PATHOMORPHOLOGICAL STUDIES ON SPONTANEOUS LUNG LESIONS IN SLAUGHTERED SHEEP

By

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Certificate

Ms. M. AMARAVATHI *has satisfactorily prosecuted the course of research and that the thesis entitled* ***" PATHOMORPHOLOGICAL STUDIES ON SPONTANEOUS LUNG LESIONS IN SLAUGHTERED SHEEP"*** *submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.*

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This is to certify that the thesis entitled ***"PATHOMORHOLOGICAL STUDIES ON SPONTANEOUS LUNG LESIONS IN SLAUGHTERED SHEEP"*** *submitted in partial fulfillment of the requirements for the degree of* ***"MASTER OF VETERINARY SCIENCE"*** *of the Sri Venkateswara Veterinary University, Tirupati, is a record of the bonafide research work carried out by* ***Ms. M. AMARAVATHI*** *under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.*

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigations have been duly acknowledged by the author of the thesis.

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I ***Ms. M. AMARAVATHI*** *hereby declare that the thesis entitled* ***"PATHOMORPHOLOGICAL STUDIES ON SPONTANEOUS LUNG LESIONS IN SLAUGHTERED SHEEP"*** *submitted to Sri Venkateswara Veterinary University, Tirupati for the degree of MASTER OF VETERINARY SCIENCE is the result of original research work done by me. I also declare that the materials contained in this thesis have not been published earlier.*

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LIST OF ABBREVIATIONS

BHI	-	Brain Heart Infusion
cm	-	Centimetre
DIC	-	Disseminated Intravascular Coagulation
EMB	-	Eosin Methylene Blue
Fig	-	Figure
MSA	-	Mannitol Salt Agar
mm	-	Millimetre
OPA	-	Ovine Pulmonary Adenocarcinoma
PPR	-	Peste des Petits Ruminants
TEM	-	Transmission Electron Microscopy
%	-	Per cent

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ABSTRACT

Sheep are prone to various infectious and non-infectious diseases that cause significant lesions in the lungs of which pneumonia is a major health problem. Hence, the present study was undertaken in slaughtered sheep to know the incidence and to describe the lesions noticed in the lungs and also to isolate various bacterial agents in possible cases.

A total of 988 sheep of either sex and of different age groups from slaughter houses, postmortems and from field mortalities were screened for gross abnormalities in the lungs and representative samples were collected for further studies.

Among 988 lungs screened, 187 (18.93%) lungs revealed definite lesions on gross and histopathological examination that were broadly grouped into abnormalities of inflation 22 (11.76%), circulatory disturbances 37 (19.79%), inflammatory conditions 116 (62.03%), parasitic conditions 7 (3.74%), neoplastic conditions 2 (1.07%) and miscellaneous conditions in 3 (1.61%) cases.

Abnormalities of inflation comprised of pulmonary emphysema (9.09%) and atelectasis (2.67%). In emphysema, lungs showed pale areas that projected from the surface grossly and giant alveoli microscopically. In atelectasis, dark and depressed areas were noticed grossly and slit-like alveoli microscopically.

Circulatory disturbances encountered in the present study included congestion and haemorrhage (10.16%) and edema (9.63%). Congestion and haemorrhage were evidenced by red, patchy areas and haemorrhages grossly that showed engorged blood vessels and haemorrhages in the alveoli microscopically. In edema, frothy fluid on cut section grossly and eosinophilic fluid in the alveoli microscopically were noticed.

The incidence of the inflammatory conditions was 62.03% in the present study which constituted various types of pneumonias and pleuritis.

Pneumonic changes accounted for 58.82% of lesions that were grouped into bronchopneumonia (56.15%) and interstitial pneumonia (2.67%). Bronchopneumonia was further subdivided into suppurative and fibrinous types. In suppurative bronchopneumonia, cranio-ventral consolidation was seen with mucopurulent exudation on cut section. Chronic cases revealed multiple abscesses. Microscopically, the lumen of the airways was filled with inflammatory exudates comprising of polymorphs in acute cases and mononuclear cells and abscesses in chronic cases. Fibrinous bronchopneumonia was characterized by cranio-ventral consolidation, fibrinous exudation and pleural thickening of lungs besides adhesions to thorax in two cases. Microscopically, the lesion revealed fibrino-cellular exudates in alveoli, bronchi and bronchioles. Interstitial pneumonia was characterized by lungs with rib impressions on the surface grossly and thickened alveolar septa with foetalization of alveolar epithelium microscopically.

Pleuritis was observed in 6 (3.21%) cases characterized grossly by excessive thickening and microscopically by fibrosis.

An incidence of 3.74% of parasitic conditions that included pulmonary hydatidosis and fasciolosis in the present study. Grossly, hydatidosis revealed a single unilocular cyst deeply embedded in the parenchyma that revealed brood capsules on cut section. Microscopically, an eosinophilic, acellular thick laminated layer lined by degenerated germinal epithelium and slight eosinophilic fluid was seen enclosed by an inflammatory zone and fibrous connective tissue. Pulmonary fasciolosis was characterized grossly by haemorrhagic areas that showed flukes in the parenchyma on cut section. Microscopically, sections of the fluke were observed in the alveolar and bronchial lumen surrounded by mononuclear cells, eosinophils and a few polymorphs.

Ovine Pulmonary Adenocarcinoma was observed in 2 cases (1.07%) that showed grayish white lungs grossly and meaty appearance on cut section. Histologically, papillary growths of tumor cells both in alveoli and bronchi into the lumen along with infiltration of mononuclears and neutrophils and variable amounts of fibrous connective tissue were noticed.

In the present study, lung lesions with sheep pox (1.07%) and PPR (0.54%) were included under miscellaneous conditions.

In sheep pox, grossly, lungs revealed numerous circular, discrete to coalescing pock nodules of varying sizes distributed throughout the parenchyma. Histologically, proliferative alveolitis and bronchiolitis, focal areas of necrosis and presence of sheep pox cells were observed. Ultrastructural changes by TEM showed macrophages with indented nucleus, distorted endoplasmic reticulum and electron dense immature virus-like particles in the cytoplasm. Nuclear changes included karyorrhexis and margination of chromatin.

In PPR, grossly, lungs revealed patchy areas of consolidation in apical and diaphragmatic lobes and froth filled trachea on cut section. Numerous giant cells were noticed in the alveoli and bronchi microscopically.

In the present study, swabs from 52 suitable lung samples with pneumonic lesions were subjected for bacterial isolation. In 50 lung samples, 8 isolates of bacteria were obtained viz. *S. aureus, Klebsiella spp., E. coli, Enterobacter spp., Edwardsiella spp., P. aeruginosa, Pasteurella spp. and Serratia spp.* The major pathogen isolated from lungs was *S. aureus*.

The present investigation revealed various lung lesions in slaughtered sheep with an incidence of 58.82%, of these, bronchopneumonia was the predominant lesion causing losses in sheep.

CHAPTER - I

INTRODUCTION

In an agriculture based country like India, livestock plays a vital role in the upliftment of economically weaker sections of the society. Among various livestock sectors, sheep husbandry with multifaceted utility contributes sustainable and dependable income to the shepherds and sheep entrepreneurs by providing mutton, wool, manure and hides. India, with an estimated sheep population of 75 million (FAO, 2012) ranks third in the world next to China and Australia. About five million households in the country are engaged solely in rearing small ruminants and allied activities (Rao, 2008). Also, sheep rearing holds considerable potential for commercialization and is also one of the major contributors to meat production in the country.

The respiratory system in general constitutes the most extensive surface and gets exposed directly to the environment. Any sudden change in the environment precipitates the infection by interfering with the local defence and rendering the system more susceptible to infections.Various infectious and non - infectious agents can damage lungs and produce significant lesions.

Under field conditions, pneumonia in sheep is a major problem and has a multifactorial etiology that includes bacteria, viruses, parasites etc. acting either alone or in concert. Among infectious agents, bacteria like *Pasteurella spp.,* viruses like PPR and parasites like *Echinococcus spp.* are the most commonly encountered etiological agents of pneumonia in sheep. *Pasteurella* organisms are the commensals of respiratory tract and with prior predisposition cause the most

common enzootic disease of sheep of all ages leading to fibrinous bronchopneumonia and mortality. *Staphylococci* another commensal seen in the mucosa of upper respiratory tract causes suppurative lesions in the lung whereas septicemic organisms like *E.coli* have special attributes for resisting host defence mechanism and they invade blood stream causing systemic lesions in lungs and death due to endotoxic shock. Opportunistic pathogens like *Pseudomonas spp.* produce various toxins and enzymes which promote tissue invasion and damage aided by antiphagocytic properties (Quinn *et al.*, 2002). PPR is a severe infectious and contagious viral disease of sheep causing respiratory distress, pneumonia and death whereas sheep pox, also a highly contagious, host specific, often fatal and economically important viral disease of sheep is characterized by systemic illness with formation of typical pox nodules in the lungs (Singh *et al.*, 2007). Parasites such as lung worms, either large or small, cause reduced weight gain, respiratory problems such as bronchopneumonia and even mortality (Soulsby, 2005) and hydatid cysts caused by *E.granulosus* are responsible for organ condemnation and zoonotic implication. Non-infectious conditions like abnormalities of inflation and circulatory disturbances are also seen commonly in the lungs. Further, primary neoplastic conditions in the lung like Jaagsiekte, a malignant tumor of epithelial origin caused by an exogenous retrovirus is also seen though metastatic lung tumors are very rare in sheep.

Diseases of the respiratory system are a threat to small ruminant farming causing high morbidity, mortality and decreased production leading to huge economic losses. A detailed study on the nature and type of pulmonary lesions

contributes to better understanding of the pathogenic mechanisms involved in many of the respiratory diseases that helps in the development of diagnostic methods and treatment. The literature available on ovine pulmonary lesions in the area under study is scanty and requires further exploration.

Hence, keeping this in view, the present study was planned with the following objectives:

1. To study the incidence of various spontaneously occurring lung lesions in sheep in selected places of Andhra Pradesh.
2. To describe the gross and histopathological changes in different pathological lesions of lungs.
3. To isolate various bacterial agents causing lesions in lungs of sheep from possible cases.

CHAPTER - II
REVIEW OF LITERATURE

The literature available on spontaneous lung lesions in sheep with regard to incidence, pathomorphological changes and bacteriological studies is reviewed briefly.

2.1 INCIDENCE

Rahman and Iyer (1979) examined 790 slaughtered sheep aged between 3-5 years at Military Butchery, Bareilly from September 1976 to May 1977 and recorded 209 cases (26.45%) of various pathological manifestations in the lungs whereas Chattopadhyay *et al.,* (1986) investigated 675 lungs with apparent gross lesions of which 392 cases (58.07%) showed significant microscopic lesions.

An incidence of 8.62% was recorded by Kamil and Parihar (1990) in an investigation on pathological abnormalities in sheep lungs.

In an abattoir study conducted by Beytut *et al.,* (2002), a total of 145 (5.84%) lungs revealed gross pneumonic changes out of 2482 sheep examined.

Kumar *et al.,* (2005) screened 991 sheep lungs at Chennai, Avikanagar, Mecheri and Bareilly and recorded specific and non-specific gross pathological changes in 175 (17.66%) lungs.

An abattoir survey on sheep slaughtered during 2005-2007 in Arusha municipality, Tanzania by Mellau *et al.,* (2010) revealed pathological lesions in 4668 (7.58%) out of 61551 lungs examined.

