challenges usually occur while evaluating a dog with respiratory disease. Palpation and the visual evaluation of the respiratory tract is complex, because the majority part of it is enclosed within the bony structures. Clinical diagnosis of the respiratory diseases is difficult until the disease becomes fairly severe (Miller 2007). Majority of dogs with respiratory diseases are presented with chief complaint of sneezing, nasal discharge, coughing, epistaxis, laboured breathing or exercise intolerance. Other less common signs include syncope, regurgitation, dysphagia, dysphonia, collapse or cachexia (Corcoran 2000, Fuentes 1998).

Mean age of dogs in the diseased group was 4.38±0.47 years (middle age) in our study (Table 25) with variation in dogs with nodular interstitial pattern (7.5±2.76 years) (older age) (Table 23). The clinical course of illness was longer in allergic rhinitis group with duration of 90 days followed by bacterial bronchopneumonia group with 82.5±29.51 days. Only about 30 percent of diseased animals had fever. Weight loss was evident in 27.5% of the diseased dogs whereas exercise intolerance was observed in 52.5% of the diseased dogs. In this study out of 40 dogs, 57% were male and 43% were females (Fig. 70). The occurrence of respiratory diseases didn't get influenced by the sex of the animal (Ayodhya *et al* 2013). Observation in our study might be because of overrepresentation of male dogs in small animal clinics of the university.

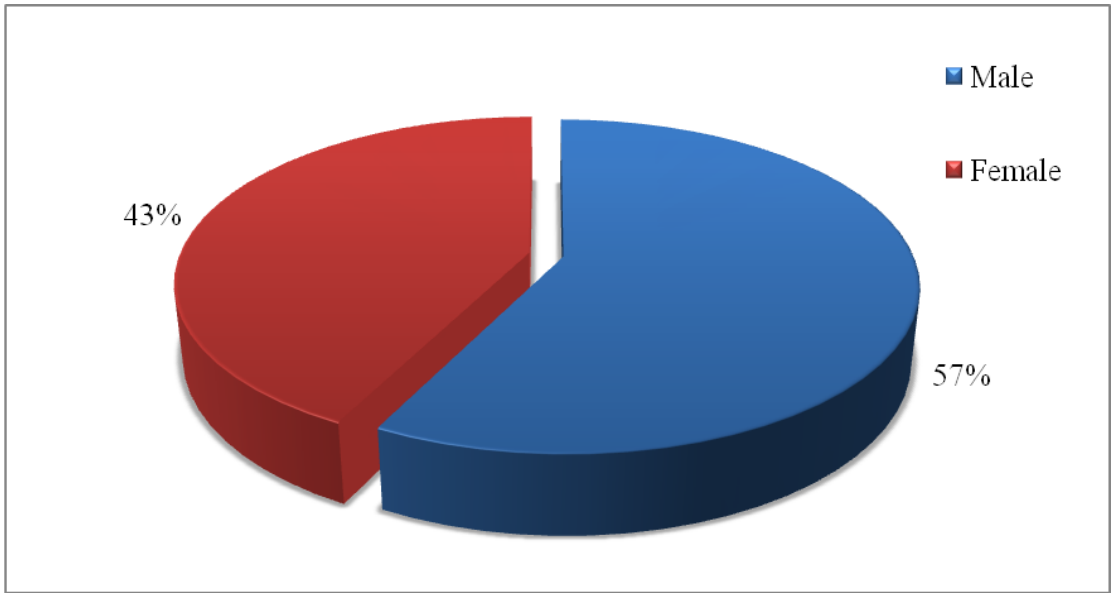

Fig. 70: Disease Distribution in Dogs

Table 23: Common historical findings in diseased dogs

Clinical signs		Chronic bronchitis (n=11)	Non-specific Interstitial pneumonia (n=9)	BP (n=7)	Undiagnosed nodular interstitial (n=3)	Anthracosis (n=2)	Lung tumors (n=2)	EBP (n=1)	Pulmonary abscessation (n=1)	Nasal SCC (n=3)	Allergic rhinitis (n=1)
Mean age (years)		3.64±0.93 (0.8-9)	2.94±0.62 (0.75-7)	4.8±1.02 (1-9)	7.5±2.76 (4.5-13)	5±2.01 (3-7)	5.5±2.50 (3-8)	5	4	4.17± 1.96 (1.5-8)	10
Mean duration of illness (days)		34±11.92 (12-122)	24.63±13.10 (3-120)	82.5±29.51 (30-240)	20.33±5.79 (10-30)	22.5±0.12 (15-30)	6.5±2.50 (4-9)	25	60	50±10.00 (30-60)	90
Duration of onset	**Acute (3-4 days)**	0	2	0	0	0	0	0	0	0	0
	Subacute (4-28 days)	4	6	0	2	1	2	1	0	0	0
	Chronic (>28days)	7	1	7	1	1	0	0	1	3	1
History of fever		0	5	1	1	1	2	1	0	1	0
Weight loss		5	0	2	1	1	1	0	1	0	0
Exercise intolerance		5	5	5	2	1	2	0	1	0	0
Sneezing		0	0	1	0	0	0	0	0	1	1
Inappetance		1	2	3	2	0	1	0	0	1	0

Table 24: Clinical manifestations in diseased dogs

Clinical signs		Chronic bronchitis (n=11)	NSIP (n=9)	BP (n=7)	Undiagnosed nodular interstitial (n=3)	Anthracosis (n=2)	Lung tumors (n=2)	EBP (n=1)	Pulmonary abscessation (n=1)	Nasal SCC (n=3)	Allergic rhinitis (n=1)	Overall (n=40)
Cough	Productive	0	2	7	1	0	0	0	0	0	0	10 (25%)
	Non productive	9	7	0	2	1	2	0	1	1	0	23 (57.5%)
Nasal discharge	Serous	3	1	1	1	0	0	0	1	0	0	7 (17.5%)
	Mucopurulent	1	0	3	0	0	0	0	0	0	0	4 (10%)
	Epistaxis	0	0	0	0	0	0	0	0	3	1	4 (10%)
Inducible cough reflex		7	8	6	2	2	0	1	0	1	0	27 (67.5%)
Breathing pattern	Dyspnea	3	2	3	1	0	1	0	1	0	0	11 (27.5%)
	Tachypnea	1	2	3	1	0	1	0	0	1	0	9 (22.5%)
Lethargy		1	0	3	2	1	1	0	1	2	0	11 (27.5%)
Dehydration		4	0	4	1	2	0	1	1	1	0	14 (35%)

4.2.3.1 Clinical manifestations in diseased dogs

Common clinical signs recorded on physical assessment in respiratory diseases in previous studies were nasal discharge, cough, exercise intolerance, increased respiratory effort or apparent respiratory distress and other systemic signs of illness such as lethargy, anorexia and fever (John *et al* 2000, Clercx *et al* 2003, Cadore 2011, Centil *et al* 2012).

In the present study, coughing was evident in nearly 82.5 percent of dogs in diseased group. Non-productive cough was observed in 57.5 percent (23/40) and productive cough was seen in 25 percent (10/40) of the diseased dogs. Abnormal breathing pattern was evident in 50 percent of the diseased dogs in this study. Respiratory distress was evident in 11 out of 40 dogs (27.5%) whereas tachypnea was observed in 9 out of 40 dogs (22.5%) in the present study (Table 24). Affected dogs exhibited varied type of nasal discharge depending on the cause. Seven out of 40 diseased dogs (17.5%) showed serous nasal discharge while only 4 diseased dogs (10%) displayed mucopurulent nasal discharge which was mainly exhibited by dogs suffering from bacterial bronchopneumonia (Sykes 2013) (Table 24). Three out of 40 dogs showed unilateral epistaxis whereas one dog had bilateral epistaxis, mainly shown by dogs suffering with squamous cell carcinoma and allergic rhinitis. Inducible cough reflex was found to be positive in 67.5 percent (27/40) diseased dogs. Dehydration was present in 14/40 dogs (35%). Mean body temperature of diseased dogs was found to be 102.71 ± 0.20^{0}F. Mean heart rate in diseased dogs was 109.13±3.61 beats/min which was higher than the healthy group (99.2±5.23 beats/min). Mean respiration rate was found to be significantly higher in diseased group (53.63±3.71 breaths/min) as compared to the healthy group (27.6±2.29breaths/min; $P<0.05$) (Table 25).

Abnormal lung sounds (harsh in 22, wheezes in 4 and crackles in 6) was observed in 32 out of 40 diseased dogs (80%) on auscultation. Miller (2007) described that crackles are the short explosive, nonmusical sounds characteristically produced by the delayed opening of small airways caused by an abnormal fluid air interface (i.e., pneumonia, pulmonary edema and bronchitis) whereas wheezes are musical sounds mainly generated by airway narrowing, obstruction or stenosis. Lung sounds

can be normal, even if history or physical evidence showed respiratory involvement (Miller 2007).

Table 25: Comparison of age and physiological parameters (mean ± SE) between healthy and diseased dogs

Parameters	Healthy (n=10)	Diseased (n=40)
Age (years)	3.8±0.52 (4.00, 1.5-7)	4.38±0.47 (3.75, 0.75-13)
Body weight (Kg)	24.68±2.36 (27.20, 13.7-35)	24.11±1.72 (22.20, 5.5-45)
RT (ºF)	102.12±0.20 (102, 101.4-103.2)	102.71±0.20 (102.8, 100-105)
HR (bpm)	99.2±5.23 (98, 76-136)	109.13±3.61 (104, 72-172)
RR (breaths/min)	27.6±2.29[b] (26, 18-40)	53.63±3.71[a] (52, 14-110)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at P<0.05

4.2.3.2 Hemato-biochemical parameters in diseased dogs

It is usually said that hematology and biochemical profiles show nonspecific changes in dogs with respiratory diseases (Miller 2007).

Mean hemoglobin concentration of diseased group was 12.34±0.38g/dl. No significant difference was recorded in hemoglobin concentrations of respiratory disease dogs (Piva *et al* 2010, Ayodhya *et al* 2013). Mean TLC count of diseased group was higher than the healthy group (14692±1142 vs 11376±1372 count/µl, respectively). Mean absolute neutrophil count of diseased group was found to be significantly higher as compared to the healthy group (12091±1020 vs 7724±1114 count/µl, respectively; P<0.05). Neutrophilia was recorded as the main hematological finding in dogs suffering from respiratory diseases in a recent study (Amrute *et al* 2009). Absolute lymphocyte count of diseased dog was 2038±200 count/µl which was significantly lower than the healthy dogs (3243±790 count/µl; P<0.05). Maden *et al* (2000) observed the significant increase in total leukocyte counts whereas macrophage and lymphocyte counts were found to be decreased.

Table 26: Comparison of age and physiological parameters (mean ± SE) between healthy and diseased groups

Parameters	Healthy (n=10)	Squamous cell carcinoma (n=3)	Allergic rhinitis (n=1)	Chronic bronchitis (n=11)	BP (n=7)	Lung tumors/Nodular interstitial pattern (n=6)	NSIP (n=9)	Anthracosis (n=2)	EBP (n=1)
Age (years)	3.8±0.52 (4.00, 1.5-7)	4.17±1.96 (1.5-8)	10	3.64±0.93 (2.5, 0.8-9)	4.8±1.02 (5, 1-9)	6.25±1.51 (3-13)	2.94±0.62 (2.5, 0.75-7)	5±2.00 (3-7)	5
Body weight (Kg)	24.68±2.36 (27.20, 13.7-35)	26±6.24 (17-38)	35	16.77±2.45 (16, 6.8-35)	24.36±5.87 (22, 5.5-45)	24.17±3.70 (14-38)	30.3±3.03 (32, 13-39)	29.35±6.67 (22.7-36)	20
RT (°F)	102.12±0.20 (102, 101.4-103.2)	102.33±0.88 (101-104)	102.2	101.89±0.34 (101.80, 100.4-103.6)	102.57±0.54 (102.8, 100-104.8)	103.03±0.40 (101.4-104)	103.58±0.28 (103.6, 102-105)	102.5±1.30 (101.2-103.8)	104.8
HR (bpm)	99.2±5.23 (98, 76-136)	110.67±14.67 (96-140)	88	105.27±8.13 (96, 72-172)	115.86±6.77 (120, 85-140)	115.33±12.45 (88-156)	108±6.99 (104, 80-140)	110±10.03 (100-120)	92
RR (breaths/min)	27.6±2.29 (26, 18-40)	34.67±7.06 (24-48)	52	48.55±6.35 (36, 28-84)	70.1±10.0 (64, 34-105)	69±9.82 (40-110)	46.22±6.54 (50, 14-76)	52±4.01 (48-56)	56

Values in parenthesis depicts range

Mean total protein of diseased group was 6.34±0.17 g/dl. Mean globulin concentration of the diseased group was 3.70±0.14g/dl. Mean plasma fibrinogen concentration of diseased group was significantly higher than the healthy control group (0.69±0.03 and 0.32±0.04g/dl, respectively; P<0.05) (Table 27). Plasma fibrinogen was considered as a potent biomarker of the respiratory diseases due to the association between plasma fibrinogen and pulmonary function (Shibata *et al* 2013).

Table 27: Comparison of hemato-biochemical parameters (mean ± SE) between healthy and diseased dogs

Parameters	Healthy (n=10)	Diseased (n=40)
Hb (g/dl)	13±0.78 (13.55, 9.3-16.5)	12.34±0.38 (13.05, 7.4-16.8)
TEC (count/µl × 10^6)	6.03±0.32[a] (6.24, 4.5-7.47)	4.93±0.19[b] (4.78, 2.47-7.31)
TLC (count/µl)	11376±1372 (10030, 6790-19100)	14692±1142 (14170, 5750-39500)
PCV (%)	39.76±2.13 (40.85, 31.5- 49.9)	37.52±1.13 (40.50, 22.4-50.8)
Platelets (count/µl×10^3)	228.8±39.9 (193.5, 119-542)	236.5±16.7 (51-532)
Absolute neutrophils (count/µl)	7724±1114[b] (6502, 2717-12926)	12091±1020[a] (10649, 4255-33180)
Absolute lymphocytes (count/µl)	3243±790[a] (2219, 1161-9168)	2038±200[b] (1890, 348-5530)
Absolute eosinophilia (count/µl)	409±120 (467, 0-901.8)	597±144 (224, 0-3391)
Total protein (g/dl)	6.38±0.08 (6.45, 5.9-6.8)	6.34±0.17 (6.4, 3.9-8.8)
Albumin (g/dl)	2.77±0.11 (2.7, 2.4-3.4)	2.64±0.08 (2.7, 1.3-3.4)
Globulin (g/dl)	3.61±0.10 (3.6, 3.1-4.1)	3.70±0.14 (3.65, 2.2-6)
A : G ratio	0.78±0.05 (0.77, 0.58-1.10)	0.75±0.03 (0.78, 0.37-1.27)
Fibrinogen (g/dl)	0.32±0.03[b] (0.4, 0.2-0.4)	0.69±0.03[a] (0.6, 0.4-1.2)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at P<0.05

Table 28: Comparison of hemato-biochemical parameters (mean ± SE) between healthy and diseased groups

Parameters	Healthy (n=10)	Squamous cell carcinoma (n=3)	Allergic rhinitis (n=1)	Chronic bronchitis (n=11)	Bacterial bronchopneumonia (n=7)	Pulmonary Neoplasia (n=2)	Nodular interstitial (n=3)	Pulmonary abscessation (n=1)	NSIP (n=9)	Anthracosis (n=2)	EBP (n=1)
Hb (g/dl)	13±0.78 (13.55, 9.3-16.5)	11± 1.83 (8.6-14.6)	12.9	12.11±0.74 (11.4, 8.4-15.1)	11.96±1.10 (13.8, 7.4-14.5)	9.25±0.25 (9-9.5)	12±1.71 (10-15.4)	16.8	13.16±0.44 (13.5, 10.1-14.5)	13.35±1.15 (12.2-14.5)	14.2
TEC (count/µl × 10^6)	6.03±0.32 (6.24, 4.5-7.47)	5.29± 1.11 (3.27-7.11)	6.87	5.12±0.34 (4.97, 3.37-7.31)	4.68±0.55 (4.80, 2.47-6.87)	3.08±0.08 (3-3.17)	5.00±1.09 (3.33-7.04)	5.6	4.78±0.20 (4.63, 4.17-6.22)	5.43±0.60 (4.83-6.03)	4.73
TLC (count/µl)	11376±1372 (10030, 6790-19100)	20996.7± 9386.7 (9000-39500)	15250	13985±1236 (14130, 8680-20900)	14844±2325 (13870, 6770-26620)	8630±530.08 (8100-9160)	19113.33±3258.52 (15400-25600)	16600	14313±3089 (13000, 5800-35310)	8075±2331.95 (5750-10400)	15520
PCV (%)	39.76±2.13 (40.85, 31.5-49.9)	33.27± 5.72 (26.2-44.6)	41.3	37.03±2.16 (35.60, 27.5-45.6)	36.17±3.28 (41.4, 22.4-43.5)	28.5±1.50 (27-30)	36.63±5.44 (30-47.4)	50.8	39.61±1.30 (40.50, 30.7-43.5)	41.25±2.26 (39-43.5)	42.6
Platelets (count/µl×10^3)	228.8±39.9 (193.5, 119-542)	218.67± 16.60 (196-251)	285	274.5±34.8 (305, 129-532)	255.7±58.50 (190, 51-462)	194.5±59.51 (135-254)	233.67±78.05 (106-375)	189	216.11±18.77 (206, 95-287)	262±41.12 (221-303)	62
Absolute neutrophils (count/µl)	7724±1114 (6502, 2717-12926)	17723.6± 7964.74 (6660-33180)	10370	11564±1378 (10928, 6423-20064)	12270±1898 (11096, 5687-21828)	7471±143.02 (7328-7614)	17249.2±2677.84 (13860-22528)	14608	11254±2710 (8422, 5330-31073)	6391.5±2142.89 (4255-8528)	11640