Belkhiri *et al.,* (2012) recorded a high frequency of ovine pulmonary lesions in 803 (28.05%) cases out of 2863 inspected over a period of two years in Tiaret slaughter house, West Algeria.

Dar *et al.,* (2013a) screened 1385 sheep slaughtered at different abattoirs in Kashmir Valley and recorded an overall prevalence of 24.18% (335 cases) in the lungs with at least one type of gross pathological lesion.

Priyadarshi *et al.,* (2013) examined 1080 slaughtered sheep for spontaneous pulmonary lesions and observed grossly appreciable lesions in 165 (15.28%) sheep at Palanpur of North Gujarat whereas Belkhiri *et al.,* (2014) recorded pathological lesions in 799 lungs (27.90%) out of 2863 ovine lungs examined at Batna and Tiaret Slaughter houses, Algeria.

2.2. PATHOMORPHOLOGY OF LUNG LESIONS

2.2.1 ABNORMALITIES OF INFLATION

Sriraman and Rama rao (1980) recorded 584 cases (17.35%) of emphysema and 34 cases (1.01%) of atelectasis out of 3364 sheep examined in different organized sheep farms of Andhra Pradesh from 1960 to 1979.

Chattopadhyay *et al.,* (1986) recorded emphysema, acute congestion and oedema in 283 cases (41.92%) out of 675 lungs from local abattoirs examined whereas Kamil and Parihar (1990) examined 342 sheep lungs and recorded 20 cases (5.84%) of emphysema.

Kumar *et al.,* (2005) examined 175 sheep lungs with gross pathomorphological lesions and observed 9 cases (5.14%) of emphysema and atelectasis. Grossly, the emphysematous areas appeared as sharply defined foci involving one or more lobes slightly projecting from the neighbouring areas. Distended alveoli and ruptured interalveolar septae were noticed microscopically. The atelectatic foci were depressed from the neighbouring areas grossly and shrunken alveoli with narrow lumen microscopically.

Dar *et al.*, (2013a) recorded emphysema and atelectasis in 18 cases (5.37%) out of 335 sheep lungs examined at slaughter in Kashmir Valley, India.

Priyadarshi *et al.,* (2013) reported pulmonary emphysema in 23 cases (13.94%) out of 165 sheep lung lesions examined. Grossly, lungs were voluminous, pale and puffy with imprints of ribs and histopathologically there was dilatation and distension of alveoli with occasional rupture of alveolar wall along with compensatory atelectasis.

An abattoir survey to determine the major causes of organs and carcasses condemnation in sheep in Central Ethiopia was conducted by Regassa *et al.,* (2013) who reported 98 cases (21.72%) of emphysema out of 451 lungs examined.

Belkhiri *et al.,* (2014) examined 2863 ovine lungs and recorded 55 cases of pulmonary emphysema and 73 cases of atelectasis with an incidence of 1.92% and 2.55% respectively.

2.2.2 CIRCULATORY DISTURBANCES

Sriraman and Rama rao (1980) recorded 738 cases (21.94%) of congestion and edema and 181 cases (5.38%) of pulmonary edema out of 3364 sheep examined in different organized sheep farms of Andhra Pradesh during 1960-1979.

Kumar *et al.,* (2005) encountered 5 cases (2.85%) of pulmonary hemorrhages and congestion and 8 cases (4.57%) of pulmonary edema out of 175 slaughtered sheep lungs examined. Macroscopically, pulmonary haemorrhage and congestion was characterized by patchy areas of haemorrhage and congestion and microscopically, engorgement of blood vessels in interalveolar and interlobular septae and peribronchial areas. Also alveoli and interalveolar septae were partly or completely flooded with erythrocytes. In pulmonary edema, lungs were oedematous, voluminous, heavy and doughy in consistency on gross examination whereas pinkish proteinaceous transudate was observed in interalveolar septae and

alveolar lumen along with alveolar emphysema in the adjoining areas and serous fluid in bronchi and bronchioles on microscopic examination.

Dar *et al.*, (2013a) found at least one gross pathological lesion in 335 out of 1385 sheep lungs screened in different abattoirs of Kashmir Valley out of which 11 cases (3.28%) of hemorrhages, 22 cases (6.56%) of congestion and 17 cases (5.07%) of froth was recorded.

Priyadarshi *et al.*, (2013) encountered 24 cases (14.55%) of pulmonary hemorrhages and 18 cases (10.90%) of pulmonary edema out of 165 sheep lung lesions examined. Grossly, patchy distribution of dark reddish areas of petechial to ecchymotic hemorrhages of varying size were observed in all the lobes and microscopically diffuse accumulation of erythrocytes in the alveoli and interstitium were noticed. In pulmonary edema, grossly the lungs were heavy, wet and did not collapse. The sub-pleural and interstitial tissues were edematous and on cut section white foamy fluid escaped upon pressure. Microscopically, homogenous eosinophilic protein rich exudates filling the alveoli were noticed.

An abattoir survey was conducted from 2009 to 2010 to determine the major causes of organs and carcasses condemnation in Central Ethiopia by Regassa *et al.*, (2013) who recorded 4 cases (0.89%) of haemorrhage out of 451sheep lungs examined.

Belkhiri *et al.*, (2014) examined 2863 ovine lungs and recorded 7.30% of pulmonary congestion and 4.16% of pulmonary edema at Batna and Tiaret Slaughter houses, Algeria.

2.2.3. INFLAMMATORY CONDITIONS

2.2.3.1 Pneumonia

Pneumonias in animals are classified on the basis of etiology, type of exudates, distribution of lesions, morphologic features and geographical distribution etc.

2.2.3.1.1 Incidence of pneumonia

Bhagwan and Singh (1972) observed 35 cases (30.97%) of pneumonia out of 113 sheep lungs examined at slaughter from October 1970 to March 1971 and categorized them under exudative and non - exudative types. Among exudative type, acute suppurative pneumonia was seen in 31 cases (88.5%) while under non-exudative type interstitial pneumonia was recorded in 2 cases (5.71%), verminous pneumonia in one (2.85%) case and maedi like lesions in one (2.85%) case only.

Among 135 lungs of slaughtered sheep examined Rahman and Iyer (1979) noticed 32 cases (23.70%) of bacterial pneumonia, 11 cases (8.15%) of viral pneumonia, 3 cases (2.22%) of Chlamydial pneumonia, 7 cases (5.19%) of fungal pneumonia and 73 cases (54.07%) of parasitic pneumonia.

Sriraman and Rama rao (1980) recorded 1643 cases (48.84%) of pneumonia out of 3364 sheep examined in different organized sheep farms of Andhra Pradesh during 1960 - 1979.

In an investigation to categorize pneumonia, Chattopadhyay *et al.,* (1986) observed acute bronchopneumonia in 125 cases (18.52%), suppurative necrotizing pneumonia in 120 cases (17.78%), fibrinous bronchopneumonia with *Pasteurella* infection in 84 cases (12.44%), interstitial pneumonia in 10 cases (1.48%), verminous pneumonia in 5 cases (0.74%), mycotic pneumonia in 3 cases (0.44%) and aspiratory pneumonia in 1 case (0.15%) out of 675 sheep lungs examined at local abattoirs.

Kamil and Parihar (1990) recorded 91 cases (26.60%) of acute bronchopneumonia, 15 cases (4.38%) of subacute bronchopneumonia, 16 cases (4.69%) of suppurative pneumonia, 10 cases (2.92%) of interstitial pneumonia and 8 cases (2.34%) of chronic fibrosing pneumonia on examination of 342 slaughtered sheep lungs.

In an abattoir study on sheep lungs, 145 (5.84%) lungs with pneumonia out of 2482 examined were classified microscopically as catarrhal purulent bronchopneumonia (1.69%), necrotic bronchopneumonia with abscess (1.32%), interstitial pneumonia (0.76%) and fibrinous pneumonia (0.24%) by Beytut *et al.,* (2002).

Kumar *et al.,* (2005) observed acute bronchopneumonia in 46 cases (26.28%), fibrinous bronchopneumonia in 24 cases (13.71%), chronic bronchopneumonia in 48 cases (27.42%), viral pneumonia in 31 cases (17.71%) and bronchioalveolar carcinoma in 1 case (0.57%) out of 175 sheep lungs that showed gross lesions.

Oruc (2006) classified 262 ovine pneumonic lungs histologically as acute-catarrhal (46 cases), catarrhal - purulent (38 cases), purulent-necrotic (25 cases), fibrinous (70 cases), fibrinous – necrotic (15 cases), interstitial (48 cases) and verminous (20 cases) with an incidence of 17.56, 14.50, 9.54, 26.72, 5.73, 18.32 and 7.63 respectively.

Inspection of 10129 sheep lungs condemned at Ziaran abattoir between 2005- 2006 revealed pneumonia in 257 cases (2.54%) which were categorized as purulent bronchopneumonia (0.51%), purulent interstitial bronchopneumonia (0.17%), purulent pleuritis/ pleuropneumonia (0.07%), purulent fibrinous bronchopneumonia (0.04%), purulent pneumonia (0.09%) and progressive pneumonia (0.01%) by Ezzi *et al.*, (2007).

Mellau *et al.,* (2010) noticed pneumonia in 1495 (32.02%) out of 4668 sheep lungs examined.

Yesuf *et al.,* (2012) recorded bronchopneumonia, interstitial pneumonia, combination of the two and granulomatous pneumonia in 41 (56.93%), 17

(23.61%), 12 (16.67%) cases and 2 (2.78%) cases respectively in a study conducted on 72 pneumonic lungs of slaughtered sheep.

Azizi *et al.,* (2013) found 42 pneumonic lungs out of 1000 slaughtered native sheep examined and classified them histopathologically as suppurative bronchopneumonia in 19 cases (45.24%), fibrinous pneumonia in 3 cases (7.14%), interstitial pneumonia in 9 cases (21.42%), bronchointerstitial pneumonia in 5 cases (11.90%) and embolic pneumonia in 1 case (2.38%).

Based on histopathology, the lesions observed in 335 sheep lungs screened were classified as acute bronchopneumonia in 121 cases (36.11%), fibrinous pneumonia in 32 cases (9.55%), chronic bronchopneumonia in 51 cases (15.22%), suppurative pneumonia in 36 cases (10.74%), interstitial pneumonia in 19 cases (5.67%), verminous pneumonia in 10 cases (2.98%) and lung abscesses in 27 cases (8.05%) by Dar *et al.,* (2013a).

Dar *et al.,* (2013b) examined 257 lungs of slaughtered sheep and noticed 127 cases (49.41%) of acute bronchopneumonia, 49 cases (19.06%) of chronic bronchopneumonia, 30 cases (11.67%) of fibrinous bronchopneumonia, 33 cases (12.94%) of suppurative bronchopneumonia and 18 cases (7.0%) of interstitial pneumonia.

Priyadarshi *et al.,* (2013) recorded 98 cases (59.39%) of pneumonia out of 165 sheep lungs examined, of which 9 cases (5.45%) were of acute suppurative

bronchopneumonia, one case (0.61%) of acute fibrinous bronchopneumonia, 58 cases (35.15%) of non suppurative pneumonia and 9 cases (5.45%) of interstitial pneumonia.

Regassa *et al.,* (2013) recorded 58 cases (12.86%) of pneumonia out of 451 sheep lungs examined in an abattoir survey conducted during 2009-2010 to determine the major causes of organs and carcasses condemnation in Central Ethiopia.