Parameters	Healthy (n=10)	Squamous cell carcinoma (n=3)	Allergic rhinitis (n=1)	Chronic bronchitis (n=11)	Bacterial bronchopneumonia (n=7)	Pulmonary Neoplasia (n=2)	Nodular interstitial (n=3)	Pulmonary abscessation (n=1)	NSIP (n=9)	Anthracosis (n=2)	EBP (n=1)
Absolute lymphocytes (count/µl)	3243±790 (2219, 1161-9168)	2386.47± 889.62 (869.4-3950)	3965	1812±276 (1903, 772-3579)	1977±373 (1939, 640-3727)	884.2±398.26 (486-1282.4)	1505.47±406.30 (980.4-2304)	1992	2677±630 (2189, 348-5530)	1338.5±535.09 (805-1872)	1552
Absolute eosinophillia (count/µl)	409±120 (467, 0-901.8)	886.6±746.42 (0-2370)	2440	608±326 (188, 0-3391)	597±362 (0, 0-2560)	274.8±274.84 (0-549.6)	358.67±223.41 (0-768)	0	362±203 (0, 0-1788)	345±346.03 (0-690)	2328
Total protein (g/dl)	6.38±0.08 (6.45, 5.9-6.8)	6.67± 0.24 (6.2-7)	8.2	6.42±0.34 (6.4, 4.8-8.8)	6.01±0.46 (6.2, 3.9-7.6)	5.3±0.20 (5.1-5.5)	5.93±0.97 (4-7)	7.5	6.39±0.34 (6.2, 5-8.3)	7±0.10 (6.9-7.1)	5.5
Albumin (g/dl)	2.77±0.11 (2.7, 2.4-3.4)	2.9± 0.06 (2.8-3)	2.2	2.66±0.12 (2.7, 2-3.3)	2.27±0.29 (2.4, 1.3-3.2)	2.25±0.65 (1.6-2.9)	2.2±0.40 (1.5-2.9)	2.6	2.87±0.12 (2.8, 2.2-3.3)	2.55±0.15 (2.4-2.7)	2.7
Globulin (g/dl)	3.61±0.10 (3.6, 3.1-4.1)	3.77± 0.26 (3.3-4.2)	6	3.75±0.28 (3.7, 2.8-6)	3.74±0.37 (3.5, 2.6-5.2)	3.05±0.45 (2.6-3.5)	3.73±0.63 (2.5-4.6)	4.9	3.52±0.32 (3.6, 2.2-5.5)	4.45±0.05 (4.4-4.5)	2.8
A : G ratio	0.78±0.05 (0.77, 0.58-1.10)	0.78±0.06 (0.67-0.88)	0.37	0.74±0.05 (0.77, 0.47-0.93)	0.64±0.09 (0.63, 0.26-0.94)	0.79±0.33 (0.46-1.12)	0.60±0.10 (0.48-0.70)	0.53	0.87±0.09 (0.84, 0.51-1.27)	0.57±0.04 (0.53-0.61)	0.96
Fibrinogen (g/dl)	0.32±0.03 (0.4, 0.2-0.4)	0.47± 0.07 (0.4-0.6)	0.6	0.62±0.03 (0.6, 0.4-0.8)	0.88±0.11 (0.9, 0.4-1.2)	0.7±0.10 (0.6-0.8)	0.73±0.07 (0.6-0.8)	0.6	0.69±0.06 (0.8, 0.4-0.9)	0.8±0.20 (0.6-1)	0.8

Values in parenthesis depicts range

4.2.3.3 Blood gas analysis

Median value of partial pressure of oxygen (PaO_2) in diseased group (64 mmHg) was significantly lower than the healthy group (86.5 mmHg; $P<0.05$) suggesting hypoxemia as the main alteration in blood gases appreciated in respiratory diseases. Mean oxygen saturation (SO_2) of diseased group was 88.26±1.92 percent (Table 29).

Table 29: Comparison of blood gas parameters (mean ± SE) between healthy and diseased dogs

Parameters	Healthy group (n=6)	Diseased group (n=26)
pH	7.44±0.01 (7.44, 7.38-7.48)	7.42±0.01 (7.44, 7.29-7.53)
$PaCO_2$ (mmHg)	27.17±1.25 (27.5, 23-32)	31.77±1.60 (30, 23-57)
HCO_3 (mmol/L)	17.17±0.34 (17.3^b, 15.6-17.9)	19.21±0.49 (18.85^a, 13.7-24.9)
AnGap (mmol/L)	16.67±0.70 (16.3^b, 14.4-18.7)	20.71±0.90 (20.9^a, 11.4-30.9)
tCO_2 (mmol/L)	18±0.36 (18.2^b, 16.3-18.8)	20.08±0.53 (19.4^a, 14.4-26.7)
BE (mmol/L)	-3.85±0.43 (-3.7, -5.5- -2.5)	-3.24±0.40 (-2.75, -7.9- -0.6)
$stHCO_3$ (mmol/L)	20.68±0.30 (21, 19.7-21.5)	21.64±0.33 (21.95, 17.3-24.2)
st pH	7.34±0.01 (7.34, 7.32-7.35)	7.35±0.01 (7.36, 7.26-7.41)
cH+ (nmol/L)	35.98±1.32 (35.65, 33.1-41.9)	37.79±1.10 (36.10, 29.7-52.5)
PaO_2 (mmHg)	87.17±1.74 (86.5^a, 81-92)	64.96±3.80 (64^b, 33-91)
tHb (g/dL)	12.57±0.67 (12.15, 11-15.3)	11.61±0.34 (12, 7.5-14.4)
SO_2 (%)	93±1.06 (93, 89-97)	88.26±1.92 (89, 60-97)
Na+ (mmol/L)	135.17±3.71 (139, 119-142)	143.62±1.93 (147.5, 121-164)
K+ (mmol/L)	3.41±0.12 (3.45, 3-3.78)	3.67±0.16 (3.45, 2.4-6.4)
Cl- (mmol/L)	105.17±2.94 (107.5, 92-111)	107±1.11 (107, 93-115)

Values in parenthesis depicts range

Values having different superscript in the same row differ significantly at $P<0.05$

Table 30: Comparison of blood gas parameters (mean ± SE) between healthy and diseased groups

Parameters	Healthy group (n=6)	Nasal SCC (n=2)	Allergic rhinitis (n=1)	Chronic bronchitis (n=6)	BP (n=6)	Pulmonary Neoplasia (n=2)	Nodular interstitial (n=2)	Pulmonary abscessation (n=1)	NSIP (n=7)	Anthracosis (n=1)	EBP (n=1)
pH	7.44±0.01 (7.44, 7.38-7.48)	7.47±0 (7.47-7.47)	7.47	7.40±0.03 (7.40, 7.29-7.48)	7.40±0.02 (7.39, 7.37-7.44)	7.34±0.04 (7.3-7.39)	7.44±0.04 (7.41-7.48)	7.37	7.45±0.02 (7.47, 7.34-7.53)	7.48	7.44
$PaCO_2$ (mmHg)	27.17±1.25 (27.5, 23-32)	27±1.63 (25-29)	28	34.8±5.62 (30, 27-57)	32.75±2.25 (33, 27-38)	35±5.00 (30-40)	25±2.01 (23-27)	46	30.86±3.25 (30, 23-49)	30	27
HCO_3 (mmol/L)	17.17±0.34 (17.3, 15.6-17.9)	18.25±0.94 (17.1-19.4)	19.1	20.28±1.17 (19.3, 18.4-24.9)	19.15±0.94 (18.5, 17.8-21.8)	17.55±0.65 (16.9-18.2)	16.1±2.41 (13.7-18.5)	24.4	19.5±0.90 (18.6, 17.1-24.3)	20.6	17.1
AnGap (mmol/L)	16.67±0.70 (16.3[b], 14.4-18.7)	17.45±2.82 (14-20.9)	21.4	22.1±3.20 (23, 11.4-30.9)	20.95±1.83 (21.95, 16-23.9)	14.15±0.95 (13.2-15.1)	18.7±1.40 (17.3-20.1)	23.2	21.83±1.44 (23.3, 16-25.6)	25.3	20.9
tCO_2 (mmol/L)	18±0.36 (18.2[b], 16.3-18.8)	19.1±0.98 (17.9-20.3)	20	21.08±1.45 (20.2, 18.6-26.7)	19.8±1.07 (18.8[a], 18.6-23)	18.65±0.85 (17.8-19.5)	16.85±2.46 (14.4-19.3)	25.8	20.43±0.98 (19.6, 17.9-25.7)	21.5	18
BE (mmol/L)	-3.85±0.43 (-3.7, -5.5- -2.5)	-2.9±0.41 (-3.4- -2.4)	-1.8	-2.8±0.58 (-2.7, -4.1- -0.9)	-4.38±0.99 (-4.85, -6.1- -1.7)	-6.6±0.70 (-7.3- -5.9)	-5.35±2.56 (-7.9- -2.8)	-0.9	-2.21±0.48 (-2.2, -4.2- -0.6)	-0.6	-4.2
$stHCO_3$ (mmol/L)	20.68±0.30 (21, 19.7-21.5)	21.65±0.53 (21-22.3)	22.5	22.1±0.44 (22.2, 20.9-23.2)	21.02±0.81 (20.6, 19.7-23.2)	19.2±0.30 (18.9-19.5)	19.55±2.26 (17.3-21.8)	24.2	22.33±0.41 (22.3, 21-23.7)	23.7	20.5
st pH	7.34±0.01 (7.34, 7.32-	7.36±0.01	7.38	7.36±0.01 (7.37, 7.31-	7.33±0.02 (7.32, 7.31-	7.31±0.01	7.31±0.05	7.41	7.37±0.01 (7.37, 7.34-	7.40	7.33

Parameters	Healthy group (n=6)	Nasal SCC (n=2)	Allergic rhinitis (n=1)	Chronic bronchitis (n=6)	BP (n=6)	Pulmonary Neoplasia (n=2)	Nodular interstitial (n=2)	Pulmonary abscessation (n=1)	NSIP (n=7)	Anthracosis (n=1)	EBP (n=1)
	7.35)	(7.34-7.37)		7.39)	7.39)	(7.30-7.31)	(7.26-7.36)		7.40)		
cH+ (nmol/L)	35.98±1.32 (35.65, 33.1-41.9)	33.7±0.16 (33.5-33.9)	33.8	39.4±3.44 (36.10, 32.9-52.5)	40.05±1.49 (40.80, 36.1-42.5)	45.5±5.00 (40.5-50.5)	36±2.61 (33.4-38.6)	42.9	35.54±1.97 (33.50, 29.7-45.7)	33.2	36.3
PaO_2 (mmHg)	87.17±1.74 (86.5, 81-92)	88.5±1.22 (87-90)	71	62.2±10.8 (67, 33-90)	55.25±6.80 (61, 35-64)	38±0 (38-38)	62.5±1.50 (61-64)	64	71.14±8.21 (84, 33-91)	84	62
tHb (g/dL)	12.57±0.67 (12.15, 11-15.3)	10.35±1.10 (9-11.7)	12.9	13.04±0.48 (13, 12-14.4)	10.7±1.09 (11.6, 7.5-12.1)	9.3±1.00 (8.3-10.3)	9.95±0.05 (9.9-10)	13.3	11.97±0.51 (12.2, 9.8-13.5)	12.7	12
SO_2 (%)	93±1.06 (93, 89-97)	94.5±1.22 (93-96)	93	87.75±4.89 (90, 74-97)	88±1.53 (89, 85-90)	64±4.00 (60-68)	89±0 (89-89)	88	92.33±1.76 (94, 86-96)	96	89
Na+ (mmol/L)	135.17±3.71 (139, 119-142)	140±7.35 (131-149)	148	149±6.04 (151, 127-164)	146.25±4.21 (149, 134-153)	125.5±4.50 (121-130)	143.5±3.51 (140-147)	150	141.57±2.49 (139, 134-149)	147	150
K+ (mmol/L)	3.41±0.12 (3.45, 3-3.78)	3.45±0.04 (3.4-3.5)	4.3	3.44±0.33 (3.3, 2.7-4.7)	4.32±0.74 (3.85, 3.2-6.4)	3.05±0.55 (2.5-3.6)	4.65±0.05 (4.6-4.7)	3.8	3.36±0.24 (3.2, 2.4-4.4)	3.8	3.2
Cl- (mmol/L)	105.17±2.94 (107.5, 92-111)	103±1.63 (101-105)	112	109.4±2.60 (111, 100-114)	110.75±2.66 (112.5, 103-115)	96.5±3.50 (93-100)	108±2.01 (106-110)	104	105.86±1.49 (105, 100-111)	105	115

Values in parenthesis depicts range

No significant difference was found in the other parameters between the diseased and healthy groups. Hypoxemia can be caused by low inspired oxygen fraction, hypoventilation, thickened respiratory barrier, shunting of pulmonary blood and physiologic dead space (West 1990, Rozanski and Chan 2005). In case of alveolar edema and atelectatic lung, shunting of pulmonary blood occurs due to the inadequate ventilation of the blood flowing through the alveolar capillaries (Miller 2007).

4.2.3.4 Fecal examination

Fecal examination done in all the diseased dogs by fecal flotation method for lungworms larvae or ova/cyst and was found to be negative in all the cases except one chronic bronchitis dog with absolute eosinophilia which was found positive for hookworms after repeated fecal examination in the 2^{nd} week. Low prevalence of different lungworms had been indicated in the fecal floatation examination surveys performed in thousand of samples in dogs with respiratory problems (Jordan *et al* 1993, Nolan and Smith 1995, Blagburn *et al* 1996, Rembiesa and Richardson 2003). Faecal flotation is the most commonly used diagnostic technique for detection of parasitic infection in veterinary practice (Flick 1973, Dryden *et al* 2005).

4.2.3.5 Radiography

Thoracic radiography is a good diagnostic tool for lower airway diseases in dogs (Sharp and Rozanski 2013).

Lung patterns are mainly classified on the basis of the location (bronchioles, interstitium, or alveoli) of the pathological change (Miller 2007). Types of lung patterns include alveolar, bronchial, vascular and interstitial (nodular and diffuse) (Spasov *et al* 2018).

In the present study, thoracic radiographic findings of dogs with bacterial bronchopneumonia showed alveolar pattern along with the interstitial pattern. In majority of dogs mixed pattern (interstitial and alveolar) was observed. Pulmonary edema was evident in cranial and caudal lobes of the most of dogs. An alveolar lung pattern is obtained due to the replacement of air in the alveoli with exudate, haemorrhage or oedema fluid (high density material) and is mainly characterized by presence of air bronchograms and lobar sign (Spasov *et al* 2018). Most common cause of occurrence of this type pattern includes bacterial and aspiration pneumonia (severe

inflammatory diseases) and pulmonary contusion, pulmonary thromboembolism, neoplasia, fungal pneumonia and systemic coagulopathy (Haemorrhage) (Nelson and Couto 2014, Spasov *et al* 2018).

In the present study, mild to moderate bronchial pattern in cranial and caudal lobe along with bronchial thickening and multiple donuts was observed in all the chronic bronchitis dogs along with non-structural (diffused) interstitial pattern in most of dogs. Bronchial pattern is usually obtained due to the thickening of the bronchial walls because of inflammation (doughnuts or tram lines or train tracks) or due to the replacement of peribronchial space by cells or fluid leading to enhanced radiographic visualization of the bronchial tree (Miller 2007,Nelson and Couto 2014 and Spasov *et al* 2018). Most common causes of this pattern are canine chronic bronchitis, allergic bronchitis, canine infectious tracheobronchitis and bacterial and parasitic infection (rarely) (Nelson and Couto 2014, Spasov *et al* 2018).

In the present study, severe nodular interstitial pattern was observed in dog suffering with pulmonary abscessation. In dog suffering with histiocytic sarcoma, moderate alveolar and bronchial pattern was observed along with air bullae (pneumobulla) seen in left caudal lung lobe. One collapsed lung lobe was seen with increased opacity of the parenchyma on both sides. In dog suffering with diffuse bronchioloalveolar carcinoma, mild to moderate bronchial pattern, moderate alveolar pattern in caudal lung lobe and severe interstitial pattern along with increased radiopacity around perihilar region and edema around secondary bronchus was observed. In our study, all the dogs suffering with ILDs, interstitial pattern was observed on chest radiography. An interstitial pattern is depicted as the linear densities giving a hazy appearance to the lung field and making the visualization of the vasculature unclear (Miller 2007). Abnormal interstitial pattern include reticular (unstructured), nodular or reticulonodular appearance (Nelson and Couto 2014). Common causes of reticular interstitial pattern include infection (viral, bacterial, mycotic and toxoplasmosis), idiopathic interstitial pneumonia and neoplasia whereas common causes of nodular pattern includes mycotic infections, tumors and abscesses (Nelson and Couto 2014, Spasov *et al* 2018).

4.2.3.6 Transtracheal wash cytology

TTW cytology reveals increased cell counts and proportion of neutrophils and increased amount of mucus in diseased group as compared to the healthy group. Mean cell number (cells/HPF) of diseased dogs was significantly higher than the healthy animals (79.73±14.48 and 8.35±2.68, respectively; P<0.05) (Table 31).

Median value of macrophage count of diseased group was significantly lower than the healthy control group (19 and 51 %, respectively; P<0.05). Mean neutrophil count of diseased group (55.32±3.68 %) was significantly higher than the healthy control group (11.43±1.00 %; P<0.05). Mean lymphocyte count of diseased group was 6.24±1.86 percent. Mean respiratory epithelial cells in diseased dogs was significantly lower than the healthy control group (9.36±1.36 and 29.14±7.13 %, respectively; P<0.05) (Table 31).

Table 31: Comparison of tracheal wash cytology (mean ± SE) between healthy and diseased dogs

Parameters	Healthy group (n=7)	Diseased group (n=25)
Macrophages (%)	49.57±5.28 (51[a], 28-66)	23.88±2.63 (19[b], 8-53)
Neutrophils (%)	11.43±1.00 (11[b], 8-15)	55.32±3.68 (58[a], 18-81)
Lymphocytes (%)	7.71±2.25 (7, 0-18)	6.24±1.86 (3, 0-41)
Eosinophils (%)	0.14±0.14 (0, 0-1)	3.16±2.53 (0, 0-63)
Respiratory epithelial cells (%)	29.14±7.13 (32[a], 9-60)	9.36±1.36 (8[b], 2-32)
Others (%)	2±0.79 (2, 0-5)	2.04±0.61 (1, 0-15)
Mean cell number (cells/HPF)	8.35±2.68 (5.8[b], 1.85-21.4)	79.73±14.5 (54.1[a], 6.7-239.7)

Values in parenthesis depicts range
Values having different superscript in the same row differ significantly at P<0.05

Table 32: Comparison of tracheal wash cytology (mean ± SE) between healthy and diseased groups

Parameters	Healthy group (n=7)	Chronic bronchitis (n=8)	Bacterial bronchopneumonia (n=5)	Pulmonary Neoplasia (n=2)	Nodular interstitial (n=1)	Pulmonary abscessation (n=1)	NSIP (n=5)	Anthracosis (n=2)	EBP (n=1)
Macrophages (%)	49.57±5.28 (51, 28-66)	30.63±4.84 (33.50, 13-53)	12.8±1.66 (12, 8-17)	38±4.00 (34-42)	29	13	26±6.36 (31, 8-43)	15.5±3.51 (12-19)	9
Neutrophils (%)	11.43±1.00 (11, 8-15)	48.5±6.48 (47, 26-80)	73±2.98 (74, 63-81)	40.5±0.50 (40-41)	59	80	59.6±6.79 (62, 36-78)	47±5.01 (42-52)	18
Lymphocytes (%)	7.71±2.25 (7, 0-18)	8.62±4.81 (3, 0-41)	2.4±0.24 (2, 2-3)	7±2.00 (5-9)	3	1	4.8±2.13 (4, 1-13)	16.5±11.53 (5-28)	0
Eosinophils (%)	0.14±0.14 (0, 0-1)	1.25±1.11 (0, 0-9)	1±1 (0, 0-5)	0±0 (0-0)	0	0	0±0 (0, 0-0)	0.50.±0.50 (0-1)	63
Respiratory epithelial cells (%)	29.14±7.13 (32, 9-60)	9.25±2.23 (8, 3-16)	9.4±2.20 (8, 5-16)	4.5±0.50 (4-5)	9	6	8.8±2.44 (8, 2-17)	17.5±14.54 (3-32)	10
Others (%)	2±0.79 (2, 0-5)	1.75±0.49 (2, 0-4)	1.4±0.24 (1, 1-2)	10±5.00 (5-15)	0	0	0.8±0.37 (1, 0-2)	3±1.00 (2-4)	0
Mean cell number (cells/HPF)	8.35±2.68 (5.8, 1.85-21.4)	69±18.6 (64.8, 12.3-148.2)	192.5±24.2 (226.1, 132.2-239.7)	24.68±9.43 (15.25-34.1)	28.35	90.25	44.2±18 (34.1, 6.7-99.8)	40.05±14.09 (26-54.1)	10.1

Values in parenthesis depicts range

On TTW cytology, neutrophilic or the mixed inflammation is the most common abnormality found in dogs with spontaneous respiratory disease (Finke 2013). Neutrophilic inflammation is observed in majority of dogs with bacterial infection and in few dogs intracellular bacteria and degenerative changes in neutrophils can also be seen (Hawkins *et al* 1995, McCullough and Brinson 1999 and Dunn 2010). In chronic bronchitis dogs, most commonly a mixed inflammatory or relatively mild neutrophillic inflammation along with increased mucus production is recorded (McCullough and Brinson 1999, Dunn 2010 and Finke 2013). Similar findings were observed in the present study. In eosinophilic bronchopneumopathy dogs, increased count of eosinophils was observed (Clercx *et al* 2000). Activated macrophages engulf the phagocytized organisms or red blood cells. Bronchial tree is mostly involved in the primary lung tumors and lymphosarcomas resulting in exfoliation of cells in tracheal wash or BAL samples (Dunn 2010). Pleomorphic epithelial cells observed in TTW in case of Bronchioloaveolar carcinoma in the present study are highly suggestive of this exfoliation.

4.3 Therapeutic management of canine respiratory affections

The main causes of respiratory affections are viral, bacterial, fungal or parasitic infections, trauma, inflammation, aspiration, neoplasia, anomalies, systemic immunodeficiency or any other cause leading to impaired local defence mechanisms (Cohn and Reinero 2007, Vieson *et al* 2012). Treatment protocol must aim to solve the underlying problem along with control of the infectious component (Vieson *et al* 2012).

Respiratory diseases mainly involve the abnormal production of secretions and exudates and reduction in ability to remove them. Main aim of the therapy is to reduce the volume and viscosity of the secretions and to assist their elimination which can be done by control of infection and inflammation, alteration of the secretions and also if possible improve the postural drainage and mechanically remove the material (Ettinger and Feldman 2010, Aiello and Moses 2016). Therapeutic protocols include alteration of the inspired air and administration of expectorants, bronchodilators, antimicrobials, antitussives, diuretics and other drugs.

Maintain the hydration of airways to facilitate the mucociliary clearance (Nelson and Couto 2014, Kuehn 2016). Humidification of inhaled air facilitates the

elimination of airway secretions (Nelson and Couto 2014, Kuehn 2016). Expectorants can be used for liquefying the secretions in combination with other additional respiratory therapy such as improved postural drainage, mild exercise and thoracic percussion (Aiello and Moses 2016). Nebulization help to loosen the excessive accumulations of secretions in tracheobronchial diseases, that can be simply done with sterile saline accepted as the mucolytic agent (Ettinger and Feldman 2010). In severe airway obstruction cases mechanical removal of firm and viscid secretions by aspiration can be followed (Aiello and Moses 2016). Antitussive agents mainly relieve the distress related with non-productive coughing (Ettinger and Feldman 2010) but are contraindicated in case of excessive mucus secretion in airways (Nelson and Couto 2014).

Methylxanthines such as theophylline and aminophylline are effective bronchodilators in dogs (Aiello and Moses 2016) and can also help in suppressing the cough through their ability to prevent bronchospasm (Ettinger and Feldman 2010). Theophylline is found to have positive effects on respiratory tract such as increasing the contraction strength of the diaphragm and ciliary clearance and have diuretic effect (Aubier and Roussos 1985, Aiello and Moses 2016). Kuehn (2016) recorded that the therapeutic index of theophylline was comparatively narrow and are found to be less effective than the β_2-agonists. Sympathomimetic drugs such as terbutaline and albuterol are selective for β_2-adrenergic receptors, therefore decreasing their cardiac effects and are preferably can be used as bronchodilators (Nelson and Couto 2014). Use of antihistamines is recommended to lessen the bronchoconstriction caused by release of histamine (Aiello and Moses 2016).

Glucocorticoids administered orally or via inhalation reduce inflammation which ultimately reduces cough especially in case of chronic bronchitis (Rozanski 2014). Most commonly used glucocorticoid is prednisone (@ 1 to 2 mg/ kg/d initially and then tapered to the lowest effective dose that controls the clinical signs) (Rozanski 2014). Inhalation therapies with glucocorticoids are beneficial and cause very few to no adverse effects; although dogs are needed to be trained to tolerate the face mask (an aerosol delivery device) (Rozanski 2014, Kuehn 2016). Bexfield *et al* (2006) recorded the benefits of inhaled corticosteroid therapy with fluticasone (125 mg twice daily) in 13 dogs with respiratory disease. Corticosteroids are greatly

effective in allergic conditions, but systemic use can result in undesirable effects (Aiello and Moses 2016).

First-line treatment of in emergency situations and the cases in which CST cannot be done, must be based on the empirical data available on the bacterial prevalence and antibiotic susceptibility (Ford 2009). Escherichia coli and Pseudomonas species are identified with high percentage of resistant isolates (Steinfeld *et al* 2012, Johnson *et al* 2013). Gram-negative enteric bacteria were found to be the main causative bacteria in the animals with severe respiratory disease needing positive pressure ventilation and these isolates were found less susceptible to the commonly used antibiotics (Epstein *et al* 2010). Proulx *et al* (2014) also showed that dogs with bacterial pneumonia commonly harboured bacteria resistant to antimicrobials used during a 4-week period before tracheal wash sampling. Rheinwald *et al* (2014) recorded the best susceptibility pattern shown by enrofloxacin against all gram negative and gram positive bacteria (86 per cent of all isolates and 87 percent of Gram-negative bacteria were susceptible) and amoxicillin/clavulanic acid showed the best susceptibility pattern in Gram-positive bacteria (92 per cent). So, in dogs with bacterial lower respiratory tract infection, these antibiotics can be suggested for first-line treatment (Rheinwald *et al* 2014). Other antibiotics used for respiratory diseases in dogs include cephalosporins, aminoglycosides, fluoroquinolones, macrolides, trimethoprim-sulfamethoxazole and tetracyclines (Kuehn 2016). Multiple antimicrobial agents should only be used, with full knowledge of the probable drug interactions.

The hypoxemia caused by most of respiratory diseases can usually be corrected by oxygen therapy (Sumner and Rozanski 2013, Nelson and Couto 2014). Though, the continuous administration of high concentrations of oxygen can also increase the tendency for local resorption atelectasis (reduction of the volume of oxygen in alveoli due to more absorption in the blood), therefore worsening the hypoxemia and can also cause its own pneumonitis (Aiello and Moses 2016).

In acute respiratory failure and comatose or apneic dogs, endotracheal intubation and mechanical ventilation are often required (Nelson and Couto 2014, Aiello and Moses 2016). Arterial blood gas analysis is very helpful in monitoring the treatment in these conditions. In pulmonary edema cases, diuretics are usually

recommended, but not in hypovolemic patients (Nelson and Couto 2014). The osmotic diuretics (mannitol) show nominal action on diuresis whereas carbonic anhydrase inhibitors (e.g. acetazolamide) have a modest effect and loop diuretics (e.g. furosemide) have a profound effect (Aiello and Moses 2016).

In veterinary practice, primary chemotherapy is usually used for hematopoietic tumors such as leukemias, lymphomas and multiple myelomas whereas chemotherapy for dogs with carcinomas and sarcomas (metastatic solid tumors) is hardly ever curative and better to be replaced with palliative therapy. Adjuvant chemotherapy done after surgery may be given to slow down the progress of metastatic disease or to possibly provide a cure. The most favourable time to administer primary chemotherapy is when the dog has microscopic disease, rather than after gross metastases. The platinum compounds and doxorubicin are being used as a part of adjuvant veterinary chemotherapy successfully for canine osteosarcoma after surgery (Ettinger and Feldman 2010).

In curative treatment of small malignant solid tumors, radiation therapy plays an important role. It is used in the management of benign tumors which include oral epulides in dogs, adenomas of the pituitary and perianal glands. Surgery is a restricted treatment. Surgical excision is the best treatment for primary lung tumors that are confined to only one lobe. In contrast radiotherapy provides loco regional treatment covering a wider area (Ettinger and Feldman 2010).

Cannon *et al* (2015) followed this chemotherapeutic protocol for management of canine histiocytic sarcoma. Chemotherapy was started using lomustine @ 70 mg/m^2 orallyin first week followed by doxorubicin @30 mg/m^2 or 1 mg/kg (<12kg) intravenously(IV) on third week. After 3 days of doxorubicin treatment, cyclophosphamide was administered at a total dose of 250 mg/m^2 orally divided over 3 days. This protocol was proposed to continue up to maximum six cycles (because of potent cumulative cardiotoxicity from doxorubicin) or until disease progression.

4.5.1 Squamous cell carcinoma (n=3)

Three dogs were diagnosed for nasal squamous cell carcinoma. One male German shepherd survived for two months after the owner refused to undertake chemotherapy in this case whereas the other male dog (non-descript) died within 2

days of diagnosis before ensuing any medical or surgical treatment. The only female dog with SCC whose owner also declined to commence chemotherapy, is still surviving (80 days of diagnosis) after antibiotic course for 10 days along with oral styptic and vitamin C for one month. A mean survival time of 3 to 6 months after diagnosis of nasal cavity tumors without any treatment was reported by Lana and Withrow (2001). Lascelles *et al* (2000) used surgical resection, radiation and combination of both in six, four and seven dogs, respectively with SCC of the nasal planum and concluded that surgical resection gave the most encouraging results provided the excision of the neoplastic tissue was with complete margins. Radiation and combination of radiation with surgical resection were not found satisfactory as the recurrence was noted in 3 of the 4 dogs undergoing radiation and all the seven dogs following combination therapy. On the contrary, radiation therapy was recognized as the most effective treatment alone or in combination with surgical excision (Lana and Withrow 2001). However, Adams *et al* (2005) observed significantly longer survival times in dogs undergone radiation therapy along with surgical resection. Though different studies gave varied versions about the therapeutic protocols followed in canine patients with SCC but need is to undertake further studies on medical management in such cases keeping in view the cosmetic outlook of the patient after surgery in mind and chances of recurrence. However, in a previous study, it was discovered that dogs with nasal tumors have poor prognosis with mean survival time of 3 to 5 months after surgery, chemotherapy, cryotherapy or no therapy (Patnaik 1989). Recent case reports also revealed non-significant difference in patient survival time whether surgery is performed or no treatment is offered (Lana and Withrow 2001).

4.5.2 Allergic rhinitis (n=1)

Allergic rhinitis has been rarely reported n dogs. However, around 20–30 percent of Indian population of human beings, suffers from allergic rhinitis and out of which 15 percent develop asthma (Varshney and Varshney 2015).

The only affected dog in our study was treated with the chlorpheniramine orally @ 4mg/dog every 12 hour for 7 days followed by every 24 hour till the signs subsided (i.e. for 1 month) as per described by Nelson and Couto (2014) along with

Celin 500 (vitamin C) for 7 days. Complete blood count was done after the clinical recovery of the dog which showed significant decrease in absolute eosinophilia from 2440 to 348 cells/µl. Hemoglobin concentration was found to be 12.7 g/dl with no significant difference. Total leucocyte count and total erythrocytic count was 8700 cells/µl and 5.52 cells/µl x 10^6, respectively. Absolute neutrophils decreased from 10370 to 5916 cells/µl. Marked decrease in eosinophils was observed between pre and post treatment (Table 33). Greater reduction in TLC was observed post treatment as compared with the pre treatment value. No significant difference was observed in other hematological parameters. Nasal radiography was found normal pre and post treatment.

Table 33: Comparison of hematological parameters in allergic rhinitic dog pre and post treatment

Parameters	Pre treatment	Post treatment
Hb (g/dl)	12.9	12.7
TEC (count/µl × 10^6)	6.87	5.52
TLC (count/µl)	15250	8700
PCV (%)	41.3	38.2
Platelets (count/µl × 10^3)	285	211
Absolute neutrophils (count/µl)	10370	5916
Absolute lymphocytes (count/µl)	3965	2436
Absolute eosinophilia (count/µl)	2440	348

Prognosis was good in the allergic rhinitic dog as it showed complete clinical recovery after treatment with the antihistaminic along with the avoidance of contact with the allergen source (suspected to be the newly brought furniture). In human beings, treatments in allergic rhinitic patients are recommended to follow ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines and usually include combination of pharmacotherapy, allergen avoidance and allergen immunotherapy (Varshney and Varshney 2015). However, intranasal corticosteroids have been observed as the most valuable approach for treating allergic rhinitis. Bilastine, a new novel H1 antihistamine has been found highly effective in the treatment of allergic rhinitis and observed as good as cetrizine or fexofenadine in *in vitro* trials (Kowal and

DuBuske 2014). It is a piperidine derivative with favourable pharmacokinetic and pharmacodynamic profile and is normally well tolerated. It has also been verified with dose-dependent antihistaminic and antiallergic effects in animal studies (Kowal and DuBuske 2014). In our study, chlorpheniramine resulted in excellent recovery after one week though it was continued for one month until clinical signs resolve and dog recuperates completely. Cetrizine, a second generation antihistamine, @ 1mg/kg every 24 hourly, was found more successful in cats (Papich *et al* 2008), not in dogs in which glucocorticoids (Prednisone @0.25 mg/kg orally every 12 hourly) can be used when antihistamines are ineffective (Nelson and Couto 2014).