2.2.3.1.2 Common types of pneumonia

2.2.3.1.2.1 Bronchopneumonia

Chronic pneumonia was described microscopically by Al-Sultan and Zubaidy (1978) in slaughtered sheep. Thickened alveolar septa, alveolar atelectasis, intraluminal aggregates of neutrophils, activated intrapulmonary lymphoid tissue and cuffing around the blood vessels and lymphatics were noticed. Bronchiolar epithelium and bronchial glands showed hyperplasia.

Sriraman and Rama rao (1980) recorded 1483 cases (90.27%) of bronchopneumonia, 11 cases (0.67%) of chronic pneumonia and 55 cases (3.34%) of pulmonary abscess out of 1643 sheep in different organized sheep farms of Andhra Pradesh during 1960 - 1979.

In an abattoir and farm study involving 175 sheep lungs with gross lesions, Kumar *et al.*, (2005) recorded 118 cases (67.42%) of bronchopneumonia which were classified into acute and chronic types. Acute bronchopneumonia was seen in 46 cases (26.28%) characterized grossly by patchy to diffuse areas of consolidation with reddish brown to grayish red discoloration and oozing of straw yellow colored fluid from bronchi and bronchioles on cut section. Histologically, pulmonary edema, infiltration with a few erythrocytes, neutrophils and mononuclear cells along with hyperplasia of epithelial cells in bronchi, bronchioles and thickened interalveolar and inter lobular septa were noticed. Chronic bronchopneumonia was seen in 48 cases (27.42%) with severe infiltration of mononuclear cells in the bronchi, bronchioles and in the alveolar lumen, marked hyperplasia of bronchi, bronchiolar epithelium and peribronchiolar lymphoid tissue.

Ezzi *et al.,* (2007) stated that gross pneumonic lesions were restricted to cranio-ventral portions and were seen as raised and consolidated areas on the affected lungs in a study conducted on slaughtered sheep. Histologically, scattered polynuclear cells and whorls of neutrophil cells (oat shaped leukocyte) within the alveoli were described in purulent pneumonia.

Grossly severe consolidation of the lungs with haemorrhages was seen in bronchopneumonia by Zhang *et al.,* (2009). Histopathological examination showed expansion of pleural connective tissue due to increased amounts of fibrous connective tissue heavily infiltrated by mixed mononuclear cells including lymphocytes, histiocytes, a few plasma cells and neutrophils.

2.2.3.1.2.1.1 Suppurative bronchopneumonia

Grossly lungs with acute suppurative pneumonia showed varying degrees of consolidation with reddish-blue to red or gray to grayish white coloration, congestion and hepatization and the cut surface was granular. Histologically, the alveoli contained thick, acidophilic, PAS- positive exudate with neutrophils and mononuclear cells in most of the cases. In few cases fibrinous exudate admixed largely with polymorphs was observed. In some cases, the alveolar lumen was filled with large number of neutrophils mixed with large lymphocytes and a few plasma cells (Bhagwan and Singh, 1972).

Sriraman and Rama rao (1980) recorded 88 cases (5.36%) of suppurative pneumonia out of 1643 sheep of various breeds in different districts of Andhra Pradesh during 1960 to 1979.

Oruc (2006) recorded 46 cases (17.56%) of acute catarrhal bronchopneumonia, 38 cases (14.50%) of catarrhal purulent bronchopneumonia and 25 cases (9.54%) of purulent necrotic bronchopneumonia out of 262 pneumonic sheep lungs investigated. Grossly, in acute catarrhal bronchopneumonia there was hyperemia and linear consolidated areas on cranial lobes and microscopically, capillary hyperemia, edema and a few neutrophilic exudations in the alveolar and bronchiolar lumina were seen. In catarrhal purulent bronchopneumonia, grossly, there was a multifocal and severely consolidated area on the cranial lobes and microscopically, moderate neutrophilic exudations in

alveolar and bronchiolar lumina, alveolar macrophages within the alveoli and desquamation of bronchial epithelium. In purulent necrotic bronchopneumonia grossly there was more extensive consolidation and necrotic changes and histologically large amounts of neutrophils and necrosis along with severe desquamation of bronchial and bronchiolar epithelium were found.

Dar *et al.,* (2012b) recorded 275 cases (18.90%) of lung abscesses out of 1455 lungs of sheep slaughtered in different organized abattoirs in Kashmir Valley. Macroscopically, abscesses in one or more lung lobes, either single or multiple, from pea to walnut sized were observed. Histologically, the abscesses were characterized by focal areas of suppuration with central caseo-necrotic core surrounded by pyogenic membrane with infiltration of polymorphonuclear cells and few mononuclear cells and macrophages. In chronic abscesses, fibrous tissue proliferation around the pyogenic membrane was noticed.

Yesuf *et al.,* (2012) recorded purulent bronchopneumonia in 21 cases (29.16%) out of 72 pneumonic sheep lungs by histopathological examination and observed exudates containing abundant neutrophils and few macrophages in the alveoli, bronchioles and bronchi. Associated bronchial lymphoid tissue revealed congestion, hemorrhages and hyperplasia.

Azizi *et al.,* (2013) recorded 19 cases (45.24%) of suppurative bronchopneumonia and 5 cases (11.90%) of lung abscesses out of 42 pneumonic sheep lungs examined and considered it as the most common type. Grossly, dark

red irregular areas of consolidation with lobular pattern in cranial, middle and accessory lobes was noticed that showed moist surface with leakage of purulent exudate from small airways on section. In acute cases, numerous small, greenish-yellow purulent foci were scattered throughout the affected cranio-ventral lobes. Microscopically, neutrophil-rich exudates in the alveolar spaces, bronchi, and bronchioles were seen. In chronic cases, grossly, abscesses of different sizes surrounded by fibrous capsule were observed in affected lobes and microscopically thickening of alveolar walls with infiltration of lymphocytes and macrophages, cellular infiltration in the alveolar lumen comprising macrophages, lymphocytes and neutrophils; hyperplasia of goblet cells of bronchiolar epithelium and extensive peribronchiolar lymphoid accumulation were seen. Pulmonary abscesses observed were 2-10 cm in diameter, either single or multiple and seen in one or more lobes. Some of the abscesses were very large occupying an entire pulmonary lobe and contained viscous white-yellow odorless pus. Chronic abscesses were surrounded by reactive fibrous walls.

Dar *et al.*, (2013b) observed patchy to diffuse, reddish brown to grayish red areas of consolidation and hepatization in apical, cardiac and antero-ventral portions of diaphragmatic lobes with oozing of straw-yellow colored fluid from bronchi and bronchioles on cut section in acute bronchopneumonia. Microscopic lesions observed were serous exudates in the bronchiolar lumen and infiltration of neutrophils around the bronchi and bronchioles and proliferation of alveolar septal cells. In chronic bronchopneumonia, the lungs were firm, hard, slightly shrunken and adhered to diaphragm and thoracic wall with pleural thickening. Histologically

focal to diffuse areas of fibrosis around bronchi, bronchioles, alveoli and blood vessels, varying degrees of mononuclear cell infiltration and pleural thickening with fibrous tissue proliferation were observed. In case of suppurative bronchopneumonia, the lungs were red to gray, solid and pale with suppurative exudates in the bronchi and bronchioles along with small pea to walnut sized abscesses scattered throughout the lung parenchyma. Microscopically, caseo-necrotic core surrounded by pyogenic membrane with infiltration of polymorphonuclear cells, few mononuclear cells and macrophages, presence of purulent material in bronchi and bronchioles along with infiltration of polymorphonuclear cells were recorded.

Ozyildiz *et al.*, (2013) observed 66% of lobular pneumonia, 16% of lobar pneumonia and 17% of both types in a study conducted on 110 suspected pneumonic lungs of slaughtered sheep. Macroscopically, swollen and dark red consolidated areas were seen which on cut section revealed fine foamy fluid or creamy suppuration that was yellowish or gray in color. Microscopically, infiltration of neutrophils and mononuclear cells in the bronchi, bronchiole, alveolar lumen and pleura, along with presence of multinucleated syncytial cell formations with spindle shaped oat cells in the alveolar lumen in the lungs were observed with catarrhal, purulent and fibrinous bronchopneumonia. In case of purulent necrotic bronchopneumonia widespread neutrophilic infiltration, coagulation necrosis and diffuse mononuclear cell infiltration in and around the bronchus and bronchioles was observed.

Priyadarshi *et al.,* (2013) categorized 89 cases (53.94%) of bronchopneumonia as acute in 41.21% and sub acute to chronic in 12.73% out of 165 sheep lung lesions. Acute bronchopneumonia was further classified into suppurative, non suppurative and fibrinous bronchopneumonia. Grossly, in acute bronchopneumonia the affected lungs revealed varying degrees of cranioventral consolidation with red hepatization along with petechial to ecchymotic hemorrhages. Histologically, in the initial stages, congestion of blood vessels, scattered hemorrhages, pronounced pulmonary edema and multifocal to diffuse infiltration of inflammatory cells predominantly of neutrophils and few lymphocytes in the alveoli were noticed. In the later stages the lungs showed desquamation of bronchiolar epithelium with accumulation of cell debris in the bronchiolar lumen along with peribronchiolar and perivascular infiltration. In sub acute to chronic form, macroscopically the affected lungs were grayish white to pale pink color with varying degrees of consolidation. Microscopically, marked infiltration of mononuclear cells in the alveoli and peribronchiolar region were observed.

In an abattoir survey to determine the major causes of organs and carcasses condemnation in Central Ethiopia Regassa *et al.,* (2013) recorded pulmonary abscess in 10 cases (2.22%) out of 451 sheep lungs examined.

Belkhiri *et al.,* (2014) examined 2863 ovine lungs and recorded 10 cases (0.35%) of suppurative pneumonia at Batna and Tiaret Slaughter house, Algeria.

2.2.3.1.2.1.2 Fibrinous bronchopneumonia

Sriraman and Rama rao (1980) recorded 9 cases (0.55%) of fibrinous bronchopneumonia out of 1643 sheep in different organized sheep farms of Andhra Pradesh during 1960 to 1979.

Kumar *et al.,* (2005) recorded 4 cases (2.29%) of fibrinous bronchopneumonia and 24 cases (13.71%) of serofibrinous bronchopneumonia out of 175 sheep lungs examined. Grossly, in serofibrinous bronchopneumonia patchy to diffuse areas of consolidation and thickening of pleura and on cut section focal to diffuse irregular necrotic areas in parenchyma were seen. Microscopically interlobular septae and pleura were distended and infiltrated by fibrino – cellular exudate. Bronchi and bronchioles revealed desquamation of epithelial cells and presence of cellular exudate in the lumen predominantly of leukocytic cells. Similar exudate was seen in alveoli also. In fibrinous bronchopneumonia microscopically, focal and wide irregular areas of necrosis infiltrated by disintegrated and intact neutrophils with fibrin mesh and surrounding vascular engorgement were observed.

On gross examination, Odugbo *et al.,* (2006) observed thin layer of fibrin covering the pneumonic parts, patchy areas of red to tan lesions and purulent material in the bronchi. Microscopically, the striking lesion observed was a mixture of macrophages and neutrophils in the alveoli, abscesses and vascular thrombosis.