4.5.3 Chronic bronchitis (n=6)

Treatment in chronic bronchitis includes bronchodilators, glucocorticoids and cough suppressants along with avoidance of aggravating factors like allergens, irritants (smoke, perfumed products) and improvement of air quality at home. Antibiotics are often recommended in CB patients, though it is good to go for confirmation of infection after culture and antibiotic sensitivity (Nelson and Couto 2014) on transtracheal specimen.

In the present study, it was possible to take TTW aspiration, only in 4 out of 6 cases at owners' consent and culture was positive in two cases. However, a predetermined treatment protocol was followed irrespective of the fact that whether TTW was possible or not or whether culture was either positive or not. Treatment protocol used involved Amoxicillin and Clavulanic acid combination @ 15 mg/kg PO q12h for first 3 days followed by antibiotic selected on the basis of CST in positive cases up to 2 weeks along with bronchodilators terbutaline @ 1.25-5 mg/dog PO q8-12h for 2 weeks. However, bronchodilator was continued for 2 more weeks seeing the mild cough and no side effect of the drug. Cetrizine @5-10mg/dog PO q12h was used in two cases. Absolute eosinophilia was found in one dog. Fecal sample in this dog was positive for hookworms in the 2^{nd} week of the course of disease, which was followed by two shots of Ivermectin @ 0.2 mg/kg SC in this dog two weeks apart. Eosinophilia resolved after 2 weeks of start of Ivermectin therapy. All the cases recovered with complete resolution of coughing after one month as per reported by dog owners. Glucocorticoids (Prednisone @ 1 to2 mg/kg/d initially and then tapered to the lowest effective dose) are generally described as the mainstay of treatment in

canine chronic bronchitis because of their anti-inflammatory effect. However, Nelson and Couto (2014) reported the potent negative effects of glucocorticoids in dogs which include increased susceptibility towards infection due to decreased airway clearance, hepatomegaly, predisposition towards obesity and muscle weakness (affecting ventilation) and can also cause pulmonary thromboembolism. Though, inhaled glucocorticoids like fluticasone @125 mg twice daily is being widely accepted in dogs with CB provided dogs are trained to tolerate the face mask, due to rare side effects (Bexfield *et al* 2006).

Terbutaline, a ß$_2$-agonist was used in the present study as a part of preset protocol and also to study its side effects, seeing the adverse effects of glucocorticoids. Moreover, terbutaline available in India or used in the present study was a combination with Ambroxol and Guaifenesin. Ambroxol is known for its anti-inflammatory (De Mey *et al* 2016) and mucolytic effect and also has local anaesthetic effect. Guaifenesin increases the volume and reduces the viscosity of secretions in the tracheobronchial tree and improves mucociliary clearance (Albrecht *et al* 2017). That could be the reason for recovery in all the cases of chronic bronchitis in the present study. Although, limited evidence of efficacy of bronchodilators is available in dogs with chronic bronchitis and terbutaline is considered less effective in dogs because of side effects such as anxiety and restlessness (Kumrow and Rozanski 2012, Rozanski 2014).

Table 34: Comparison of age and vital parameters pre and post treatment in chronic bronchitis dogs (n=6)

Parameters	Pre Treatment (n=6)	Post Treatment after two weeks (n=6)
Body Weight (Kg)	18.88±4.17 (14.35, 10-35)	18.22±4.02 (14, 9.7-34.6)
RT (ºF)	101.67±0.42 (101.7, 100.4-102.8)	102.20±0.36 (102.20, 100.8-103.4)
HR (bpm)	102±8.25 (100, 72-128)	96±6.20 (92, 84-124)
RR (breaths/min)	51±12.1 (50, 18-84)	34±5.63 (32, 20-52)

Values in parenthesis depicts range

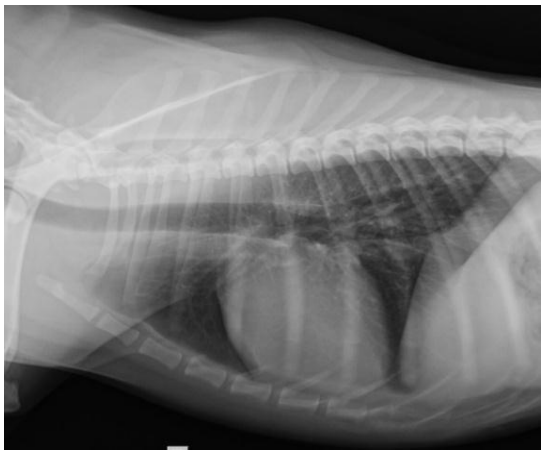

Fig. 66: Lateral thoracic radiograph showing moderate bronchial pattern in cranial and caudal lung lobe, bronchial wall thickening and increased opacity of cranial and caudal lung lobe. Tram lines and donuts also seen in a dog with chronic bronchitis

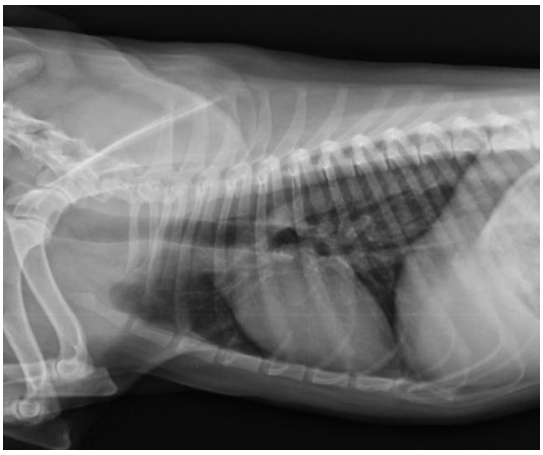

Fig. 67: Improved radiopacity and mild bronchial pattern observed on lateral thoracic radiograph after clinical recovery in the same dog

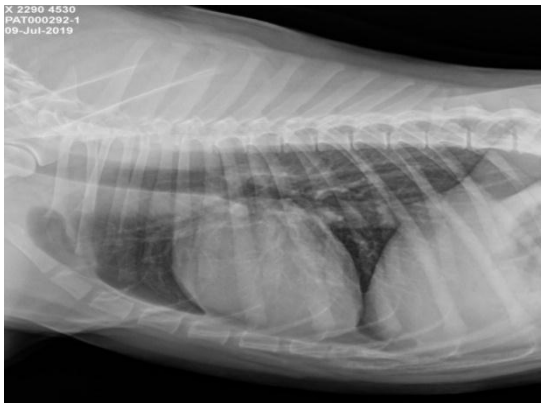

Fig. 68: Increased radiopacity and moderate interstitial pattern, soft tissue density around hilar area seen in dog with ILDs

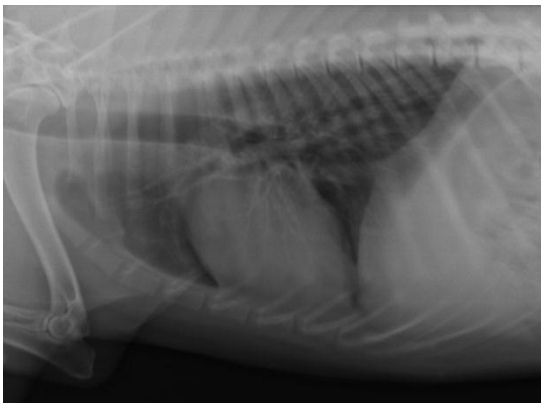

Fig. 69: Decrease in radiopacity along with mild interstitial pattern in the same dog with ILDs

Marked decrease in the respiration rate was observed 2 weeks post treatment whereas no significant difference was observed in rectal temperature and heart rate (Table 34). No significant difference was observed in hematology between pre and post treatment levels (Table 35).

Table 35: Comparison of hematological parameters in chronic bronchitis dogs pre and post treatment

Parameters	Pre treatment (n=6)	Post treatment after two weeks(n=6)
Hb (g/dl)	13.25±0.83 (14, 10.1-15.1)	14.02±0.41 (13.95, 12.7-15.6)
TEC (count/µl × 10^6)	5.05±0.53 (4.82, 3.37-7.31)	6.64±0.21 (6.57, 6.08-7.32)
TLC (count/µl)	14787±1809 (13570, 9390-20900)	12652±1512 (12205, 7670-19100)
PCV (%)	40.17±2.44 (42.40, 30.3-45.6)	42.27±1.17 (42.55, 38.1-46.6)
Platelets (count/µl× 10^3)	326.7±47.2 (316.5, 190-532)	313.67±43.5 (297.5, 189-510)
Absolute neutrophils (count/µl)	12428±2223 (9904, 7700-20064)	9975±1269 (9702, 5829-15280)
Absolute lymphocytes (count/µl)	1442±255 (1351, 772-2261)	2445±396 (2440, 1163-3438)
Absolute eosinophils (count/µl)	917±583 (94, 0-3391)	189.78±92.8 (116.3, 0-524)

Values in parenthesis depicts range

Thoracic radiography revealed decrease in the radiopacity in all lung lobes post treatment, with improvement in the bronchial pattern from moderate or severe to mild (Fig. 66, 67). Rozanski (2014) and McKiernan (2000) reported that the clinical signs usually improve after treatment, however, the persistence of the ongoing airway disease and some form of cough is expected. Appropriate remedial management usually alleviate clinical signs and also impede or slows down the progression of bronchial damage. Episodic relapses of cough are not uncommon and they require modifications in the treatment protocol, such as a transitory increase in glucocorticoids or addition of bronchodilators, antibiotics or cough suppressants, till

the clinical signs subside. Regular assessment and making a treatment plan specific for individual dogs can provide the best result to clinicians. The development of newer diagnostic techniques for early detection of chronic bronchitis and more effective treatment will definitely improve knowledge about the disease and limit their long-term effects on dogs.

4.5.4 Bacterial bronchopneumonia (n=7)

Successful treatment in bacterial bronchopneumonia comprised of antibacterial along with supportive care. Therapeutic considerations followed in bacterial pneumonia dogs usually include antibiotics, airway hydration, bronchodilators and oxygen supplementation.

Seven cases were diagnosed with bacterial bronchopneumonia. Treatment protocol recommended in all the cases of bronchopneumonia, in the present study includes Amoxicillin/clavulanic acid @15mg/kg PO or Amoxicillin- Sulbactum @ 15 mg/kg IM q12h for first 3 days followed by antibiotic selected on the basis of CST along with bronchodilators terbutaline @ 1.25-5 mg/dog PO q8-12h for one to two weeks depending on the severity of case. Six out of 7 cases did not respond to treatment due to resistance to most of the commonly used antibiotics and late presentation of most of these cases in small animal clinics. Only one case survived. It was reported that the prognosis was seen more guarded in dogs having underlying problems that predispose them to infection (Nelson and Couto 2014). Lung fibrosis was observed in 1 case on post-mortem which may have rendered the dog to severe hypoxemia (64 mmHg) and multi-organ failure leading to death. Two dogs died within 3 days before culture report, due to sudden worsening of condition leading to respiratory distress and death even after continuation of empirical treatment. One dog which had been suffering for 8 months and having severe respiratory distress, weight loss, purulent nasal discharge have severe alveolar pattern in cranial and right middle lung lobe died due to chronic lung changes. Other 2 dogs, Pug and a Labrador having similar pulmonary parenchymatous changes and clinical signs died due to severe hypoxemia (60 mmHg, 62 mmHg respectively). One possible cause of death in 5 other cases of bacterial bronchopneumonia may be that the antibiotic given might have not penetrated adequately in the lung tissue. The life threatening deterioration of the lung function may be another factor which can occur within hours in these cases (Corcoran 2004).

4.5.5 Lung tumors/ Undiagnosed nodular interstitial pattern (n=6)

In case of histiocytic sarcoma, doxorubicin was used @30mg/m^2 but dog died after few hours of treatment. In dog with bronchioloalveolar carcinoma, metastasis occurred leading to multiorgan involvement making him a poor candidate for chemotherapy. Pulmonary abscessation was very severe in the third case that animal did not respond to any antibiotic and died within 3 days of treatment before CST results. One of the 3 cases of undiagnosed nodular interstitial pneumonia died before start of treatment based on CST whereas other one with haemoptysis died on the second day of presentation before TTW aspiration or FNAC from pulmonary tissue. Third case partially responded to empirical antibiotic treatment as the owner was not ready for TTW aspiration of his dog and died after 2 months of treatment.

Common causes of nodular pattern on radiographs include mycotic infections, tumors and abscesses (Nelson and Couto 2014, Spasov *et al* 2018). Rarely, foreign material or neoplasia was also documented as underlying cause of abscessation (Kuhajda *et al* 2015). Poor prognosis was reported in diffuse BAC in both humans and dogs (Bertazzolo *et al* 2002). In most of the studies so far, prognosis in disseminated histiocytic sarcoma was found guarded to grave as most of the chemotherapeutic regimens were seen ineffective (Skorupski 2007). Surgical lobectomy might be a good option in case there is a single pulmonary abscess (Ettinger and Feldman 2010) because it minimizes the risk of pneumothorax and pyothorax due to abscess rupture (Schweigert *et al* 2011). However, it could not be possible in multiple abscessations as in the present study. Antimicrobial therapy based on culture and sensitivity is the only solution when surgery is not possible.

Three cases remained undiagnosed for the reason that TTW was not possible in all these cases because one or the other reason, most probably due to lack of owners' consent for TTW. These cases might of any fungal or neoplastic origin which could have lead to death in all these cases similar to the other 3 cases of lung tumors (n=2) and pulmonary abscessation (n=1). Had the owner got ready for postmortem, the diagnosis would have been possible in all these 3 patients.

4.5.6 Interstitial lung diseases (n=8)

Eight dogs were treated in 7 cases of non-specific interstitial pneumonia (NSIP) and 1 case of anthracosis (n=1)) in the ILDs group.

Affected dogs (n=8) were treated with the amoxicillin and clavulanic acid combination @ 15 mg/kg PO q12h for 3 days followed by antibiotic based on CST up to 2 weeks along with bronchodilator terbutaline @ 1.25-5 mg/dog PO q8-12h as per preset protocol. Antihistaminics like cetrizine @5-10mg/dog PO q12h was used in two cases. No significant difference was observed in vital parameters in all the cases with ILDs after treatment (Table 36). Similar non-significant difference was observed in hematological indices post treatment (Table 37). However, an improvement in the radiopacity was observed in all lung lobes with reduction in severity of interstitial pattern from moderate to mild and severe to moderate (Fig. 68, 69).

There is no single treatment for ILDs because of diverse group of diseases. It is advised to address the inciting cause (fungal or parasitic) directly, when identified, along with supportive care using anti-inflammatory drugs and in patients with severe disease, oxygen therapy (Reinero and Cohn 2007). Moreover, optimal therapy has not been established yet for many ILDs in dogs and cats. In eosinophilic pneumonias, it is critical to treat the underlying infectious agent. An appropriate adulticide and microfilaricide should be used in case of *Dirofilaria immitis* infection and specific anthelmintic can be used for *Ancylostoma/Strongyloides/Toxocara* species. Pulmonary eosinophilia after a drug introduction or due to neoplasia should be treated with discontinuation of drug and specific chemotherapy, respectively. If no etiology is identified, immunosuppressants like Prednisone @ 1 to 2 mg/kg/day and in severe affected dogs, cyclophophamide and azathioprine were advocated (Noone 1986, Clercx *et al* 2000). Prognosis is fair to excellent in most of the eosinophilic pneumonias. Other causes of ILDs identified were pulmonary interstitial fibrosis, lymphocyic interstitial pneumonitis (LIP), bronchiolitis obliterans with organizing pneumonia (BOOP), endogenous lipid pneumonia (EnLP), pulmonary alveolar proteinosis (PAP), silicosis and asbestosis. Majority of them were case repots and none of these anomalies was seen in our study. In most of these cases, no information about antemortem treatment in small animals is available (Jones *et al* 2000, Raya *et al* 2006). Prednisone at immunosuppressive doses was recommended in BOOP patients however, relapse has been noticed after discontinuation, so reinstitution of drug needs to be done. A series of therapeutic large volume bronchoalveolar lavage every six monthly was used in a PAP dog to dilute and remove lipoproteinaceous material from the lungs (Jefferies *et al* 1987, Silverstein *et al* 2000).

Table 36: Comparison of age and vital parameters pre and post treatment in ILDs dogs (n=8)

Parameters	Pre Treatment (n=8)	Post Treatment (n=8)
Body Weight (Kg)	31.53±3.40 (36, 13-39)	31.24±3.41 (35.8, 13.2-38.9)
RT (ºF)	103.13±0.36 (103.5, 101.2-104.2)	102.60±.19 (102.8, 101.4-103.2)
HR (bpm)	113.50±6.84 (118, 80-140)	98.50±4.53 (94, 88-128)
RR (breaths/min)	46.00±7.30 (51, 14-76)	28.50±2.67 (28, 20-40)

Values in parenthesis depicts range

Table 37: Comparison of hematological parameters in ILDs dogs pre and post treatment

Parameters	Pre Treatment (n=8)	Post Treatment (n=8)
Hb (g/dl)	13.75±0.25 (13.8, 12.5-14.5)	13.71±0.72 (13.95, 10.4-16.5)
TEC (count/µl × 10^6)	4.81±0.22 (4.7^b, 4.17-6.22)	6.68±0.27 (6.9^a, 5-7.47)
TLC (count/µl)	13604±3335 (11700, 5800-35310)	13768±938 (13765, 9760-16690)
PCV (%)	41.41±0.72 (41.4, 37.5-43.5)	42.54±2.56 (44.2, 31.2-49.9)
Platelets (count/µl× 10^3)	225.4±11.4 (213.5, 198-287)	250.6±16.9 (243, 184-328)
Absolute neutrophils (count/µl)	10916±2973 (8475, 5330-31073)	10016±1011 (9508, 6776-15355)
Absolute lymphocytes (count/µl)	2281±592 (1830, 348-5227.2)	2942±552 (2986, 902-5082)
Absolute eosinophils (count/µl)	407±224 (130, 0-1788)	363±125 (349, 0-902)

Values in parenthesis depicts range

In the present study, most of the cases (n=7) were diagnosed as non-specific interstitial pneumonia (NSIP) that too with the cellular form in which lung parenchyma was still maintained. That could be the reason that all these cases responded well to the predetermined protocol comprising antibiotics and bronchodilators (Bjoraker *et al* 1998, Travis *et al* 2008, Travis *et al* 2013). The other dog with anthracosis (a milder type of pneumoconiosis), which occurs due to accumulation of black dust particles after chronic exposure to air pollution or inhalation of coal dust or smoke (Mirsadraee 2014), recovered completely after shifting its premises from factory area to residential locality.

No significant difference was observed in the post treatment hematological values (Table 37).

On radiographic examination in all the cases improvement in the radiopacity was observed in all lung lobes along with the improvement in the interstitial pattern from moderate and severe to mild and moderate (Fig. 68, 69).

CHAPTER V

SUMMARY AND CONCLUSIONS

Respiratory diseases are common problem in dogs. Very young and older dogs are always at a higher risk as compared to healthy adult dogs. The syndrome may vary from merely rhinitis, sinusitis to tracheal collapse, infectious tracheobronchitis, bacterial and viral pneumonia, lung lobe torsion and sinonasal tumors. Clinical signs usually include laboured breathing and coughing however, they may range from dyspnoea, costal or abdominal respirations, cough, nasal discharge and congestion, edema to consolidation of lungs, lethargy and weight loss. Till date, diagnosis was based on chest auscultation, haemato-biochemistry, radiography, arterial blood gas analysis, fecal examination and nasal swab culture in India. Now in the present study, the advanced technique like transtracheal wash is being explored for specific identification of the aetiology. The focus of the study was "Diagnosis and therapeutic management of the canine respiratory affections". The method of transtracheal wash aspiration was standardised as per the resources available in Indian conditions.

Ten clinically healthy dogs without any evidence of respiratory disease on clinical examination were placed in the control group. The animals had no history of any clinical illness for last 6 months to 1 year. Concurrently, 40 dogs presented with suggestive signs of respiratory diseases to the small animal clinics were included in the diseased group. Any dog with at least one of the specific symptom such as cough, nasal discharge, respiratory distress, exercise intolerance was involved in the study. Thorough physical examination was undertaken in all the healthy and diseased dogs.

Blood was collected for hemato-biochemical parameters in all the 50 dogs and analyzed for hematological and biochemical parameters. Arterial blood collected from femoral artery was subjected to blood gas analysis. Faecal examination was done by faecal floatation method to check presence of lungworm larvae or ova/cyst. Transtracheal wash (TTW) taken from the dogs was submitted for cytologic examination and bacterial culture for the identification of etiological agent. Culture sensitivity test was undertaken on the bacterial isolates obtained. Thoracic radiography, being an important diagnostic tool, was done in all dogs. Treatment was done empirically in all the animals followed after 3 days by protocol based on the culture sensitivity results. All the numerical data were analyzed using SAS software

using Mann-Whitney Testfor comparison of median values between the groups and Wilcoxon Signed Rank Test to compare median values between pre and post treatment groups.

In all the healthy animals, all physical and vital parameters were within the normal range. Mean cell number (cells/HPF) of transtracheal wash (TTW) fluid in healthy dogs was8.35±2.68. Mean macrophage and neutrophil cell count in TTW was 49.57±5.28 and 11.43±1.00 percent, respectively. The mean lymphocyte count in TTW fluid was 7.71±2.25 percent. Hemato-biochemical and blood gas parameters were within the normal range.

Out of the 40 diseased dogs presented to the small animal clinics, 4 were diagnosed with the upper respiratory tract affections and 36 with the lower respiratory tract affections. Diagnosis of nasal squamous cell carcinoma (n=3) and allergic rhinitis (n=1) in dogs was confirmed on the basis of nasal swab cytology and nasal radiography along with physical and clinical examination. Diagnosis of Chronic bronchitis (n=11), Bacterial bronchopneumonia (n=7), Pulmonary neoplasia (n=2), undiagnosed nodular interstitial pattern (n=3), Pulmonary abscessation (n=1) and Interstitial lung diseases (n=12) in dogs were confirmed on the basis of transtracheal wash fluid cytology and/or culture and isolation, thoracic radiography supported by physical and clinical examination.

Three dogs (2 German shepherd and 1 Non-descript) aged between 1.5 to 7 years, diagnosed with nasal squamous cell carcinoma, were having history of epistaxis, sneezing, tachypnea, fever, lethargy, decreased appetite and occasional non productive cough. Hemato-biochemistry revealed severe neutrophilic leucocytosis, mild anaemia and high fibrinogen levels. Blood gas parameters were in the normal range with mean partial pressure of oxygen was 88.5±1.22 mmHg. On nasal swab cytological examination, clusters of pleomorphic epithelial cells with abundant cytoplasm and centrally placed nuclei were observed, suggestive of squamous cell carcinoma. Radiograph of nasal cavity (DV view) was normal in two dogs and increased soft tissue density in nasal cavity and maxillary sinus was observed in one dog. Male German shepherd survived for two months after the owner refuse to undertake chemotherapy in this case whereas the other male dog (non-descript) died within 2 days of diagnosis before ensuing any medical or surgical treatment. The only

female dog with SCC, is still surviving after 80 days of diagnosis and symptomatic treatment.

Allergic rhinitis was diagnosed in a 10 year old female German shepherd presented with history of epistaxis and sneezing from 3 months. Dog was alert and active with normal appetite. After taking detailed history from the owner it was found that new furniture was brought to the house, which could be a source of allergy due to being recently varnished. Hemato-biochemical parameters revealed absolute eosinophilia (2440 cells/µl), high total protein (8.2 g/dl), low albumin (2.2 g/dl) and high fibrinogen level (0.6 g/dl). Slight decrease in PaO_2 (71 mmHg) was observed. On cytological examination of nasal swab, scattered neutrophils along with eosinophils were observed. Nasal cavity radiograph (DV view) was found normal. Treatment was done with chlorpheniramine orally @ 4mg/dog every 12 hour for first 7 days followed by every 24 hourly treatment, till the signs subsided (1 month in the present case) along with Celin 500 (vitamin C) for 7 days. Complete blood count was done after the clinical recovery of the dog which showed significant decrease in eosinophils count.

Seven dogs with bacterial bronchopneumonia were presented to small animal clinics with history of cough, bilateral nasal discharge, exercise intolerance, dyspnea, fever, lethargy, weight loss, dehydration and decreased appetite. Abnormal lung sounds were heard during auscultation in all the dogs. Hemato-biochemical parameters revealed absolute neutrophilia (12270±1898 cells/µl) and high fibrinogen count (0.88±0.11 g/dl). Severe hypoxemia noticed on blood gas analysis. Mixed interstitial and alveolar pattern was observed on thoracic radiography in majority of dogs. Right middle lung lobe congestion was observed in 2 dogs along with pulmonary edema in cranial and caudal lobes of 4 dogs. TTW fluid cytology revealed markedly increased cellularity with majority of neutrophils (degenerative and non-degenerative) and few macrophages and occasional sloughed epithelial cells suggestive of purulent pneumonia. *Staphylococcus aureus* (n=3) and *E. coli*(n=2) organisms were isolated from culture on specific media i.e., Baird Parker and EMB, respectively. Treatment was done as per preset protocol with Amoxicillin/clavulanic acid @15mg/kg PO or Amoxicillin- Sulbactum @ 15 mg/kg IM q12h for first 3 days followed by antibiotic selected on the basis of CST along with bronchodilators terbutaline @ 1.25-5 mg/dog PO q8-12h for one to two weeks depending on the

severity of case. None of the 6 cases treated, responded, due to resistance to most of the commonly used antibiotics and late presentation of most of these cases.

In chronic bronchitis, eleven dogs were presented to the small animal clinics with complaints of coughing, respiratory distress and exercise intolerance along with positive inducible cough reflex. Abnormal lung sounds were heard in 9 affected dogs and normal lung sounds in 2 dogs. Hemato-biochemistry showed absolute neutrophilia and high fibrinogen level (0.62±0.03 g/dl). Hypoxemia was observed on blood gas analysis. Thoracic radiography showed mild to moderate bronchial pattern in cranial and caudal lobe along with bronchial thickening and multiple donuts observed in all the dogs suffering with chronic bronchitis. Non-structural (diffused) interstitial pattern was observed in 6 dogs on thoracic radiography. TTW cytology revealed increased cellularity along with moderate increase in neutrophils, presence of mucus and hyperplastic epithelial cells in all the dogs suffering from chronic bronchitis. *Staphylococcus aureus* (n=2), *E. coli* (n=2) and *Klebsiella* sp. (n=1) organisms were isolated from culture on specific media i.e., Baird Parker, EMB and MLA, respectively. On histopathology of lung tissue in one dog, sloughing of tracheal epithelium along with lung emphysema and thickening of interlobular septa was recorded. Treatment protocol used involved Amoxicillin and Clavulanic acid combination @ 15 mg/kg PO q12h for first 3 days followed by antibiotic selected on the basis of CST up to 2 weeks along with bronchodilators terbutaline @ 1.25-5 mg/dog PO q8-12h for 2 weeks. Cetrizine @5-10mg/dog PO q12h was used in two cases. Absolute eosinophilia was found in one dog. Fecal sample in this dog was positive for hookworms in the 2nd week of the course of disease. Absolute eosinophilia resolved after two shots of Ivermectin @ 0.2 mg/kg SC in this dog two weeks apart. Radiographic recovery was noticed in all the six cases of chronic bronchitis after 2 weeks of treatment.

Six dogs diagnosed with histiocytic sarcoma (HS) (n=1), bronchioloalveolar carcinoma (BAC) (n=1), pulmonary abscessation (n=1) and undiagnosed nodular interstitial group (n=3). Dogs were presented with a complaint of coughing (non-productive), respiratory distress and exercise intolerance. However, the additional sign seen in HS was lameness. High rectal temperature was recorded in BAC. Haemato-biochemical parameters revealed anaemia in HS and BAC affected dogs;

and neutrophilic leukocytosis in undiagnosed nodular interstitial group dogs. Absolute neutrophilia was observed in the dog with pulmonary abscessation, along with hyperfibrinogenemia in all the 6 dogs. Severe hypoxemia was observed in pulmonary neoplasia group (n=2) dogs with PaO_2 38mm Hg. FNAC from the nodules in lung parenchyma demonstrated large number of neutrophils along with few macrophages suggestive of predominant suppurative inflammation. In histiocytic sarcoma, pleomorphic large cells with round to oval eccentric placed nuclei, resembling macrophages were observed in the fine needle aspirate taken from the cavitory lesion of the diseased dog. Lymph node aspiration in this case revealed reactive hyperplasia of the lymphocytes indicating metastasis of the tumor. In case of Histiocytic sarcoma, doxorubicin was used @ 30mg/m^2 but dog died after few hours of treatment. In dog with bronchioloalveolar carcinoma, malignancy occurred leading to multiorgan involvement making it a poor candidate for chemotherapy. Pulmonary abscessation was so severe that animal did not respond to any antibiotic and died within few days of treatment. One of the 3 cases of undiagnosed nodular interstitial pneumonia died before start of treatment based on CST whereas other one with haemoptysis died on the second day of presentation before TTW aspiration or FNAC from pulmonary tissue. Third case partially responded to empirical antibiotic treatment as the owner was not ready for TTW aspiration of his dog and died after 2 months of treatment.

Interstitial lung diseases (ILDs) were diagnosed in 12 dogs, which include anthracosis (n=2), eosinophillic bronchopneumopathy (EBP) (n=1) and non-specific interstitial pneumonia (NSIP) (n=9). Dogs were presented with history of coughing, exercise intolerance, tachypnea, dyspnea, fever, lethargy, decreased appetite and positive inducible cough reflex. Harsh lung sounds were recorded in majority of the affected dogs. Hemato-biochemistry revealed absolute neutrophilia in NSIP dogs and absolute eosinophilia in EBP dog and high fibrinogen count in all the dogs. Hypoxemia was only observed in NSIP and EBP dogs. Thoracic radiography showed mixed bronchial and interstitial pattern in all the affected dogs. TTW cytology examination in anthracosis dog revealed increased number of neutrophils and macrophages with engulfed blackish pigment and lymphoid cells suggesting long standing chronic active inflammation. In our study, severe eosinophilia (63%) with mean cell count of 10.1cells/HPF was observed in tracheal wash of eosinophilic bronchpneumopathic dog. In NSIP dogs, increased number of non degenerative

neutrophils and mucus was observed on TTW cytology. *Staphylococcus aureus* was identified in two dogs with positive culture. Affected dogs (n= 8) were treated with the amoxicillin and clavulanic acid combination @ 15 mg/kg PO q12h for 3 days followed by antibiotic based on CST up to 2 weeks along with bronchodilator terbutaline @ 1.25-5 mg/dog PO q8-12h for 2 weeks. Antihistaminics like cetrizine @5-10mg/dog PO q12h was used in two cases.

Overall mean cell number (cells/HPF) in transtracheal wash (79.73±14.48) in diseased dogs was more as compared to healthy animals. Marked increase in cellularity and the number of neutrophils was appreciated in the TTW cytology of bacterial bronchopneumonia and pulmonary abscessation. Blood gas analysis revealed hypoxemia as the only important finding in most of the dogs suffering with respiratory diseases. Absolute neutrophilia and hyperfibrinogenemia was the most common finding in dogs affected with respiratory diseases.

CONCLUSIONS

- Transtracheal wash procedure can be easily performed in dogs more than 10kg body weight, using a disposable 14 G cannula and 4 FG dog tube.
- Squamous cell carcinoma is common tumour of nasal cavity in dogs and should be ruled out in cases of epistaxis in dogs.
- Chronic bronchitis is most common respiratory affection followed by non-specific interstitial pneumonia, bacterial bronchopneumonia and lung neoplasia/nodular lung pattern, anthracosis and eosinophilic bronchopneumopathy.
- TTW mean and differential cell count can be used as a differentiating feature to diagnose bacterial bronchopneumonia from chronic bronchitis.
- Terbutaline @ 1.25-5mg/dog is effective in dogs suffering with chronic bronchitis and interstital lung diseases.
- Radiography is a good ancillary diagnostic aid for diagnosis of respiratory affections.
- TTW cytology can be added to the diagnostic protocol for lower respiratory affections in dogs.

REFERENCES

Adams W M, Bjorling D E and McAnulty J E. 2005. Outcome of accelerated radiotherapy alone or accelerated radiotherapy followed by exenteration of the nasal cavity in dogs with intranasal neoplasia: 53 cases (1990–2002). *Journal of American Veterinary Medical Association* **227**: 936–41.

Adaszek L, Gorna M, Zietek J, Kutrzuba J and Winiarczyk S. 2009. Bacterial nosocomial infections in dogs and cats. *Zycie Weterynaryjne* **84**: 805-08.

Affolter V K and Moore P F. 2002. Localized and disseminated histiocytic sarcoma of dendritic cell origin in dogs *Veterinary Pathology* **39**: 74-83.

Aiello S E and Moses M A. 2016. The Merck Veterinary Manual, John Wiley & Sons, USA.

Albrecht H H, Dicpinigaitis P V and Guenin E P. 2017. Role of guaifenesin in the management of chronic bronchitis and upper respiratory tract infections. *Multidisciplinary respiratory medicine* **12(1)**: 31.

Amrute M P, Muley V D, Dighe D G, Velhankar R D and Keskar D V. 2009. Chronic bronchopneumonia in a Great Dane pup. *Veterinary World* **2**(9): 358-59.

Anusz K. 2005. Some respiratory diseases in ageing dogs. *Veterinary Practice* **2**: 14-17.