Oruc (2006) recorded 70 cases (26.72%) of fibrinous pneumonia and 15 cases (5.73%) of fibrinous necrotic bronchopneumonia out of 262 pneumonic sheep lungs. Grossly, lungs with fibrinous bronchopneumonia showed diffuse or lobular hepatization in the entire lobe along with fibrinous exudates on cut surface. Microscopically, the interlobular septa and pleura were expanded due to fibrinous or neutrophilic exudation and lymphatic capillaries were obstructed with fibrin thrombosis. The histological picture of fibrinous necrotic bronchopneumonia was characterized by prominent neutrophilic exudation, necrosis, enlarged interlobular septa and pleuritis due to fibrinous exudation.

On gross examination of the affected lungs with fibrinous pneumonia, Dar *et al.,* (2012d) observed pleuritis and patchy to diffuse areas of consolidation covered by a layer of fibrin, mostly in the apical, cardiac and antero-ventral portion of diaphragmatic lobes. On cut section, well circumscribed hemorrhagic areas with red tinged mucopurulent discharge oozing out from bronchi and bronchioles were noticed. Microscopically, alveoli filled with fibrin and thickened interlobular septae with infiltration of fibrinocellular exudates comprising of mononuclear cells were noticed. Bronchi and bronchioles showed epithelial desquamation and the lumen were plugged with inflammatory exudates and cellular debris. Large number of macrophages in the form of basophilic spindle shaped cells or oat shaped cells in the lumen of alveoli was also seen.

Yesuf *et al.,* (2012) examined 72 pneumonic sheep lungs and recorded fibrinous bronchopneumonia and fibrinopurulent bronchopneumonia in 16 cases

(22.22%) and 4 cases (5.56%) each. Histologically, exudates with abundant fibrin, few neutrophils and macrophages were seen in fibrinous bronchopneumonia while equal amounts of neutrophils and fibrin were seen in fibrinopurulent bronchopneumonia.

Lobar type lesion with antero-ventral distribution was described by Azizi *et al.,* (2013). The apical and cardiac lobes were most affected followed by diaphragmatic lobe in severe cases. Grossly, the affected portion was consolidated, dark red and firm. Microscopically presence of multifocal areas of necrosis and variable amounts of fibrinous exudates in the lumen of alveoli and bronchioles were the most predominant and conspicuous lesions observed. The interlobular septa were thickened by fibrin, neutrophils, edema and some of the necrotic areas were surrounded by 'oat cells'. Fibrinous pleurisy with or without adhesion, wide spread and extensive vascular thrombosis in small blood vessels, capillaries and lymphatics were also noticed.

Epithelial desquamation in bronchi and bronchioles with inflammatory exudates in the lumen, large number of macrophages in the form of oat shaped cells in the lumen of alveoli and pleural thickening with neutrophilic fibrinous exudates were noticed by Dar *et al.*, (2013b) in fibrinous bronchopneumonia.

Priyadarshi *et al.*, (2013) recorded a single case (0.61%) of fibrinous pneumonia out of 165 sheep lungs examined based on histopathology. Grossly the affected lungs were characterized by patchy to diffuse areas of consolidation

mostly in the apical, cardiac, antero-ventral portion of diaphragmatic lobes, especially on right side and the cut surface showed focal to diffuse, consolidated firm areas. Microscopically the interlobular septae were thickened with fibrino-cellular exudates predominantly comprising of mononuclear cells and occasional neutrophils.

2.2.3.1.2.2 Interstitial pneumonia

Sriraman and Rama rao (1980) recorded 19 cases (1.15%) of interstitial pneumonia out of 1643 sheep examined in different districts of Andhra Pradesh during 1960 to 1979.

Bhagwan and Singh (1972) recorded interstitial pneumonia in 2 cases (5.71%) out of 35 pneumonic sheep lungs. Voluminous, reddish blue lungs were seen grossly and infiltration of mononuclear cells in the interalveolar septa, peribronchial and peribronchiolar regions and around the blood vessels were seen microscopically.

Chattopadhyay *et al.,* (1986) investigated 675 sheep lungs and recorded 10 cases (1.48%) of interstitial pneumonia with features of epitheliolization and lymphocytic cuffing.

Oruc (2006) detected 48 cases (18.32%) of interstitial pneumonia in an investigation on 262 pneumonic sheep lungs. Grossly, lungs appeared voluminous

with rib impressions on the surface. Microscopically mononuclear cells in the interalveolar septa and within the alveolar lumen were observed. In some cases, peribronchial and peribronchiolar proliferation of lymphocytes were also seen. In addition, in most of the cases fibrous tissue proliferation was prominent in the interalveolar septa. In some cases, there was thickening of alveolar septa and neutrophilic exudation within the lumen which was ascribed as atypical interstitial pneumonia or bronchointerstitial pneumonia.

Ezzi *et al.,* (2007) observed the microscopic changes in interstitial pneumonia and described infiltration of neutrophil, lymphocyte and few plasma cells with thickening of the inter-alveolar septa.

Yesuf *et al.,* (2012) examined 72 pneumonic lungs and reported 17 cases (23.61%) of interstitial pneumonia in a study on slaughtered sheep. Histologically thickening of alveolar wall due to mononuclear cell infiltration and / or fibroblast proliferation were noticed.

Azizi *et al.,* (2013) described interstitial pneumonia in sheep. Macroscopically, lungs showed severe involvement of dorso - caudal region, diffuse red to pale color and had rubbery consistency. Rib impressions were seen on the costal surfaces of diaphragmatic lobes and the lungs failed to collapse. Histopathologically, a marked increase in mononuclear cells and mild fibrosis were seen in interalveolar septa. Hyperplasia of type II pneumocytes and absence of exudates in the alveolar spaces and airways were also observed.

Dar *et al.,* (2013a) observed 19 cases (5.67%) of interstitial pneumonia out of 335 affected lungs of slaughtered sheep screened in different abattoirs in Kashmir Valley, India.

Dar *et al.,* (2013b) noticed 18 cases (7.0%) of interstitial pneumonia on examination of 257 slaughtered sheep lungs. Grossly, the affected lungs were voluminous and microscopically, there was proliferation and desquamation of the alveolar epithelial cells and thickening of interalveolar septae with infiltration of lymphocytes, neutrophils and macrophages. Bronchial and bronchiolar epithelial hyperplasia was evident along with perivascular and peribronchiolar lymphoid aggregates.

Priyadarshi *et al.,* (2013) observed interstitial pneumonia in 9 cases (5.45%) out of 165 lung lesions in slaughtered sheep. Macroscopically, lesions were seen in all the lobes which were sunken, red to pale grey to mottled, elastic and had granular appearance under pleura and on cut surface. Microscopically, thickened alveolar septa due to infiltration of mononuclear cells were noticed.

Belkhiri *et al.,* (2014) examined 2863 ovine lungs and recorded 99 cases (3.46%) of interstitial pneumonia at Batna and Tiaret Slaughter house, Algeria.

2.2.3.2 Pleuritis

Chattopadhyay *et al.,* (1986) recorded pleuritis in 3 cases (0.44%) out of 675 lungs examined in an investigation on sheep lungs at local abattoirs whereas

one case (2.38%) of pleuritis out of 42 pneumonic lungs of sheep slaughtered in Shahrekord slaughter house, Iran was reported by Azizi *et al.,* (2013).

Dar *et al.,* (2012d) observed thickened pleura with fibrinous exudates associated with neutrophilic infiltration, focal or wide irregular areas of necrosis, vascular engorgement, emphysema and edema.

2.2.4 PARASITIC CONDITIONS

Rahman and Iyer (1979) observed an overall incidence of 54.07% parasitic conditions in 135 lungs of slaughtered sheep aged between 3-5 years at Military Butchery, Bareilly.

2.2.4.1 Hydatidosis

Sriraman and Rama rao (1980) recorded 30 cases (0.89%) of pulmonary hydatidosis out of 3364 sheep from organized farms examined in different districts of Andhra Pradesh during 1960 to 1979 whereas Chattopadhyay *et al.,* (1986) observed pulmonary hydatidosis in 9 cases (1.33%) out of 675 sheep lungs in an investigation on pulmonary affections at local abattoirs.

Kamil and Parihar (1990) recorded 18 cases (5.26%) of pulmonary hydatidosis out of 342 slaughtered sheep of either sex examined for pathological

abnormalities at Bareilley, Avikanagar, Srinagar and Varanasi. Grossly the cysts were single to multiple, marble to walnut sized and occasionally as big as a cricket ball and soft to touch except in a few cases which were nodular. Cysts were embedded in the lung parenchyma or visible from the lung surface. Microscopically, the lesion was characterized by foreign body reaction.

Das *et al.,* (1998) studied the prevalence of hydatidosis in five different species of food animals and found 6 cases (2.61%) of pulmonary hydatidosis out of 230 sheep slaughtered in and around Calcutta.

A total of 3661 cases (11.13%) were found to be infected out of 32,898 slaughtered sheep examined for *Echinococcus granulosus* infection by Dalimi *et al.,* (2002). The distribution of hydatid cysts was higher in lungs (59.4%) followed by liver (21.1%) and in both (19.4%).

Raman and John (2003) conducted a study on 4565 sheep slaughtered at Corporation slaughter house, Chennai and found a prevalence of 256 cases (5.61%) of hydatid cysts. Lungs (62%) were found to harbor the cysts most commonly followed by liver (23%), heart (6%) and spleen (5%).

Maraqa (2005) recorded hydatidosis in 20.31% (90/443) Awasi sheep and 0.59% (28/4680) of Australian sheep that were slaughtered in Amman Central Abattoir, Jordan during November - December 1999.

A survey conducted by Ahmed *et al.,* (2006) during 2001-2002 to determine the status of hydatidosis at Army Supply Corps abattoir, Quetta revealed 41 cases (17.37%) of hydatid lungs among 236 sheep examined.

Postmortem inspection conducted on 2948 sheep from 2001 to 2004 in rural and urban abattoirs in Morocco revealed a prevalence of 32 cases (1.08%) of cystic ecchinococcosis (Azlaf and Dakkak, 2006).

Scala *et al.,* (2006) recorded cystic echinococcosis in 580 cases (75.22%) out of 771 Sardinian sheep slaughtered in different slaughter houses, Italy between September 1998 and April 2003 and also noticed that among the positive cases, 10.3% were fertile cysts.

An overall prevalence of 5.60% of hydatidosis was reported by Sangaran and John (2009) who observed 33 cysts in lung (51.56%), 29 in liver (45.31%) and 2 (3.13%) each in lung and liver on examination of 1141 sheep at slaughter.

Belkhiri *et al.,* (2012) observed 374 cases (13.06%) of hydatid cysts and 10 cases (0.35%) of pulmonary fasciolosis out of 2863 sheep lungs examined.

In an abattoir survey of 542 sheep during June 2007 to July 2008 in Addis Ababa Abattoir, Fikire *et al.,* (2012) reported 73 cases (13.46%) harboring hydatid cysts with 91.7% of lung involvement.

Daniel Getachew *et al.,* (2012) examined 325 sheep in a cross sectional study at Modjo Luna Export Slaughter House, Ethiopia to determine the prevalence of hydatidosis in small ruminants and recorded 25 cases (7.69%) of hydatidosis. 60% of hydatid cysts were present in lungs which were mostly fertile.

A prevalence of 34 cases (8.52%) of hydatidosis was recorded by Helina Getachew *et al.,* (2012) in a cross sectional study conducted on 399 slaughtered sheep at Addis Ababa Abattoir Enterprise, Ethiopia.

Jarjees and Al-Bakri (2012) studied the occurrence of hydatid cysts in 4800 slaughtered sheep at Mosul abattoir, Iraq and recorded an incidence of 96 cases (2%) of fertile hydatid cysts in the lung whereas Iqbal *et al.,* (2012) recorded a prevalence of 8.25% of hydatidosis (198 cases) in a study conducted to observe the status of hydatidosis in 2400 sheep slaughtered at Lahore abattoir during March 2010 to February 2011.