Aspinall V. 2004. Anatomy and Physiology of the Dog and Cat 7. The Respiratory System, *Veterinary Nursing Journal* **19(2)**: 54-59.

Atkins C. 2001. Evaluation of cough in dogs with heart murmurs. *World Small Animal Veterinary Association.*

Attili A R, Cerquetella M, Pampurini F, Laus F, Spaterna A and Cuteri V. 2012. Association between enrofloxacin and N-acetylcysteine in recurrent bronchopneumopathies in dogs caused by biofilm producer bacteria. *Journal of Animal and Veterinary Advances* **11**: 462-69.

Aubier M and Roussos C. 1985. Effect of theophylline on respiratory muscle function. *Chest* **88**: 91S-7S.

Ayodhya S, Rao D T, Reddy Y N, Sundar N S and Kumar V G. 2013. Epidemiological, clinical and haematological studies on canine respiratory diseases in and around Hyderabad city, Andhra Pradesh, India. *International Journal of Current Microbiology and Applied Sciences* **2(11)**: 453-62.

Bailiff N L and Norris C R. 2002. Clinical signs, clinicopathological findings, etiology, and outcome associated with hemoptysis in dogs: 36 cases (1990–1999). *Journal of the American Animal Hospital Association* **38(2)**: 125–33.

Barr F J, Gibbs C and Brown P J. 1986. The radiological features of primary lung tumours in the dog: a review of thirty-six cases. *Journal of Small Animal Practice* **27**: 493–505.

Benson C J, Edlund M B, Gray S, Powell L, Paulin-Curlee G, Armien A and Overmann J A. 2013. The presence of diatom algae in a tracheal wash from a German Wirehaired Pointer with aspiration pneumonia. *Veterinary Clinical Pathology* **42**: 221-26.

Bertazzolo W, Zuliani D, Pogliani E, Caniatti M and Bussadori C. 2002. Diffuse bronchiolo-alveolar carcinoma in a dog. *Journal of small animal practice* **43(6)**: 265-68.

Bexfield N H, Foale R D and Davison L J. 2006. Management of 13 cases of canine respiratory disease using inhaled corticosteroids. *Journal of Small Animal Practice* **47**: 377–82.

Billen F, Day M J and Clercx C. 2006. Diagnosis of pharyngeal disorders in dogs: a retrospective study of 67 cases. *Journal of Small Animal Practice* **47**: 122-29.

Binder E, Holopainen E, Malmberg H and Salo O P. 1984. Clinical findings in patients with allergic rhinitis. *Rhinology* **22(4)**: 255-60.

Bjoraker J A, Ryu J H, Edwin M K, Myers J L, Tazelaar H D, Schroeder D R, Offord K P. 1998. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. *American Journal of Respiratory and Critical Care Medicine* **157**: 199-203.

Blagburn B L, Lindsay D S and Vaughn J L. 1996. Prevalence of canine parasites based on fecal flotation. *Compendium on Continuing Education for the Practising Veterinarian* **18**: 483-509.

Bolln G, Wolk U, Bausch M, Kresken J G and Hoptner C. 2003. Retrospective study of 143 cases (between 1997 and 2001) of chronic nasal discharge in the dog and cat. *Practical Veterinarian* **84**: 742-51.

Boothe D M and McKiernan B C. 1992. Respiratory therapeutics. *The Veterinary Clinics North American Small Animal Practice* **22**: 1231-58.

Bouda J and Jagos P. 1991. Disorders in the acid-base balance. In: Vrzgula L (Ed.) *Metabolic Disorders and their Prevention in Farm Animals*. Elsevier, Amsterdam. pp. 248-68.

Bouda J, Núñez-Ochoa L, Ávila-González E, Doubek J, Fuente-Martínez B and Aguilar-Bobadilla J. 2009. Blood acid–base and plasma electrolyte values in healthy ostriches: the effect of age and sex. *Research in Veterinary Science* **87(1)**: 26-28.

Brendan. 2006. Clinical evaluation of the patient with respiratory diseases In: Ettinger J D and Feldan (Eds.) *Textbook of Small Animal Internal Medicine*. 6th Edn. WB Saunders, Co, Philadelphia, pp. 1034-39.

Brown N L. 2010. Sneezing and nasal discharge. In: Ettinger S J and Feldman E C (Eds.) *Textbook of Veterinary Internal Medicine*, 7th Edn. pp. 1030-40.

Brownlie J, Mitchell J and Walker C A. 2013. Mycoplasmas and novel viral pathogens in canine infectious respiratory disease. *Journal of Veterinary Internal Medicine* (Seattle).

Brownlie S E. 1990. A retrospective study of diagnosis in 109 cases of canine lower respiratory disease. *Journal of Small Animal Practice* **31(8)**: 371-76.

Buonavoglia C and Martella V. 2007. Canine respiratory viruses. *Veterinary Research* **38**: 355-73.

Burkhard M J, Valenciano A and Barger A. 2001. In: Raskin R E, Meyer D J (Eds.) *Atlas of Canine and Feline Cytology*. Philadelphia: WB Saunders, pp. 135-85.

Cadore J L. 2011. Questions and answers on...canine infectious tracheobronchitis. *The New Veterinary Practitioner Canine Feline* **48**: 9-10.

Cannon C, Borgatti A, Henson Mand Husbands B. 2015. Evaluation of a combination chemotherapy protocol including lomustine and doxorubicin in canine histiocytic sarcoma. *Journal of Small Animal Practice* **56(7)**: 425-29.

Carey S A. 2009. "Bronchopneumonia in the small animal patient" International summit on Advancing Veterinary Medical care: Challenges and Strategies & 27th ISVM convention Satellite seminar on Veterinary Internal Medicine, 19-21 Feb '09, pp. 59-69.

Carey S A. 2011. Current Therapy for Canine Chronic Bronchitis.

Carter D and Egglestone J C. 1980. Tumors of the respiratory tract. In: *Atlas of Tumor Pathology*. 2nd Series, Volume 17. AFIP, Washington DC, pp.127-47.

Cave N, More G, Sowman H, Acke E, Midwinter A and Dunowska M. 2015. Acute Canine Infectious Tracheobronchitis (kennel cough) in Greyhounds and other dogs in New Zealand. *New Zealand Veterinary Journal* **66(5)**: 236-42.

Centil M, Guthardt J, Heusinger A and Muller E. 2012. Coughs, colds, hoarseness infectious diseases of the respiratory tract in dogs. *Practical Veterinarian* **93**: 896-903.

Chan E D and Iseman M D. 2016. Bronchiectasis. In: Broaddus V C,Mason R C, Ernst J D, King T E, Lazarus S C, Murray J F, Nadel J A, Slutsky A, Gotway M (Ed.) Murray and Nadel's Textbook of Respiratory Medicine, 6th Edn. St. Louis, MO: Elsevier 853–76.

Chan P C, Huang L M and Wu P S. 2005. Clinical management and outcome of childhood lung abscess: a 16-year experience. *Journal of Microbiology Immunology and Infection* **38**: 183-8.

Charkrabarthi A. 2009. *Text book of Clinical Veterinary Medicine*. 3rd Edn. pp. 327-72.

Clercx C and Peeters D. 2007. Canine eosinophilic bronchopneumopathy. *Veterinary Clinics of North America*: *Small Animal Practice* **37(5)**: 917–35.

Clercx C, Peeters D, Snaps F, Hansen P, McEntee K, Detilleux J and Day M J. 2000. Eosinophilic bronchopneumopathy in dogs. *Journal of Veterinary Internal Medicine* **14(3)**: 282-291.

Clercx C, Reichler I, PeetersD, McEntee K, German A, Dubois J, Schynts F, Schaaf-Lafontaine N, Willems T, Jorissen M and Day M J.2003. Rhinitis/ bronchopneumonia syndrome in Irish Wolfhounds. *Journal of Veterinary Internal Medicine* **17**: 843-49.

Cohn L A, Norris C R, Hawkins E C, Dye J A, Johnson C A and Williams K J. 2004. Identification and characterization of an idiopathic pulmonary fibrosis-like condition in cats. *Journal of Veterinary Internal Medicine* **18(5)**: 632-41.

Cooke K. 2005. Sneezing and nasal discharge. In: Ettinger S J and Feldman E C (Eds.) *Textbook of Veterinary Internal Medicine,* 6th Edn. vol 1. Saint-Louis: Elsevier Saunders, pp: 207-10.

Coomer A R and Liptak J M. 2008. Canine histiocytic diseases. *Compendium on Continuing Education for the Practising Veterinarian-North American Edition* **30(4)**: 202.

Corcoran B M, Cobb M, Martin M W, Dukes-McEwan J, French A, Fuentes V L, Boswood A and Rhind S. 1999. Chronic pulmonary disease in West Highland white terriers. *Veterinary Record* **144**: 611-16.

Corcoran B M, Fuentes V L and Clarke C J. 1992. Chronic tracheobroncheal syndrome in eight dogs. *Veterinary Record* **130(22)**: 485-87.

Corcoran B M. 2004. Bacterial bronchopneumonia: Diagnosis, management and treatment. In: *Proceedings of World Small Animal Veterinary Association World Congress*.

Corcoran B. 2000. Clinical evaluation of the patient with respiratory disease. In: Ettinger S J and Feldman E C (Eds.) *Textbook of Veterinary Internal Medicine.* 5th Edn. Philadelphia: WB Saunders, pp. 1034-39.

Cowell R L, Tyler R D and Baldwin C J. 1989. Transtracheal and bronchial washes. In: Cowell R L, Taylor R D, Eds. Diagnostic Cytology of the Dog and Cat. Goleta. CA: *American Veterinary Publications* 167-77.

Crapo J D, Harmsen A G and Sherman M P. 2000. Pulmonary immunobiology and inflammation in pulmonary diseases. *American Journal of Respiratory and Critical Care Medicine* **162(5)**: 1983-86.

Creevy K E. 2009. Airway evaluation and flexible endoscopic procedures in dogs and cats: laryngoscopy, transtracheal wash, tracheobronchoscopy, and bronchoalveolar lavage. *Veterinary Clinics of North America*: *Small Animal Practice* **39(5)**: 869-80.

Dahl K, Rorvik A M and Lanageland M. 2002. Bronchogenic cyst in a German shepherd dog. *Journal of Small Animal Practice* **43(10)**: 456-58.

Danik J S, Pare G and Chasman D I. 2009. Novel loci, including those related to crohn disease, psoriasis, and inflammation, identified in a genome- wide association study of fibrinogen in 17 686 women: The women's genome health study. *Circulation: Cardiovascular Genetics* **2:** 134-41.

Datz C. 2003. *Bordetella* infections in dogs and cats: treatment and prevention. *Compendium on Continuing Education for the Practising Veterinarian* **25**: 902-14.

De Lorenzi D, Bonfanti U, Masserdotti C, Caldin M and Furlanello T. 2006. Diagnosis of canine nasal aspergillosis by cytological examination: a comparison of four different collection techniques. *Journal of Small Animal Practice* **47(6)**: 316-19.

De Mey C, Koelsch S, Richter E, Pohlmann T and Sousa R. 2016. Efficacy and Safety of Ambroxol Lozenges in the Treatment of Acute Uncomplicated Sore Throat–a Pooled Analysis. *Drug research* **66(07)**: 384-92.

Dear J D. 2014. Bacterial pneumonia in dogs and cats. *Veterinary Clinics of Small Animal Practice* **44**: 143-59.

Dervisis N G, Kiupel M, Qin Q and Cesario L. 2017. Clinical prognostic factors in canine histiocytic sarcoma. *Veterinary and Comparative Oncology* **15(4)**: 1171-80.

Donaldson J C, Kaminsky D B and Elliott R C. 1978. Bronchiolar Carcinoma. *Cancer* **41**: 250-58.

Dryden M W, Payne P A and Ridley R. 2005. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Veterinary Therapeutics*: *Research in Applied Veterinary Medicine* **6**: 15-28.

Dungworth D L, Hauser B, Hanh F F, Wilson D W, Haenichen T and Harneka J R. 1999. Bronchioloalveolar Carcinoma. In: *Histological Classification of Tumors of the Respiratory System of Domestic Animals*. 2nd Series, Volume 6. AFIP, Washington DC, pp 30-31.

Dunn J. 2010. Cytological examination of the lower respiratory tract in dogs and cats. *In Practice* **32(4)**: 150-55.

Eckersall P D and Bell R. 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *The Veterinary Journal* **185(1)**: 23-27.

Elliot K M and Mayer M N. 2009. Radiation therapy for tumors of the nasal cavity and paranasal sinuses in dogs. *The Canadian Veterinary Journal* **50(3)**: 309.

Epstein S E, Mellema M S and Hopper K. 2010. Airway microbial culture and susceptibility patterns in dogs and cats with respiratory disease of varying severity. *The Journal of Veterinary Emergency and Critical Care* **20(6)**: 587–94.

Ettinger S J and Feldman E C. 2010. *Textbook of Veterinary Internal Medicine*, 7th Edn. Philadelphia, WB Saunders, pp. 1066-88.

Farzan S. 1990. Cough and sputum production. In: Walker H K, Hall W D, Hurst J W, (Eds.) *Clinical Methods*: *The History, Physical and Laboratory Examinations.* 3rd Edn. London: Butterworths; 207–10.

Finke D M. 2013. Transtracheal Wash and Bronchoalveolar Lavage. *Topics in Companion Animal Medicine* **28**: 97-102.

Flick S C. 1973. Endoparasites in cats: current practice and opinions. *Feline Practice* **3(4)**: 21-34.

Fonfara S L, de la Heras Alegret, German A J, Blackwood L, McEwan J D, Noble P J M, Burrow R D. 2011. Underlying diseases in dogs referred to a veterinary teaching hospital because of dyspnea: 229 cases (2003-2007). *Journal of American Veterinary Medical Association* **239**: 1219-24.

Ford R B. 2009. Bacterial pneumonia. In: Bonagura J D and Twedt D C (Eds.) *Kirk's Current Veterinary Therapy XIV*. 14th Edn. Saunders, pp. 658-62.

Fossum T W. 2013. Pulmonary Neoplasia. In: *Proceedings of World Small Animal Veterinary Association World Congress*.

Fox P R. 2007. Approach to the coughing and dyspneic dog. In: *Proceedings of the WSAVA*, Sydney, Australia, August 20-24, pp. 1-5.

Fuentes V L. 1998. Differential diagnosis of dyspnea. In: Fuentes V L, Swift S, editors. BSAVA manual of small animal cardiorespiratory medicine and surgery. Cheltenham: BSAVA: pp 123-8.

Fulmer A K and Mauldin G E. 2007. Canine histiocyticneoplasia: an overview. *The Canadian Veterinary Journal* **48**: 1041–43.

Gaskell C K. 1974. The radiographic anatomy of the pharynx and larynx of a dog. *Journal of Small Animal Practice* **14**: 89-100.

Greene C E and Appel M J. 1990. Canine distemper. In: Greene C E (Ed.) *Infectious Diseases of the Dog and Cat*. Philadelphia, WB Saunders, p. 226.

Guyton A C and Hall J E. 2006. *Guyton & Hall Textbook of Medical Physiology*. 11th Ed. Philadelphia, W.B. Saunders.

Harpster N K. 2004. Physical examination of the respiratory tract. In: King L G (Ed.) *Respiratory Disease in Dogs and Cats*. Philadelphia: WB Saunders; pp. 67-72.

Haskins S C. 2004. Interpretation of blood gas measurements. In: King LG (Ed.) *Respiratory Disease in Dogs and Cats*. Philadelphia: WB Saunders; 2004. pp. 181-92.

Hawkins E C and DeNicola D B. 1990 . Cytologic analysis of tracheal wash specimens and bronchoalveolar lavage fluid in the diagnosis of mycotic infections in dogs. *Journal of American Veterinary Medical Association* **197**: 79-83.

Hawkins E C, Basseches J, Berry C R. 2003. Demographic, clinical, and radiographic features of bronchiectasis in dogs: 316cases (1988–2000). *Journal of American Veterinary Medical Association* **223**: 1628-35.

Hawkins E C, Clay L D, Bradley J M and Davidian M. 2010. Demographic and historical findings, including exposure to environmental tobacco smoke, in dogs with chronic cough. *Journal of veterinary internal medicine* **24(4)**: 825-31.

Hawkins E C, DeNicola D B and Plier M L. 1995. Cytologic analysis of bronchoalveolar lavage fluid in the diagnosis of spontaneous respiratory tract disease in dogs: a retrospectivestudy. *Journal of Veterinary Internal Medicine* **9**: 386–92.

Hedlund C S. 1997. Surgery of the upper respiratory system. In: Fossum T W (Ed.) *Small Animal Surgery*. St. Louis (MO): Mosby; pp. 609-20.

Hoover J P, Martin M O H and Bahr R J. 1989. Chronic bronchitis, Bronchiectasis, bronchiolitis, bronchiolitisobliterans and bronchopneumonia in a rottweiler with primary ciliary dyskineria. *Journal of the American Animal Hospital Association* **25**: 397-304.

Jain N C. 1986. Schalm's Veterinary Haematology, Lea and Febinger, Philadelphia, USA.

Jefferies A R, Dunn J K and Dennis R. 1987. Pulmonary alveolar proteinosis (phospholipoproteinosis) in a dog. *Journal of Small Animal Practice* **28(3)**: 203-14.

Jiang H G, Li J and Shi SB. 2014. Value of fibrinogen and D-dimer inpredicting recurrence and metastasis after radical surgery for non-smallcell lung cancer. *Medical Oncology* **31**: 22.

John G H, Noel R K. Peter H A S, James T S and Stanley T W. 2000. *Bergy's Manual of Determinative Bacteriology*, 9th Edn.

Johnson E G and Wisner E R. 2007. Advances in respiratory imaging. *Veterinary Clinics of North America*: *Small Animal Practice* **37(5)**: 879-900.

Johnson L R, Johnson E G, Vernau W, Kass P H and Byrne B A. 2016. Bronchoscopy, imaging, and concurrent diseases in dogs with bronchiectasis: (2003–2014). *Journal of veterinary internal medicine* **30(1)**: 247-54.

Johnson L. 2000. Tracheal collapse. Diagnosis and medical and surgical treatment. *The Veterinary Clinics North American Small Animal Practice* **30**: 1253-66.

Johnson L R, Queen E V, Vernau W, Sykes J E and Byrne B A. 2013. Microbiologic and cytologic assessment of bronchoalveolar lavage fluid from dogs with lower respiratory tract infection: 105 cases (2001-2011). *Journal of Veterinary Internal Medicine* **27**: 259-67.

Jolivet F, Diquélou A, Trumel C, Privat S and Dossin O. 2017. Fibrinogen deficiency in a dog-a case report. *BMC Veterinary Research* **13(1)**: 183.

Jones D J, Norris C R, Samii V F and Griffey S M. 2000. Endogenous lipid pneumonia in cats: 24 cases (1985–1998). *Journal of the American Veterinary Medical Association* **216(9)**: 1437-40.

Jordan H E, Mullins S T and Stebbins M E. 1993. Endoparasitism in dogs: 21,583 cases (1981–1990). *Journal of American Veterinary Medical Association* **203**: 547-49.

Joseph C, Togawa Y, Shindo N. 2013. Bacterial and viral infections associated with influenza. *Influenza and Other Respiratory Viruses* **7(Suppl 2)**: 105–13.

Jp N A. Imanaka M and Suganuma N. 2017. Japanese workplace health management in pneumoconiosis prevention. *Journal of Occupational Health* **59**: 91–103.

Khan S A, Epstein J H, Olival K J, Hassan M M, Hossain M B, Rahman K B M A and Desmond J. 2011. Hematology and serum chemistry reference values of stray dogs in Bangladesh. *Open Veterinary Journal* **1(1)**: 13-20.

Khattab M R, Salem S I, Kubesy A A and Ali M E. 2017. Trans-tracheal wash analysis of canine distemper infected dogs. *Bioscience Research* **14(4)**: 1128-35.

King L G and Hendricks J C. 1995. Clinical pulmonary function tests. In: Ettinger S J, Feldman E C (Eds.) *Textbook of Veterinary Internal Medicine*, Vol. 1. 4th Edn. Philadelphia: WB Saunders; pp. 738-54.

King R R, Greiner E C and Ackerman N. 1990. Nasal capillariasis in a dog. *Journal of the American Animal Hospital Association* **26**: 381.

Klaassen J K. 1999. Reference values in veterinary medicine. *Laboratory Medicine* **30(3)**: 194-97.

Klahn SL, Kitchell B E and Dervisis NG. 2011. Evaluation and comparison of outcomes in dogs with periarticular and nonperiarticular histiocytic sarcoma. *Journal of the American Veterinary Medical Association* **239**: 90–96.

Kleiter M and Malarkey D M. 2004. Expression of cyclooxygenase-2 in canine epithelial nasal tumors. *Veterinary Radiology and Ultrasound* **45(3)**: 255-60.

Kneller S K. 1998. The larynx, pharynx, and trachea. In: Thrall D E (Ed.) *Textbook of Diagnostic Veterinary Radiology*. 3rd Edn. Philadelphia: WB Saunders; pp. 263-68.

Kogan D A, Johnson L R, Jandrey K E and Pollard R E. 2008. Clinical, clinicopathologic, and radiographic findings in dogs with aspiration pneumonia: 88 cases (2004–2006). *Journal of the American Veterinary Medical Association* **233**: 1742–47.

Kowal K and DuBuske L. 2014. Bilastine as a potential treatment in allergic rhinitis. *American Journal of Rhinology and Allergy* **28(4)**: 312-16.

Krimer P M. 2011. Generating and interpreting test results: test validity, quality control, reference values, and basic epidemiology. In: Latimer K S (Ed.) *Duncan and Prasse's Veterinary Laboratory Medicine*: *Clinical Pathology*. 5th Edn. Wiley-Blackwell, Chichester, pp. 365-82.

Kuehn N F. 2016. Respiratory diseases of small animals. In: Aiello S E and Moses M A (Eds.) *The Merck Veterinary Manual*. Merck & Co.

Kuhajda I, Zarogoulidis K, Tsirgogianni K, Tsavlis D, Kioumis I, Kosmidis C and Baloukas D. 2015. Lung abscess-etiology, diagnostic and treatment options. *Annals of Translational Medicine* **3(13)**: 183.

Kules J, Brkljacic M, Crnogaj M, Potocnjak D, Grden D, Torti M and Rafaj R B. 2015. Arterial blood acid-base and electrolyte values in dogs: conventional and "strong ion" approach. *Veterinarski Arhiv* **85(5)**: 533-45.

Kumrow K J and Rozanski E A. 2012. Canine chronic bronchitis. *Today's Veterinary Practice*, pp. 12-17.

Lamb C R. 1998. The canine lung. In: Thrall D E (Ed.) *Textbook of Diagnostic Veterinary Radiology*. 3rd Edn. Philadelphia: WB Saunders; pp. 366-84.

Lana S E and Withrow S J. 2001. Tumors of the respiratory system—nasal tumors. In: Withrow S J and MacEwen E G (Eds.) *Small Animal Clinical Oncology*, 3rd Edn. Philadelphia, PA, Saunders, pp. 370-77.

Lane J G. 1977. Rhinitis and sinusitis in the dog. In: Kirk R W (Ed.) *Current Veterinary Therapy VI*. Philadelphia, WB Saunders, 1977, p 220.

Lang J, Wortman J A, Glickman L T, Biery D N and Rhodes W H. 1986. Sensitivity of radiographic detection of lung metastases in the dog. *Veterinary Radiology* **27(3)**: 74-78.

Lappin M R, Blondeau J, Boothe D, Breitschwerdt E B, Guardabassi L, Lloyd D H and Weese J S. 2017. Antimicrobial use guidelines for treatment of respiratory tract disease in dogs and cats: antimicrobial guidelines working group of the International Society for Companion Animal Infectious Diseases. *Journal of veterinary internal medicine* **31(2)**: 279-94.

Larson M M. 2009. Ultrasound of the thorax (noncardiac). *Veterinary Clinics of North America*: *Small Animal Practice* **39(4)**: 733-45.

Lascelles B D X, Parry A T, Stidworthy M F, Dobson J M and White R A S. 2000. Squamous cell carcinoma of the nasal planum in 17 dogs. *Veterinary Record* **147(17)**: 473-76.

Legendre A M. 2006. Blastomycosis. In: Greene C E (Ed.) *Infectious Diseases of the Dogand Cat*. 3rd Edn. St. Louis: Elsevier Saunders, pp. 569-76.

Lichtenstein D, Goldstein I, Mourgeon E, Cluzel P, Grenier P and Rouby J. 2004. Comparative diagnostic performances of auscultation, chest radiography and lung ultrasonography in acute respiratory distress syndrome. *Anesthesiology* **100**: 9-15.

Liebow A A. 1960. Bronchiolo-alveolar carcinoma. *Advances in Internal Medicine* **10**: 329-58.

Lind L. 2003. Circulating markers of inflammation and atherosclerosis. Atherosclerosis **169:** 203-14.

Liu Y H, Lin Y C, Liang S J, Tu C Y, Chen C H, Chen H J andHsu W H. 2010. Ultrasound-guided pigtail catheters for drainage of various pleural diseases. *The American journal of emergency medicine* **28(8)**: 915-21.

Lode H S, Raffenberg T M and Mauch H. 1993. Diagnostic problems in lower respiratory tract infections. *The Journal of Antimicrobial Chemotherapy* **32**: 29-37.

Ludington L G, Verska J J, Howard T, Kypridakis G and Brewer L A. 1972. Bronchiolar carcinoma (alveolar cell), another great imitator: a review of 41cases. *Chest* **61**: 622-28.

Maden M, Birdane F M, Alkan F, Hadimli H H and Aslan V. 2000. Clinical, cytologic bacteriologic and radiographic analysis of respiratory diseases in dogs. *Journal of Veterinary Sciences* **16(1)**: 43-50.

Makino T, Shirai M, Kumagai K, Maejima T, Sehata S, Manabe S and Teranishi M. 2008. Bronchioloalveolar carcinoma in an aged beagle dog. *Journal of Toxicologic Pathology* **21(2)**: 109-13.

Mantis P, Lamb C and Boswood A. 1998. Assessment of the accuracy of thoracic radiography in the diagnosis of canine chronic bronchitis. *Journal of Small Animal Practice* **39**: 518-20.

Mathews K G. 2004. Fungal rhinitis. In: *Textbook of Respiratory Disease in Dogs and Cats*. WB Saunders pp. 284-93.

Maxie G. 2015. *Jubb, Kennedy and Palmer's Pathology of Domestic Animals-E-Book* (Vol. 2). Elsevier Health Sciences.

Mayer P, Laber Gand Walzl H. 1990. Bronchoalveolar lavage in dogs; analysis of proteins and respiratory cells. *Journal of Veterinary Medicine Series A* **37(1-10)**: 392-99.

Mazzone S B. 2005. An overview of the sensory receptors regulating cough. Cough **1**: 1–9.

McConnell F. 2008. Imaging the Nasal Cavity and Paranasal Sinuses. In proceedings: British Small Animal Veterinary Congress 2008.

McCullough S and Brinson J. 1999. Collection and interpretation of respiratory cytology. *Clinical Techniques in Small Animal Practice* **14(4)**: 220-26.

McDougal B J. 1977. Allergic rhinitis: A cause of recurrent epistaxis. *Journal ofthe American Veterinary Medical Association* **171**: 545.

McEntee M C. 2004. Neoplasms of the nasal cavity. In: King L G (Ed.) *Textbook of Respiratory Diseases in Dogs and Cats*. 1st Edn. Philadelphia: WB Saunders, pp. 293–301.

McKiernan B C. 2000. Diagnosis and treatment of canine chronic bronchitis: twenty years of experience. *Veterinary Clinics*: *Small Animal Practice* **30(6)**: 1267-78.

McMillan C J and Taylor S M. 2008. Transtracheal aspiration in the diagnosis of pulmonary blastomycosis (17 cases: 2000–2005). *Canadian Veterinary Journal* **49**: 53-55.

McNiel E A, Ogilvie G K and Powers B E. 1997. Evaluation of prognostic factors for dogs with primary lung tumors: 67 cases (1985-1992). *Journal of American Veterinary Medical Association* **211(11)**: 1422-27.

Mehlhaff C J, Leifer C E and Patnaik A K. 1984. Surgical treatment of pulmonary neoplasia in 15 dogs. *Journal of American Animal Hospital Association* **20**: 799-803.

Miller C J. 2007. Approach to the respiratory patient. *Veterinary Clinics of North America*: *Small Animal Practice* **37(5)**: 861-78.

Mirsadraee M. 2014. Anthracosis of the lungs: etiology, clinical manifestations and diagnosis: A review. *Tanaffos* **13**: 1-13.

Moulton J E, von Tscharner C and Schneider R. 1981. Classification of lung carcinomas in the dog and cat. *Veterinary Pathology* **18(4)**: 513-28.

Murphy K A and Brisson B A. 2006. Evaluation of lung lobe torsion in pugs: 7 cases (1991-2004). *Journal of the American Veterinary MedicalAssociation* **228**: 86-90.

Naranjo C, Dubielzig R R and Friedrichs K R. 2007. Canine ocular histiocytic sarcoma. *Veterinary Ophthalmology* **10**: 179-85.

Nathan L B and Norris C R. 2002. Clinical signs, clinicopathological findings, etiology, and outcome associated with hemoptysis in dogs: 36 cases (1990-1999). *Journal of the American Animal Hospital Association* **38**: 125-33.

Nelson R W and Couto C G. 2014. *Small Animal Internal Medicine-E-Book*. Elsevier Health Sciences.

Nguyen G K. 1989. Aspiration biopsy cytology of benign clear cell (" sugar") tumor of the lung. *Acta Cytologica* **33(4)**: 511-15.

Nolan T and Smith G. 1995. Time series analysis of the prevalence of endoparasitic infections in cats and dogs presented to a veterinary teaching hospital. *Veterinary Parasitology* **59**: 87-96.

Noone K E. 1986. Pulmonary hypersensitivities. *Current Veterinary Therapy IX. Philadelphia: WB Saunders* 285-92.

Norris C R, Griffey S M and Walsh P. 2002. Use of keyhole lung biopsy for diagnosis of interstitial lung diseases in dogs and cats: 13 cases (1998–2001). *Journal of the American Veterinary Medical Association* **221(10)**: 1453-59.

Norris C R, Griffey S M, Samii V F, Christopher M M and Mellema M S. 2001. Comparison of results of thoracic radiography, cytologic evaluation of bronchoalveolar lavage fluid, and histologic evaluation of lung specimens in dogs with respiratory tract disease: 16 cases (1996-2000). *Journal of the American Veterinary Medical Association* **218**: 9, 1456-1461.

Ogilvie G K, Haschek W M and Withrow S J. 1989. Classification of primary lung tumors in dogs: 210 cases (1975-1985). *Journal of American Veterinary Medical Association* **195(1)**: 106-08.

Olgilvie G and Moore A. 2006. Nasal tumors. In: *Managing the Canine Cancer Patient*. Pennsylvania: Veterinary Learning Systems 406–11.

Olsen J D. 2000. Rational antibiotic therapy for respiratory disorders in dogs and cats. *The Veterinary Clinics North American Small Animal Practice* **30**: 1337-55.

Oskouizadeh K, Selk-Ghafari M, Zahraei-Salehi T andDezfolian O. 2011. Isolation of Bordetella bronchiseptica in a dog with tracheal collapse. *Comparative Clinical Pathology* **20(5)**: 527-29.

Padrid P A, Hornof W J, Kurpershoek C J and Cross C E. 1990. Canine Chronic Bronchitis. *Journal of Veterinary Internal Medicine* **4(3)**: 172–180.

Padrid P and Amis T C. 1992. Chronic tracheobronchial disease in the dog. *Veterinary Clinics North America*: *Small Animal Practice* **22(5)**: 1203-29.

Padrid P. 1992. Chronic lower airway disease in the dog and cat. *Problems in Veterinary Medicine* **4(2)**: 320-44.

Pajas A M G A, Lozano R B, Oronan R B, Flores M L S and Gicana K R B. 2015. Abnormalities in lateral thoracic radiographs of domestic dogs with coughing. *Philippine Journal of Veterinary and Animal Sciences* **40(1)**: 15-18.

Pande A, Nasir S and Rueda A M 2012. The incidenceof necrotizing changes in adults with pneumococcalpneumonia. *Clinical Infectious Diseases* **54**: 10-6.

Papich M G, Schooley E K and Reinero C R. 2008. Pharmacokinetics of cetirizine in healthy cats. *American journal of veterinary research* **69(5)**: 670-74.

Patel A K and Nagpal T P. 2014. Comparison of blood absolute eosinophil count and nasal smear eosinophils with symptoms and severity of clinical score in patients of allergic rhinitis. *Indian Journal of Allergy, Asthma and Immunology* **28(2)**: 74.

Patil N R. 2014. 'Use of endoscopy in respiratory diseases of dogs'. M.V.Sc. Thesis, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India.

Patnaik A K. 1989. Canine sinonasal neoplasms: Clinicopathological study of 285 cases. *Journal of the American Animal Hospital Association* **25**: 103.

Peeters D and Clercx C. 2007. Update on canine sinonasal aspergillosis. *Veterinary Clinics of North America*: *Small Animal Practice*, **37(5)**: 901-16.

Peeters D E, McKiernan B C and Weisiger R M. 2000. Quantitative bacterial cultures and cytological examination of bronchoalveolar lavage specimens in dogs. *Journal of Veterinary Internal Medicine* **14**: 534-41.

Peeters D, Day M J and Clercx C. 2005. An immunohistochemical study of canine nasal aspergillosis. *Journal of comparative pathology* **132(4)**: 283-88.

Peltola V T and McCullers J A. 2004. Respiratory viruses predisposing to bacterial infections: Role of neuraminidase. *Pediatric Infectious Disease Journal* **23**: S87–S97.

Peters M A, Teerds K J, Van der Gaag I, De Rooij D G and Van Sluijs F J. 2001. Use of antibodies against LH receptor, 3beta-hydroxysteroid dehydrogenase and vimentin to characterize different types of testicular tumour in dogs. *Reproduction-Cambridge* **121(2)**: 287-96.