Kouidri *et al.,* (2012) examined 1973 sheep at Tiaret abattoir, Algeria and recorded 75 cases (3.80%) of hydatidosis of which 17.3% showed pulmonary hydatidosis. Fertile cysts were found to be 72.45%.

Priyadarshi *et al.,* (2013) recorded pulmonary hydatidosis in 2 cases (0.18%) out of 1080 sheep examined at necropsy and the gross changes observed were single to multiple grayish white cysts of variable size in the middle of left diaphragmatic lobes. The cysts were soft to touch, filled with 3-4 ml clear fluid and

deeply embedded in lung parenchyma. Microscopically, large cystic spaces with the outer capsule of fibroblastic proliferation were observed.

An abattoir survey was undertaken by Regassa *et al.,* (2013) to determine the major causes of organs and carcasses condemnation in Central Ethiopia and one case (0.22%) of hydatidosis was recorded out of 451 sheep lungs examined.

In a postmortem study of 5889 slaughtered sheep, ecchinococcosis was detected in 579 cases (9.83%) sheep in Kazakhsthan during the years 2010-12 by Valiyeva *et al.,* (2013). Grossly unilocular cysts were seen in all lung lobes particularly in the right lung. Histologically, the wall of cyst consisted of an interior nucleating (germinal layer) and exterior chitinous (cuticular layer) membranes, surrounded by fibrous capsule (rarely calcified) and adjacent areas of lung showed atelectasis and emphysema.

Belkhiri *et al.,* (2014) examined 2863 ovine lungs and recorded 374 cases (13.06%) of hydatidosis at Batna and Tiaret Slaughter house, Algeria.

2.2.4.2 Pulmonary fasciolosis

Pandit *et al.,* (1991) observed 10 sheep with acute hepato-pulmonary fascioliasis at Jammu and Kashmir and large numbers of liver flukes were collected from liver and lungs and identified as mature *Fasciola gigantica.*

Out of 342 sheep lungs examined at Bareilly, Avikanagar, Srinagar and Varanasi, Kamil and Parihar (1990) recorded one case (0.29%) of pulmonary fascioliasis with grayish-red areas of consolidation along with a few pea-sized grayish-white abscess-like structures in the diaphragmatic lobe. Histopathologically, cross section of parasite structure without any body cavity and with a spiny cuticle was observed in the exudate.

Rao and Madhubala (1998) observed hepato-pulmonary fascioliasis in sheep with heavy mortality in certain parts of Andhra Pradesh during March and April 1992. On postmortem examination, the lungs were found inflamed with presence of blood clots and occasional immature flukes.

Yoshihara *et al.*, (1998) observed erratic parasitism in the lungs of sheep experimentally infected with *Fasciola gigantica*. Macroscopically, severe hyperaemia was noticed on the surface of the lungs with presence of juvenile flukes. Microscopically a slight inflammatory reaction was observed and occasionally, a mixed thrombus with many eosinophils was found in the blood vessels adjacent to the bronchi.

A prevalence of 3.17% (15/473) of *Fasciola hepatica* infection in the lungs of Romania sheep in Amman Central Abattoir, Jordan was recorded by Maraqa (2005) from November to December, 1999.

Soundararajan and Iyue (2005) investigated the cause of sudden death of 8 (5.29%) Nilgiri sheep in a flock of 151 in Tamil Nadu and observed inflamed lungs with swollen necrotic areas over left apical lobe. Immature flukes were recovered from necrosed area.

2.2.4.3 Dictyocaulosis and Muelleriosis

On histopathological examination of 675 lungs with apparent lesions Chattopadhyay *et al.*, (1986) observed 5 cases (0.74%) of verminous pneumonia with cross section of nematode parasite (*Dictyocaulus sps.*) surrounded by granulomatous inflammatory zone.

Ayana and Chanie (2013) recorded 221 cases of lung worm infection with an overall prevalence of 57.55% out of 384 apparently healthy slaughtered sheep in Ethiopia and opined that *Dictyocaulus filaria* was found to be most prevalent followed by *Muellerius capillaris* and *Protostrongylus rufescens*. Grossly, localized lesions on caudal lobes within dorsal side characterized by atelectasis, emphysema, multifocal patches, and firm consistency were seen. Mucus and foamy blood tinged exudates in the bronchi and bronchioles besides long whitish colored worms in moderate and heavy infestations were also observed. Microscopically, alveolar emphysema, desquamated bronchial and bronchiolar epithelial cells with occlusion of lumen by mass of fibrous tissue and diffuse infiltrations with inflammatory cells were noticed. Parasitic granulomas with calcified center infiltrated with eosinophils and neutrophils were commonly seen.

Of the 1455, a total of 387 cases (26.6%) revealed lung worm infection caused by *D. filariae* in a study conducted by Dar *et al.*, (2012c) in indigenous sheep slaughtered from July 2010 to October 2011 in different regions of Kashmir valley. Grossly, the lungs revealed depressed, consolidated areas and elevated emphysematous patches on the surface of diaphragmatic lobes with copious foamy froth in trachea-bronchial tree with slender thread like worms. Histopathologically, the sections revealed bronchitis and bronchiolitis with cross sections of the adult worms in the lumen. The bronchial and bronchiolar epithelium was markedly hyperplastic and showed desquamation. Peribronchiolar lymphoid hyperplasia, vasculitis and perivasculitis were the other changes observed. Tracheal fluid revealed numerous adults, larvae and eggs of the lung worm.

2.2.5 NEOPLASTIC CONDITIONS

2.2.5.1 Ovine Pulmonary Adenocarcinoma

Damodaran (1960) recorded 4 cases of OPA in the lung sections of sheep maintained in the Hosur Cattle Farm, Karnataka. Grossly numerous grayish – white circumscribed nodules about a millimeter to 2.5 cm in diameter were seen under the pleura and in the parenchyma of both the lungs. Several small nodules coalesced to form larger nodules which resembled abscesses but were firm and did not easily break down on incision. Microscopically, the alveolar wall was lined by a layer of non-ciliated cuboidal to columnar epithelial cells. Some alveoli were filled with epithelium without any lumen and were seen scattered here and there. Hyperplastic groups of cells forming papillary tufts with a central core of vascular

connective tissue were frequently found projecting into the alveolar lumina. The lumen was either empty or contained cellular exudates consisting exclusively or varying proportions of desquamated epithelial cells, mononuclear cells, neutrophils and lymphocytes. Numerous macrophages showing marked vacuolation were seen in some of the affected alveoli. Similar changes were also noticed mostly in the bronchioles and occasionally in the smaller bronchi. The lining cells were of columnar type, rarely ciliated, and usually larger than those of the normal respiratory bronchioles. The papillary tufts of epithelium often coalesced to present an adenomatous appearance. The alveoli appeared to be predominantly affected than the bronchioles. The interalveolar connective tissue in the nodular area was considerably thickened by lymphocytes, mononuclear cells and occasionally neutrophils. Nodular lymphoid hyperplasia was rarely observed but diffused lymphocytic infiltration with several plasma cells was invariably noticed.

Rahman and Iyer (1979) recorded an incidence of 6 cases (4.44%) of Jaagsiekte in 135 slaughtered sheep aged between 3-5 years at Military Butchery, Bareilly. Grossly patchy to diffuse areas of consolidation with translucent and grayish white nodules from a pinhead to 2-3 cm diameter were seen in the OPA affected lungs. Microscopically neoplastic transformation of alveolar epithelium into cuboidal or columnar type was observed that showed proliferation resembling papilloma. Identical changes were seen in bronchi/bronchioles. The thickened interalveolar septae showed myxomatous changes with triangular stellate cells.

Sriraman and Rama rao (1980) recorded 90 cases (2.66%) of Jaagsiekte out of 3364 sheep in different organized sheep farms of Andhra Pradesh during 1960 to 1979.

In an investigation on pulmonary affections of sheep at local abattoirs Chattopadhyay *et al.,* (1986) recorded 16 (2.38%) cases of Jaagsiekte out of 675 sheep examined histopathologically. The lesions were characterized grossly by focal to multicentric areas of adenomatosis and thickening of interalveolar septa.

Attia *et al.,* (1997) recorded macroscopically gray areas of 2 mm diameter or more scattered in the anterior cardiac and diaphragmatic lobes. Histologically, both type II pneumocytes and epithelial cells in the terminal bronchioles showed hypertrophy and hyperplasia to cuboidal and columnar epithelium. Papillary projections extended from alveolar septa into the alveoli.

Rama Devi *et al.,* (2001) recorded a case of OPA in a severely emaciated carcass of Nellore breed ewe and observed patchy to diffuse areas of consolidation and grayish white nodular foci up to 1-2 mm in apical, cardiac and diaphragmatic lobes. Microscopically, several alveoli were lined by single or more layers of cuboidal to low columnar epithelial cells giving adenomatous appearance. In addition, intrabronchiolar papillary processes into the lumen composed of irregular masses of columnar cells was observed along with thickening of interalveolar septa and infiltration of mononuclear cells. The alveolar lumina were filled with macrophages, lymphocytes and few polymorphonuclear cells.

Kumar *et al.,* (2005) recorded 13 cases (7.43%) of Jaagsiekte out of 175 sheep lungs that showed pathological lesions. Grossly, the affected lungs were grayish to light purple, enlarged and heavy with granular to meaty consistency along with presence of variable sized nodules, 1.5 to 2 cm diameter, which on cut section revealed grayish exudate. Microscopically, in early stages presence of distinctly cuboidal to columnar type of alveolar epithelial cells and in extreme cases papillomatous proliferation of alveolar epithelium with distinct connective tissue cores were observed. Bronchial and bronchiolar lining cells revealed hyperplastic changes rarely forming papillary projections and obstructing the lumen partially or completely. Interalveolar septae, peribronchial, peribronchiolar and perivascular areas were infiltrated with lymphocytes and plasma cells.

In OPA, Beytut *et al.,* (2009) observed enlarged and heavier lungs due to extensive lesions in the dependent areas of cranial, medial and caudal lobes which failed to collapse. On cut Section, affected areas appeared consolidated and clear, foamy fluid exuded from the cut surface and airways. The tumor masses varied in size, grey in color, firm and clearly demarcated from the unaffected dorso-ventral lung. Microscopically, neoplastic foci composed of epithelial cells with acinar to papillary growth pattern were noticed with compressed adjacent alveoli that contained large alveolar macrophages with foamy cytoplasm. Bronchioles contained areas of epithelial hyperplasia, papillary growth of the lining epithelium or tumor nodules. The tumor cells were cuboidal to columnar with a clear or vacuolated cytoplasm. Neoplastic epithelial cells were supported by a connective tissue stroma within which there was infiltration of mononuclear cells and a few

granulocytes. Occasional abscessation with prominent neutrophilic infiltration was also noticed. Lymphoid proliferation was consistently noticed within the alveolar interstitium and around the bronchioles adjacent to neoplastic foci and in some cases myxomatous nodules were also noticed.