Pietra M, Spinella G, Pasquali F, Romagnoli N, Bettini G and Spadari A. 2010. Clinical findings, rhinoscopy and histological evaluation of 54 dogs with chronic nasal disease. *Journal of veterinary science* **11(3)**: 249-55.

Piva S, Zanoni R G, Specchi S, Brunetti B, Florio D and Pietra M. 2010. Chronic rhinitis due to *Streptococcus equi* subspecies *zooepidemicus* in a dog. *Veterinary Record* **67(5)**: 177-78.

Pradeep M. 2014. Application of acute phase proteins as biomarkers in modern veterinary practice. *Indian Journal of Veterinary Animal Science Research* **43(1)**: 1-13.

Priestnall S L, Erles K, Brooks H W, Cardwell J M, Waller A S, Paillot R, Robinson C, Darby A C, Holden M T G and Schonigerm S. 2010. Characterization of pneumonia due to *Streptococcus equi* subsp. *Zooepidemicus* in dogs. *Clinical and Vaccine Immunology* **17(11)**: 1790-796.

Priestnall S L, Mitchell J A, Walker C A, Erles K andBrownlie J. 2014. New and emerging pathogens in canine infectious respiratory disease. *Veterinary pathology* **51(2)**: 492-504.

Proulx A, Hume D Z, Drobatz K J and Reineke E L. 2014. *In vitro* bacterial isolate susceptibility to empirically selected antimicrobials in 111 dogswith bacterial pneumonia. *Journal of Veterinary Emergency and Critical Care* **24**: 194–200.

Quinn P J, Carter M E, Markey B and Carter G R. 1994. *Veterinary Clinical Microbiology*. Wolfe Publication, London UK, pp. 254-58.

Radhakrishnan A, Drobatz K J, Culp W T and King L G. 2007. Community-acquired infectious pneumonia in puppies: 65 cases (1993–2002). *Journal of the American Veterinary Medical Association* **230**: 1493-97.

Raya A I, Fernández-de Marco M, Núñez A, Afonso J C, Cortade L E and Carrasco L. 2006. Endogenous lipid pneumonia in a dog. *Journal of Comparative Pathology* **135(2-3)**: 153-55.

Reece W O. 2004. *Functional Anatomy and Physiology of Domestic animals*, 3rd Edn. pp. 230-68. Blackwell publishing, UK.

Reece W O. 2015. Overview of the respiratory system. *Dukes' physiology of domestic animals* 203.

Reichle J K and Wisner E R. 2000. Non-cardiac thoracic ultrasound in 75 feline and canine patients. *Veterinary Radiology and Ultrasound* **41(2)**: 154–62.

Reinero C R and Cohn L A. 2007. Interstitial lung diseases. *Veterinary Clinics of North America*: *Small Animal Practice* **37(5)**: 937-47.

Rembiesa C and Richardson D J. 2003 Helminth parasites of the house cat, Felis catus, in Connecticut, USA. *Comparative Parasitology* **70**: 115-19.

Rheinwald M, Hartmann K, Hahner M, Wolf G, Straubinger R K and Schulz B. 2014. Antibiotic susceptibility of bacterial isolates from 502 dogs with respiratory signs. *Veterinary Record* **176**: 357.

Rief J S, Bruns C and Lower K S. 1998. Cancer of the nasal cavity and paranasal sinuses and exposure to environmental tobacco smoke in pet dogs. *American journal of epidemiology* **147(5)**: 488-92.

Rizzi T E, Meinkoth J H and Clinkenbeard K D. 2011. Normal hematology of the dog. In: Weiss D J and Wardrop K J (Eds.) *Schalm's Veterinary Hematology*. John Wiley & Sons.

Roedler F S, Pohl S and Oechtering GU. 2013. How does severe brachycephaly affect dog's lives? Results of a structured preoperative owner questionnaire. *Veterinary Journal* **198(3)**: 606–10.

Rose B D and Post T W. 2001. Acid-base physiology. In: *Clinical Physiology of Acid-Base and Electrolyte Disorders*. New York: McGraw-Hill; pp. 299-325.

Roudebush P. 1990. Bacterial infections of the respiratory system. In: Greene C E (Ed.) *Infectious Diseases of the Dog and Cat*. Philadelphia, WB Saunders, pp. 114.

Rozanski E A, Bach J F and ShawS P. 2007. Advances in respiratory therapy. *The Veterinary Clinics North American Small Animal Practice* **37**: 963-74.

Rozanski E and Chan D L. 2005. Approach to the patient with respiratory distress. *VCNA* **35**: 307-17.

Rozanski E. 2014. Canine chronic bronchitis. *Veterinary Clinics*: *Small Animal Practice* **44(1)**: 107-16.

Rudd R G and Richardson D C. 1985. A diagnostic and therapeutic approach to nasal disease in dogs. *Compendium on Continuing Education for the Practising Veterinarian* **7**: 103.

Rudorf H, Barr F J and Lane J G. 2001. The role of ultrasound in the assessment of laryngeal paralysis in the dog. *Veterinary Radiology and Ultrasound* **42**: 338.

Ruslander D. 2011. Multimodality approach to soft tissue sarcomas in dogs and cats (Proceedings).

Saunders H M and Keith D. 2004. Thoracic imaging. In: King L G (Ed.) *Respiratory Disease in Dogs and Cats*. Philadelphia: WB Saunders, pp. 72-92.

Schalm O W, Jain N C and Carrol E J. 1975. *Veterinary Hematology*. 3rd Edn. Lea and Febiger Publisher, Philadelphia, United States of America, pp. 51-81.

Schultz RM, Puchalski S M, Kent M and Moore PF. 2007. Skeletal lesions of histiocytic sarcoma in nineteen dogs. *Veterinary Radiology & Ultrasound* **48**: 539-43.

Schwartz A R, Smith P L and Kashima K H. 1994. Respiratory function of the upper airways. In: Murray J F and Nadel J A (Eds.) *Textbook of Respiratory Medicine*. Vol. 2, 2nd Edn. Philadelphia: WB Saunders; pp. 1451–70.

Schweigert M, Dubecz A, Stadlhuber RJ. 2011. Modern history of surgical management of lung abscess: from Harold Neuhof to current concepts. *Annals of Thoracic Surgery* **92**: 2293-97.

Seo H, Cha S I and Shin K M. 2013. Focal necrotizing pneumonia is a distinct entity from lung abscess. *Respirology***18**: 1095-100.

Sestini P, Rottoli L, Gotti G, Miracco C and Luzi P.1985. Bronchoalveolar lavage diagnosis ofbronchiolo-alveolar carcinoma. *European Journal of Respiratory Diseases* **66**: 55-58.

Sharp C R and Rozanski E A. 2013. Physical examination of the respiratory system. *Topics in Companion Animal Medicine* **28(3)**: 79-85.

Sharp N J H, Harvey C E and Sullivan M. 1991. Canine nasal aspergillosis and penicilliosis. *Compendium on Continuing Education for the Practising Veterinarian* **13**: 41.

Shibata Y, Abe S, Inoue S, Igarashi A, Yamauchi K, Aida Y and Sato K. 2013. Relationship between plasma fibrinogen levels and pulmonary function in the Japanese population: the Takahata study. *International Journal of Medical Sciences* **10(11)**: 1530.

Silverstein D, Greene C, Gregory C, Lucas S and Quandt J. 2000. Pulmonary alveolar proteinosis in a dog. *Journal of Veterinary Internal Medicine* **14(5)**: 546-51.

Skorupski K A, Clifford C A, Paoloni M C, Lara-Garcia A, Barber L, Kent M S and Couto C G. 2007. CCNU for the treatment of dogs with histiocytic sarcoma. *Journal of Veterinary Internal Medicine* **21(1)**: 121-26.

Solter P F, Hoffmann W E, Hungerford L L,Siegel J P, St Denis S H and Dorner J L. 1991. Haptoglobin andCeruloplasmin as determinants ofinflammation in dogs. *American Journal of Veterinary Research* **52**: 1738-42.

Spasov K, Kunovska M and Dimov D. 2018. Lung patterns in the dog–Normal and Pathological. *Tradition and Modernity and Veterinary Medicine*vol. 3, No **1(4)**: 7–14.

Spier AW. 2011. The Coughing Dog. In *Proceedings of the 82nd FVMA Annual Conference, Florida, USA. 83rd_Annual_Conference/Proceedings/The Coughing Dog.***1990**: 207-10.

Spuzak J. 2006. Bronchoalveolar lavage (BAL) examination in diagnosing lower airways diseases in dogs. *Veterinary Medicine* **62(6)**: 678-82.

Steinfeld A, Prenger-Berninghoff E, Bauer N, Weiss R and Moritz A. 2012. Bacterial isolates from the lower respiratory tract of diseased dogs and their current resistance situation. *Veterinary Practice Small Animals* **40**: 309-17.

Sullivan M. 1987. Rhinoscopy: A diagnostic aid? *Journal of Small Animal Practice* **28**: 839.

Sumner C and Rozanski E. 2013. Management of respiratory emergencies in small animals. *The Veterinary Clinics of North America*: *Small Animal Practice* **43(4)**: 799-815.

Sura P A and Durant A M. 2012. Trachea and bronchi. In: *Veterinary Surgery in Small Animals. St. Louis Missouri*: *Elsevier Saunders*, pp. 1734-51.

Suter P F and Lord P F. 1984. Thoracic radiography: a text atlas of thoracic diseases of the dog and cat. Wettswil, Switzerland: P.F. Suter: 517–624.

Sykes J E. 2013. Bacterial bronchopneumonia and pyothorax. In: *Canine and Feline Infectious Diseases*. Elsevier Inc. pp. 847-58.

Tappin S W. 2016. Canine tracheal collapse. *Journal of Small Animal Practice* **57**: 9-17.

Tart K M, Babski D M and Lee J A. 2010. Potential risks, prognostic indicators, and diagnostic and treatment modalities affecting survival in dogs with presumptive aspiration pneumonia: 125 cases (2005-2008). *The Journal of Veterinary Emergency and Critical Care* **20(3)**: 319-29.

Tasker S, Knottenbelt C M, Munro E A, Stonehewer J, Simpson J W and Mackin A J. 1999. Aetiology and diagnosis of persistent nasal disease in the dog: A retrospective study of 42 cases. *Journal of Small Animal Practice* **40**: 473-78.

Taubert A, Pantchev N, Vrhovec M G, Bauer C and Hermosilla C. 2009. Lungworm infections (*Angiostrongylus vasorum, Crenosoma vulpis, Aelurostrongylus abstrusus*) in dogs and cats in Germany and Denmark in 2003–2007. *Veterinary Parasitology* **159(2)**: 175-80.

Taylor S M. 2015. *Small Animal Clinical Techniques-E-Book*. Elsevier Health Sciences.

Tenwolde A C, Johnson L R and Hunt G B. 2010. The role of bronchoscopy in foreign body removal in dogs and cats: 37 cases (2000-2008). *Journal of Veterinary Internal Medicine* **24(5)**: 1063-68.

Thrall D E and Widmer W R. 1998. Radiation physics, radiation protection, and darkroom theory. In: Thrall D E (Ed.) *Textbook of Diagnostic Veterinary Radiology*. 3rd Edn. Philadelphia: WB Saunders; pp. 1-19.

Thurlbeck W M. 1988. Chronic airflow obstruction. In: Thurlbeck W M (Ed.) *Pathology of the Lung*. New York: Thieme Medical Publishers, pp. 519-75.

Travis W D, Costabel U, Hansell D M, King T E, Jr Lynch D A, Nicholson A G, Ryerson C J, Ryu J H, Selman M and Wells AU. 2013. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *American Journal of Respiratory and Critical Care Medicine* **188**: 733-48.

Travis W D, Hunninghake G, King T E, Jr Lynch D A, Colby T V, Galvin J R, Brown K K, Chung M P, Cordier J F and du Bois R M. 2008. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. *American Journal of Respiratory and Critical Care Medicine* **177**: 1338-47.

Travis W D, King T E and Bateman E D. 2002. American thoracic society/european respiratory society international multidisciplinary consensus classification of the idiopathic interstitial pneumonias, general principles and recommendations. *American Journal of Respiratory and Critical Care Medicine* **165**: 277-304.

Turek M M and Lana SE. 2007. Canine nasosinal tumors. In: Withrow S J and VailD M (Ed.) *Small Animal Clinical Oncology*. 4th Edn. Philadelphia: WB Saunders, 525–39.

Varshney J andVarshney H. 2015. Allergic rhinitis: An overview. *Indian Journal of Otolaryngology and Head & Neck Surgery* **67(2)**: 143-49.

Vegad J L and Katiyar A K. 2004. *Textbook of Veterinary Systemic Pathology*. 1st Edn. International Book Distributing Co. Lucknow, India.

Vieson M D, Piñeyro Pand Leroith T. 2012. A review of the pathology and treatment of canine respiratory infections. *Veterinary Medicine* **3**: 25-39.

Viitanen S J, Laurila H P, Lilja-Maula L I, Melamies M A, Rantala M and Rajamäki M M. 2014. Serum C-reactive protein as a diagnostic biomarker in dogs with bacterial respiratory diseases. *Journal of Veterinary Internal Medicine* **28(1)**: 84-91.

West J B. 1990. Diffusion – how gas gets across the alveolar wall. In: Satterfield T S (Ed.) *Respiratory Physiology*. 4th Edn. Baltimore: Williams and Wilkins; 1990. p. 1–30.

White R A S and Williams J M. 1994. Tracheal collapse in the dog-is there really a role for surgery? A survey of 100 cases. *Journal of Small Animal Practice* **35(4)**: 191-96.

Widdicombe J G. 2003. A brief overview of the mechanisms of cough. In: Chung K F, Widdicombe J, Boushey H A (Eds.) *Cough*: *Causes, Mechanisms and Therapy*. Oxford: Blackwell, pp. 17-23.

Williams M, Olver Cand Thrall M A. 2006. Transtracheal wash from a puppy with respiratory disease. *Veterinary Clinical Pathology* **35(4)**: 471-73.

Wilson D W and Dungworth D L. 2002. Tumors of the respiratory tract. *Tumors in Domestic Animals* **4**: 365-400.

Windsor R C and Johnson L R. 2006. Canine chronic inflammatory rhinitis. *Clinical Techniques in Small Animal Practice* **21(2)**: 76-81.

Withrow S J. 1989. Tumors of the respiratory system. In: Withrow S J and MacEwen E G (Eds.) *Clinical Veterinary Oncology*. Philadelphia, JB Lippincott, p. 215.

Withrow S J. 1996 Tumors of the respiratory system. In: Withrow S J and MacEwen E G (Eds.) *Small Animal Clinical Oncology*, 2nd Edn. W.B. Saunders, Philadelphia; pp. 268-86.

Withrow S J. 2007. Tumors of the respiratory system: lung cancer. In: Withrow S J, Vail D M (Eds.) *Withrow and MacEwen's Small Animal Clinical Oncology*. 4th Edn. Philadelphia, Pa: WB Saunders Co.; pp. 517-25.

Workman H C, Bailiff N L and Jang S S. 2008. *Capnocytophaga cynodegmi* in a Rottweiler dog with severe bronchitis and foreign-body pneumonia. *Journal of Clinical Microbiology* **46(12)**: 4099-103.

Wray J. 2006. Understanding and treating canine infectious tracheobronchitis. *Veterinary Times* **36(10)**: 14-16.

Yazbeck M F, Dahdel M., Kalra A, Browne A S and Pratter M R. 2014. Lung abscess: update on microbiology and management. *American Journal of Therapeutics* **21(3)**: 217-21.

Yen C C, Tang R B and Chen S J. 2004. Pediatric lung abscess: a retrospective review of 23 cases. *Journal of Microbiology, Immunology and Infection* **37**: 45-9.

Zapryanova, Mircheva T and Denev S A. 2013. Plasma protein profiles and fibrinogen concentrations in dogs with experimentally induced Staphylococcus aureus infection. *Revue de Medecine Veterinaire* **64**: 150-55.

Zhu B Y, Johnson L R and Vernau W. 2015. Tracheobronchial brush cytology and bronchoalveolar lavage in dogs and cats with chronic cough: 45 cases (2012–2014). *Journal of Veterinary Internal Medicine* **29(2)**: 526-32.

Zonderland J L, Störk C K, Saunders J H, Hamaide A J, Balligand M H and Clercx C M. 2002. Intranasal infusion of enilconazole for treatment of sinonasal aspergillosis in dogs. *Journal of the American Veterinary Medical Association* **221(10)**: 1421-25.

VITA

Name of the student	:	Jasleen Kaur
Father's name	:	Sh. Jatinder Pal Singh
Mother's name	:	Smt. Balvinder Kaur
Nationality	:	Indian
Date of birth	:	19-08-1994
Permanent home address	:	Near Bus Stand Mahal Kalan Distt. & Teh. Barnala, Punjab -148104

EDUCATIONAL QUALIFICATIONS

Bachelor's degree	:	B.V.Sc. & A.H.
University	:	Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana
Year of award	:	2017
OGPA/OCPA/% marks	:	7.810/10.00
Master's degree	:	M.V.Sc.
OGPA/OCPA/% marks	:	8.69/10.00