Khodakaram Tafti and Razavi (2010) observed OPA in 21 (0.22%) lungs out of 9400 sheep and recorded gross lesions of classical form of OPA as firm, white to grayish coalescing masses mostly in the cranio-ventral lobes associated with wet surface and frothy fluid in the airways especially in the tracheal lumen. Further in atypical form of OPA, small clearly demarcated, grayish white, hard nodules mostly in the dorsal aspects of diaphragmatic lobes were observed associated with dry cut surface. Histologically acinar or papillary growth of neoplastic cells in the alveoli, polypoid proliferation of bronchiolar epithelium with variable amounts of connective tissue, myxomatous foci and infiltration of lymphocytes and plasma cells in the interstitial tissue of affected alveoli were seen in both the forms.

Oda and Youssef (2011) recorded OPA in the lungs and associated lymph nodes of seven sheep. Few multifocal gray to white masses with no particular lobar distribution were described. The masses occupied less than 10% of the total lung mass. They were consolidated, slightly elevated, ranging from 0.5 to 3.0 cm diameter and the cut surface was pale and dry. Histologically, well circumscribed, multifocal, random, highly cellular and neoplstic masses composed of well differentiated epithelial cells, arranged in cystic or papillary acinar like structures

or rarely in solid aggregates were observed. Individual cells were cuboidal to low columnar with a vacuolated cytoplasm and basally located round to oval nuclei containing central nucleolus. The lumina of the neoplastic acini contained desquamated neoplastic cells, lymphocytes, plasma cells and moderate numbers of alveolar macrophages that were characterized by a foamy vacuolated cytoplasm. The neoplastic acini were supported by variable amount of dense fibrous connective tissue that containing numerous lymphocytes and plasma cells.

Amini and Tehrani (2013) recorded 132 cases (20.3%) of OPA out of 650 sheep examined. Grossly, lungs were double or triple their normal size, grey-yellow color, non collapsible with rubbery consistency and had meaty appearance mostly in the ventral parts of the lung.

Classic and atypical forms of OPA were described by Azizi *et al.*, (2014). Atypical form of OPA showed grayish-white, hard, multiple subpleural nodules of variable sizes, dispersed on dorsal surfaces of diaphragmatic lobes grossly and the cut surface was dry with no mucoid fluid in the airways. Lungs affected with classic OPA revealed multifocal nodules some of which coalesced to form larger masses in the cranio-ventral lobes. Cut surface was moist with mucoid and foamy material in the airways. Microscopically almost similar histopathological findings were noticed in both the forms that revealed papillary projections of cuboidal to low columnar neoplastic cells in the lumen of alveoli and bronchioli respectively. The neoplastic foci were supported by sparse connective tissue stroma infiltrated by numerous mononuclear cells and connective tissue in the atypical form.

2.2.5.2 Pulmonary myxoma

Paliwal and Baxi (1979) recorded a case of multiple pulmonary myxoma in a 2 year old ewe. Grossly, the lungs were heavy, consolidated, hard to cut and with extensive pleural adhesions in the right thoracic wall. Histopathologically, lung showed increase in perialveolar and peribronchial connective tissue with islands of myxomatous tissue. Multiple myxomatous areas with typical triangular or stellate cells with long branching processes in a clear homogenous matrix were seen.

Ilhan and Yener (2009) recorded pulmonary myxoma in the left lung of a 5 year old sheep at slaughter. Grossly, the tumor was pale tan, lobulated and well circumscribed mass. Microscopically, the tumor was composed of spindloid to stellate cells in a myxoid matrix.

Oryan *et al.*, (2009) reported a case of pulmonary myxoma in a 4 year old ewe. Grossly, the affected lung revealed a mass of 8x6x4 cm, embedded in the diaphragmatic lobe that was soft, white and on cut section revealed mucoid exudation. Microscopically, the tumor was comprised of lobules consisting of numerous stellate to fusiform cells and delineated by fibrous connective tissue septa in the parenchyma. The mass was encapsulated and adjacent alveoli were atelectic.

2.2.6 MISCELLANEOUS CONDITIONS

2.2.6.1 Sheep pox

Chattopadhyay *et al.,* (1986) recorded 2 cases (0.29%) of sheep pox out of 675 sheep lungs examined at slaughter and noticed sub pleural congestion and consolidation.

Afshar *et al.,* (1986) observed grey, firm large nodules throughout the lung. The subpleural nodules were round or coalescing in the apical and diaphragmatic lobes. Microscopically, proliferative bronchiolitis and alveolitis giving the appearance of gland-like structures and presence of large eosinophilic cytoplasmic inclusions in the pulmonary macrophages were seen. Transmission electron microscopy revealed sheep pox cells with vacuolated nucleus and virions with dumbbell shaped cores in the affected lungs.

Mondal *et al.,* (2004) observed nodular lesions in the lungs which are characteristic of generalized sheep pox on postmortem examination in an outbreak of sheep pox in Jammu, India.

Bhanuprakash *et al.,* (2006) recorded variable sized pock lesions in the form of large, irregular consolidated areas of chocolate color on the lungs that showed sheep pox lesions grossly. On microscopic examination, lungs revealed hyperplasia, desquamation and necrosis of bronchiolar epithelium, infiltration of septal walls with polymorphonuclear cells and serous exudates in alveolar lumen with vasculitis and ulceration. Edema, lymphocytic infiltration of subpleural interstitial connective tissue and peribronchiolar lymphoid hyperplasia were also observed.

Grossly the lesions in sheep pox lung consisted of subpleural, whitish, multiple nodules mainly on the dorso caudal lobes which were 2-3 cm in diameter surrounded by slight congestion. Histologic examination showed epithelial hyperplasia of the alveoli, bronchi and bronchioli. Alveoli contained eosinophilic material, cubic-columnar epithelial cells, many neutrophils, macrophages and sheep pox cells. Peribronchial, peribronchiolar and perivascular sheaths were infiltrated with mononuclear cells, sheep pox cells, a few neutrophils and karyorrhectic cellular debris. Bronchiolar epithelial cells and many sheep pox cells revealed presence of intracytoplasmic eosinophilic inclusion bodies (Gulbahar *et al.,* 2006).

Tarello and Kinne (2007) observed sheep pox in 145 sheep (2.63%) out of 5500 non vaccinated sheep in December 2005 in Dubai, United Arab Emirates. Grossly, the affected lungs showed numerous small grayish nodules of 3-5 mm diameter and microscopically the lesions were characterized by presence of focal catarrhal pneumonia with massive proliferation of type II- pneumocytes containing eosinophilic material.

Singh *et al.,* (2007) observed congested lungs grossly and thickened interalveolar septa with infiltration of lymphocytes, monocytes /macrophages (sheep pox cells) and engorged capillaries microscopically.

Roy *et al.,* (2008) observed nodular lesions in the lungs which were more severe in Mechrey breed whereas histopathological examination of sheep lungs by

Haligur and Ozmen (2009) revealed hyperplasia and hypertrophy of alveolar, bronchial and bronchiolar epithelial cells along with desquamation and characteristic cellules claveleuses in bronchi and bronchioles. Few eosinophilic intra-cytoplasmic inclusion bodies were also detected in the proliferative alveolar, bronchial and bronchiolar epithelial cells besides alveolar edema in some cases. Stromal tissue was infiltrated with mononuclear cells that consisted of few plasma cells, histiocytes and macrophages.

Grossly, lungs with sheep pox showed areas of congestion, edema, consolidation and often multiple firm white nodules (2-15 mm diameter) surrounded by a zone of hyperaemia disseminated throughout the lobes particularly in the caudal lobes. Histologically, the lesions were characterized by proliferative alveolitis and bronchiolitis, composed of hyperplastic type II pneumocytes with round nuclei and abundant cytoplasm. Numerous sheep pox cells with vacuolated nuclei and marginated chromatin, often containing cytoplasmic eosinophilic inclusion bodies were seen. Nodules showed necrosis, minor hemorrhages and inflammatory infiltration of neutrophils and lymphocytes and the alveolar lumina contained pale eosinophilic amorphous material. Bronchiolar epithelium often showed hyperplasia and infiltration by neutrophils. Interalveolar septal thickening and slight fibrosis were detected around some nodules whereas some pock nodules consisted solely of epithelial hyperplasia with the appearance of gland like structures and no inflammatory reaction. Ultrastructurally, the pock nodules were composed of type-II pneumocytes characterized by numerous apical microvilli and lamellar bodies appearing as clear vacuoles. The alveolar lumina were occluded by

distended hyperplastic type II pneumocytes. Numerous pox virus particles with dumb-bell shaped cores were detected in the cytoplasm of macrophages and epithelial cells with marginal chromasia in some sheep pox cells (Beytut, 2010).

Bhanuprakash *et al.*, (2010) observed nodular lesions on the lungs grossly and immature and mature virus particles with brick shape and superficial tubular structure under electron microscopy in sheep pox virus infected cell cultures.

Chanie (2011) recorded a prevalence of 799 cases (49.54%) of sheep pox out of 1615 sheep examined in a study conducted from November 2007 to December 2009 at Ethiopia. Macroscopically lungs were congested, edematous, firm and grey with few whitish nodular areas. Histologically nodules were characterized by proliferative bronchiolitis and alveolitis giving gland like appearance.

Dar *et al.*, (2012a) observed multiple, variably sized nodular areas particularly in diaphragmatic lobes. On cut section, the larger nodules were grey, firm and surrounded by edematous connective tissue. Histologically, there was proliferation and desquamation of alveolar and bronchiolar epithelial cells along with perivascular and peribronchiolar lymphoid aggregation. Proliferative bronchiolitis and alveolitis giving gland-like appearance were noticed in the parenchyma. Severe congestion, edema and thickening of interalveolar septae with infiltration of lymphocytes, neutrophils and macrophages were also seen.

Occasionally syncytial cells were observed. Eosinophilic intracytoplasmic inclusions were noticed in pulmonary macrophages and bronchial epithelial cells.

Sharma *et al.,* (2013) reported nodules studded throughout parenchyma of lungs affected with sheep pox. Microscopically, edema, areas of necrosis, proliferation of bronchial and bronchiolar epithelium and presence of sheep pox cells were observed. Bronchitis and bronchiolitis with proliferation of peribronchiolar lymphoid cells along with infiltration of neutrophils, regeneration of lining epithelial cells and cellular debris in the lumen were also seen.

Zangana and Abdullah (2013) conducted a study from October 2011 to June 2012 on skin lesions of 196 lambs and recorded an incidence of 60 cases (30.61%) of sheep pox in different areas of Duhok, Iraq. Grossly the lungs were congested, edematous and containing few whitish to red colored areas.

2.2.6.2 Peste Des Petitis Ruminants

Diffuse interstitial pneumonia with occasional eosinophilic nuclear inclusions in the pneumocytes and alveolar giant cells in the lungs of adult sheep inoculated intranasally with PPR viral isolate was reported by Bundza *et al.*, (1988).

Ettorre *et al.,* (2007) observed histopathologically desquamative alveolitis, alveolar epithelisation and the presence of giant cells in the lungs of lambs aged

less than 12 months in an abattoir study in Italy from December 2002 to April 2003.

Out of 70 pneumonic lungs, presence of PPR viral antigen was detected by Saglam and Temur (2009) in 8 lungs (11.42%) by immunohistochemistry. Histopathological examination revealed necrotic bronchitis, bronchiolitis, presence of alveolar macrophages and syncytial cells in the alveoli, intracytoplasmic inclusion bodies in alveolar, bronchial and bronchiolar epithelial cells, intranuclear inclusion bodies in the pneumocytes and desquamation of epithelial cells and mononuclear cell infiltration in the bronchial and bronchiolar lumina along with interstitial pneumonia.

Aktas *et al.,* (2011) recorded 86 cases (16%) of PPR out of 525 sheep examined. At necropsy, grossly lungs showed consolidation, hemorrhages, edema, hepatization and bronchial exudates. Microscopically intense hyperemia and petechial haemorrhages in the regions of lung, degenerative changes in the alveoli with eosinophil infiltration in the septum, perivascular lymphocytic infiltration, exudates in bronchi and bronchioles, peribronchial lymphoid infiltration and fibrocytic proliferation along with interstitial pneumonia were present.

Muse *et al.,* (2012) observed grossly, severely congested, consolidated, and hyperemic lungs with dark red, firm parenchyma in cranio-ventral lobes and purulent froth in the trachea. Microscopically, thickened interalveolar walls with

infiltration of mononuclear cells and moderate number of neutrophils were observed in PPR suspected cases.

Madboli and Ali (2012) examined 17 slaughtered ewes naturally infected with PPR and recorded grossly consolidation of different lobes of lung with oozing of large amount of blood on cut section. Microscopically, severe lymphocytic interstitial pneumonia with diffuse infiltration of mononuclear cells mainly lymphocytes and macrophages in the peribronchial and perivascular areas along with intra alveolar edematous fluid infiltration was observed.

Bronchointerstitial pneumonia with eosinophilic intracytoplasmic and intranuclear inclusions in giant cells and alveolar macrophages was observed in lungs of sheep with *Peste Des Petitis Ruminants* (Goodnight *et al.,* 2013).

2.3 BACTERIOLOGICAL STUDIES

Al-Sultan and Zubaidy (1978) isolated *Staphylococci, Streptococci*, *Klebsiella spp* and *E.coli* from 18 pneumonic sheep lungs and *Mycoplasma* from 10 diseased sheep lungs examined.

Bakr *et al.,* (1980) isolated *Streptococcus spp.* and *Staphylococcus aureus* from bronchopneumonia, *Pseudomonas aeruginosa* and *Proteus mirabilis* from fibrinous pneumonia and *Staphylococcus aureus*, *Streptococcus spp.* and *Mycoplasma arginini* from chronic interstitial pneumonia out of 140 diseased sheep lungs examined.

A total of 50 samples collected out of 60 pneumonic lungs examined revealed 72 different bacterial isolates in a study carried out by Rajiv Kumar *et al.,* (2000) *viz., Citrobacter diversus* 7 (9.72%), *Pseudomonas aeruginosa* 6 (8.33%), *Pasteurella haemolytica* 2 (2.78%), *Pasteurella multocida* 1 (1.39%), *Actinomyces pyogenes* 11 (15.28%), *Streptococcus pyogenes* 10 (13.89%), *Staphylococcal aureus* 10 (13.89%), *Micrococcus luteus* 6 (8.33%), *Staphylococcus epidermidis* 5 (6.94%) , *Corynebacterium ovis* 4 (5.55%), *Micrococcus varians* 3 (4.17%), *Bacillus megatermis* 3(4.17%), *Micrococcus kristinae* 2 (2.78%) and *Staphylococcus intermedius* 2 (2.78%).

Rhamyas *et al.,* (2001) isolated *Pasteurella hemolytica* (17), *Corynebacterium spp* (13), *Streptococcus spp* (8), and *Pseudomonas spp* (6), *Klebsiella spp* (4), *E.coli* (1), non hemolytic *Staphylococcus spp* (1) and *Yeast* (1) from 52 samples taken from sheep with chronic pneumonia.

Obasi *et al.,* (2001) isolated various bacteria such as *E. coli* in 70 cases (24.74%), *Pasteurella multocida* in 49 cases (17.31%), *Arcanobacterium pyogenes* in 42 cases (14.84%), *Streptococcus pyogenes* in 20 cases (7.06%), and *Neisseria catarrhalis* in 16 cases (5.65%) from 283 ovine lungs.

Beytut *et al.,* (2002) carried out microbiological inoculations from 65 pneumonic lungs and isolated various agents singly or in combination out of which *Staphylococcus aureus* (22.25%) was predominant.

Srinivasan *et al.,* (2003) isolated *Klebsiella spp.* in 13 (39.39%), and *Pasteurella spp.* in 12 (36.36%) followed by *Staphylococcus spp.* in 3 (9.09%), *Streptococcus spp., Escherichia spp.* and Proteus *spp.* in 1 (3.03%) each from 33 pneumonic lungs.

Kumar *et al.,* (2005) isolated *Pasteurella* organisms from fibrinous bronchopneumonia and Gram positive *Streptococci* from necrotizing pneumonia.

Oruc (2006) isolated *Mannheimia hemolytica* (56.14%), *E.coli* (24.56%) and *Pasteurella multocida* (10.52%) from pneumonic lesions in lambs whereas Odugbo *et al.,* (2006) isolated *Pasteurella multocida* (9.44%) from lung samples of sheep that showed Pasteurellosis.

In a study conducted on slaughtered sheep and goats, Ezzi *et al.,* (2007) isolated *Pasteurella multocida* from pneumonic lung tissues of slaughtered sheep whereas Zhang *et al.,* (2009) isolated *Helcococcus ovis* from a 6 month old mixed breed ewe lamb died of respiratory disease.

Yesuf *et al.,* (2012) isolated *Pasteurella* species (47.85%), *Staphylococcus* species (17.68%), *Streptococcus* species (13.44%) and other bacteria (21.03%) from 24 pneumonic lungs of slaughtered sheep.

Ozyildiz *et al.,* (2013) isolated *Pasteurella multocida* from 38 cases of lungs suspected for pneumonia whereas Azizi *et al.,* (2013) found the presence of

Pasteurella multocida (24.53%), *Staphylococcus aureus* (20.75%), *Klebsiella pneumonia* (15.09%), *Corynebacterium pseudotuberculosis* (7.55%) and *Actinomyces pyogenes* (1.89%) in 42 sheep lungs examined.

CHAPTER - III

MATERIALS AND METHODS

3.1 SOURCE

The materials for the present study consisted of tissue samples of lungs of sheep collected from various slaughter houses located in and around Vijayawada and Tirupathi apart from the animals necropsied in the Department of Veterinary Pathology, N.T.R College of Veterinary Science, Gannavaram and from field mortalities. The duration of the study lasted from June 2013 to July 2014. A total of 988 lungs from sheep of either sex and of different age groups were examined in detail at necropsy/slaughter for the presence of gross abnormalities, if any, of which 187 lung samples that showed gross lesions were collected and preserved in 10% neutral buffered formalin for further studies.

3.2 MODE OF COLLECTION OF SAMPLES

At necropsy or slaughter, a detailed examination was carried out on 988 sheep for the presence of any gross pathological lesions of the lungs. For bacterial isolation, swabs were collected in sterile containers from suitable cases by deep incisions on the lung by taking all the necessary aseptic measures. Tissue pieces from representative portions of lungs that showed definite gross lesions were collected and fixed in 10% neutral buffered formalin for histopathological examination. The parasites found in the lungs were collected in normal saline for

further studies. Few lung tissues collected from suspected cases of sheep pox were preserved in 3% glutaraldehyde for ultrastructural studies by TEM.

3.3 METHODS EMPLOYED

3.3.1 Bacterial isolation

The swabs collected in sterile test tubes were inoculated in Nutrient broth and Brain Heart Infusion (BHI) broth and incubated at 37^0C for 24 hours. Then the broth culture material was streaked on Nutrient agar and BHI agar plates prepared in the laboratory as per manufacturer's instructions. A provisional identification of bacterial growth was done based on the colony morphology and Gram's staining. Isolation and characterization of bacteria was done as mentioned in Bergey's Manual of Determanative Bacteriology (Holt *et al.,* 1994). The selective media for different bacterial species *viz.* Mac Conkey agar for gram negative bacteria, Eosin Methylene blue (EMB) agar for *E.coli*, Mannitol salt agar for *Staphylococcus spp* and Sheep Blood agar media for *Pasteurella spp* were used in the present study. The motility of the organisms was tested using the hanging drop method. The biochemical tests like Coagulase, Catalase, Oxidase, Urease, Indole, Methyl red, Voges Proskauer and Citrate tests were employed as per the methods described in the Cowan and Steels Manual for the identification of Medical Bacteria (Barrow and Feltham, 2003).

3.3.2 Histopathology

Representative tissue samples collected from different portions of the lungs that showed lesions for histopathological examination were processed by routine paraffin embedding technique. Sections of 4 to 5 μ thickness were cut and stained by Harris Haematoxylin and Eosin method (Luna, 1968). Special staining procedures like Van-Giesson's staining technique (Mc Manus and Mowry, 1964) and Masson's trichrome method (Singh and Sulochana, 1996) were employed for collagen fibers.

3.3.3 Electron microscopic studies

To observe the ultrastructural changes in lung tissues from suspected cases of sheep pox by Transmission electron microscopy (TEM), samples were rinsed in 0.1M phosphate buffer pH 7.2 (PB) to remove blood from the surface. Lung tissues greater than 2 cm long were minced into smaller pieces of approximately 3x3 mm and were fixed in 3% glutaraldehyde in 0.05 M phosphate buffer (p^H 7.2) for 3 hours. It was rinsed twice with phosphate buffer for 10 minutes for each rinse. The tissues were then fixed in 2% aqueous osmium tetraoxide in the same buffer for 2 hours. After post fixation samples were dehydrated in a graded series of ethyl alcohol, infiltrated and embedded in Araldite 6005 resin (Glauert and Glauert 1958 and Mollenhauer, 1959).

Thin sections of 600 nm and ultra thin sections of 60 nm were obtained by use of ultra microtome. Thin sections were mounted on glass slides and stained with toluedine blue for light microscopy (Olympus AX-70) and ultra thin sections

were placed on a copper 200 mesh grid and stained with saturated aqueous uranyl acetate and counter stained with 4% lead citrate (Bozzola and Russell,1998) for transmission electron microscopy (Hitachi,H-7500).

3.3.4 Identification of parasites

The parasites collected from the lungs of sheep were processed as per the standard methods (Soulsby, 2005) for identification.

CHAPTER - IV

RESULTS

4.1 INCIDENCE

In the present investigation, a total of 988 sheep of either sex and of different age groups from slaughter houses located in and around Vijayawada and Tirupathi, postmortems conducted at Department of Veterinary Pathology, N.T.R College of Veterinary Science, Gannavaram and from field mortalities were screened (Table-1) for gross pathological lesions in the lungs. Of which 187 lungs revealed various pathological changes grossly and representative tissue samples were collected from the same for further studies.

Among 988 lungs examined, 187 lungs revealed definite lesions on gross and histopathological examination with an overall incidence of 18.93% (Table-2). The lesions recorded were broadly grouped as abnormalities of inflation in 22 (11.76%), circulatory disturbances in 37 (19.79%), inflammatory conditions in 116 (62.03%), parasitic conditions in 7 (3.74%), neoplastic conditions in 2 (1.07%) and miscellaneous conditions in 3 (1.61%) lungs that are depicted in Chart 1. The details of various predominant pathological lesions observed in sheep lungs are presented in Table 3.

Table 1: Collection of samples

S.No	Sources for Sample collection	No. of samples
01	Slaughter houses located in and around Vijayawada and Tirupathi	957
02	Post mortems conducted at Department of Veterinary Pathology, N.T.R College of Veterinary Science, Gannavaram	14
03	Field mortalities	17
TOTAL		988

Table 2: Incidence of lung lesions in sheep

S.No	Total No. of sheep lungs examined	No. of lungs with lesions (%)
01	988	187 (18.93%)

Table 3: Details of various predominant pathological lesions in sheep lungs

S.No	Type of disease	Number	Percentage (%)
I	**Abnormalities of inflation**	**22**	**11.76**
	1.Pulmonary emphysema	17	9.09
	2.Atelectasis	5	2.67
II	**Circulatory disturbances**	**37**	**19.79**
	1.Pulmonary congestion and haemorrhage	19	10.16
	2.Pulmonary edema	18	9.63
III	**Inflammatory conditions**	**116**	**62.03**
	a. Types of pneumonia	**110**	**58.82**
	1.Bronchopneumonia	105	56.15
	(i) Suppurative bronchopneumonia	98	52.41
	(ii)Fibrinous bronchopneumonia	7	3.74
	2.Interstitial pneumonia	5	2.67
	b. Pleuritis	**6**	**3.21**
IV	**Parasitic conditions**	**07**	**3.74**
	1.Hydatidosis	01	0.53
	2.Pulmonary fasciolosis	06	3.21
V	**Neoplastic conditions**	**02**	**1.07**
	Ovine Pulmonary Adenocarcinoma	2	1.07
VI	**Miscellaneous conditions**	**03**	**1.61**
	1.Sheep pox	2	1.07
	2.Peste Des Petitis Ruminants (PPR)	1	0.54
	TOTAL	**187**	**100**

Chart 1: Incidence of various pathological conditions in sheep lungs

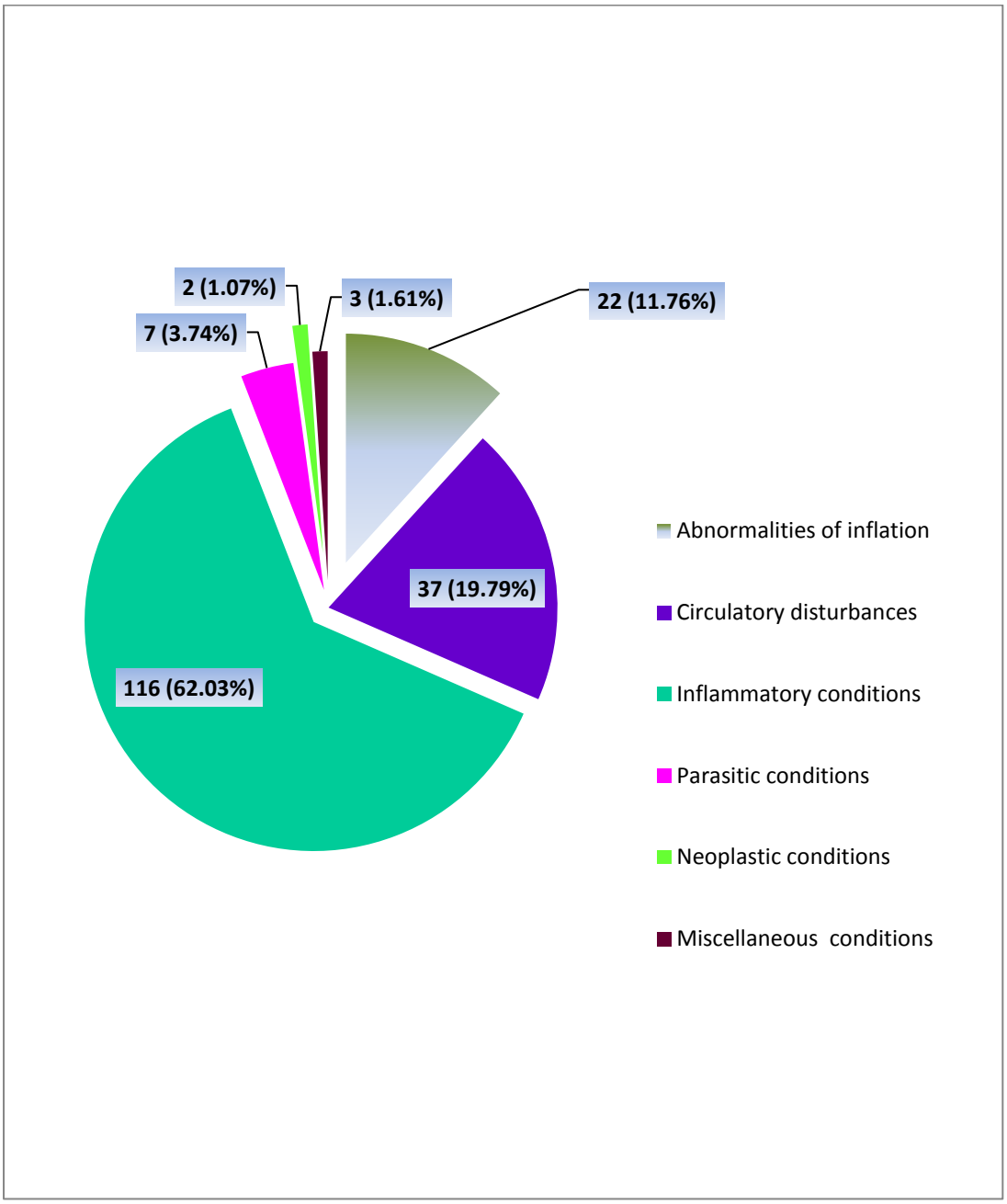
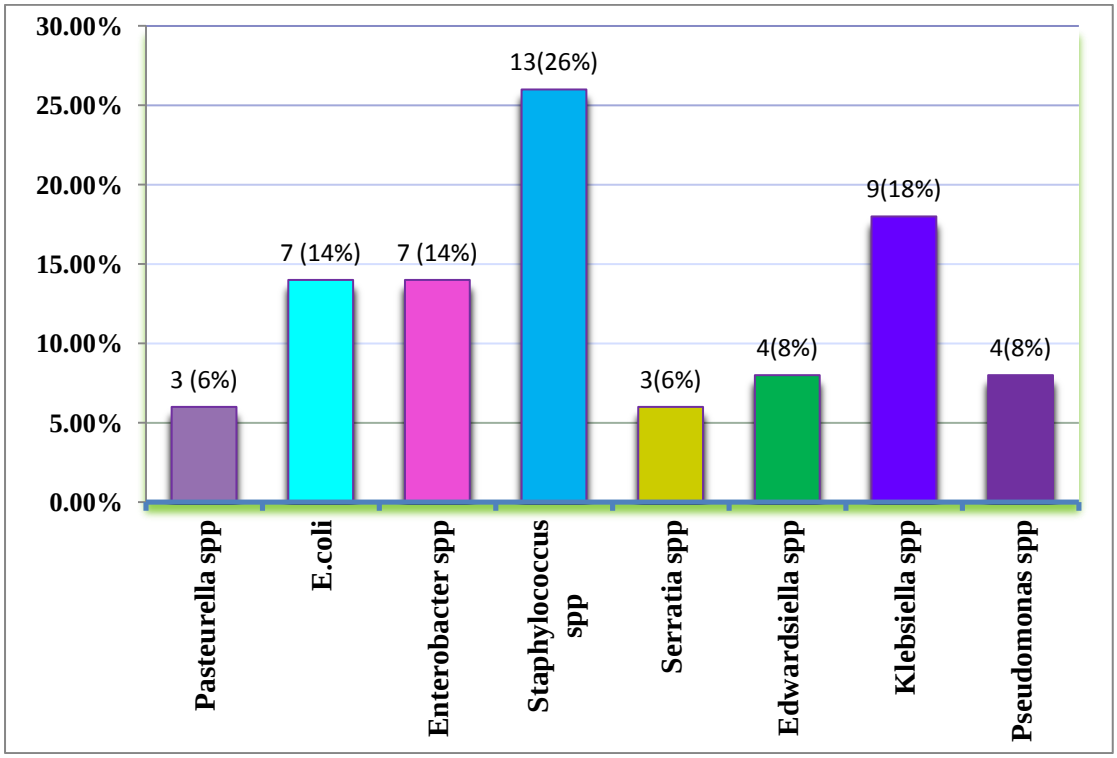


Table 4: Various bacteria isolated from sheep lung lesions

S.No	Name of the bacterium	Number	Percentage (%)
1.	*Pasteurella spp.*	03	6.0
2.	*Escherichia coli*	07	14.0
3.	*Enterobacter spp.*	07	14.0
4.	*Staphylococcus aureus*	13	26.0
5.	*Serratia spp.*	03	6.0
6.	*Edwardsiella spp.*	04	8.0
7.	*Klebsiella spp.*	09	18.0
8.	*Pseudomonas aeruginosa*	04	8.0
	TOTAL	**50**	**100**

Chart 2: Various bacteria isolated from sheep lung lesions



In the present study, swabs from 52 suitable pneumonic lung samples of sheep were subjected for bacterial isolation. Of these 50 samples revealed 8 isolates of bacteria *viz. Pasteurella spp., E.coli, Enterobacter spp., S. aureus, Serratia spp., Edwardsiella spp., Klebsiella spp.* and *P.aeruginosa* (Table- 4 and Chart 2). However no bacteria were isolated from two lung samples.

4.2 PATHOMORPHOLOGY OF LUNG LESIONS

For morphological descriptions, the lesions observed in the present study were categorized broadly into abnormalities of inflation, circulatory disturbances, inflammatory, parasitic, neoplastic and miscellaneous conditions. However, mixed lesions were also observed in some sections.

4.2.1 ABNORMALITIES OF INFLATION

In the present study, abnormalities of inflation were observed in 22 (11.76%) cases out of 187 lungs examined that included pulmonary emphysema and atelectasis.

4.2.1.1 Pulmonary emphysema

Pulmonary emphysema was seen with an incidence of 9.09%. Grossly, sharply defined foci of pale and enlarged emphysematous areas (Fig.1) involving one or more lobes of lungs slightly projecting from the neighbouring areas were observed that showed crepitation and depression upon pressure.

Microscopically, sections of lung revealed distended alveoli and ruptured interalveolar septa forming giant alveoli (Fig. 2&3).

Figure 1 Alveolar emphysema: Note well defined, pale areas in the lung parenchyma.

Figure 2 Lung: Alveolar emphysema - Section showing giant alveoli H&E x 40.

Figure 3 Lung: Alveolar emphysema - Section showing distended alveoli and ruptured interalveolar septa forming giant alveoli H&E x 100.

Figure 4 Atelectasis: Note dark red to bluish areas depressed below the surface of the lung.

Figure 5 Lung: Atelectasis - Section showing slit-like collapsed alveoli with narrow lumina H&E x 40.

Figure 6 Pulmonary congestion and haemorrhage: Note areas of congestion and petechiae scattered throughout the lungs.

4.2.1.2 Atelectasis

Atelectasis was noticed with an incidence of 2.67%. Grossly, dark red to bluish areas, depressed below the surface with firm texture were observed in one or more lobes of the lungs (Fig.4). Microscopically, atelectatic areas revealed slit-like collapsed alveoli with narrow lumen (Fig.5) and with emphysematous foci in the adjacent areas. Alveolar walls appeared parallel and in apposition with no inflammatory change either in the alveoli or in the interstitium.

4.2.2 CIRCULATORY DISTURBANCES

The circulatory disturbances observed were pulmonary congestion, haemorrhage and edema in 37 (19.79%) cases out of 187 lungs examined in the present study.

4.2.2.1 Pulmonary congestion and haemorrhage

Pulmonary congestion and haemorrhage were encountered in 19 (10.16%) cases out of 187 sheep lungs examined. Grossly, areas of congestion and either petechial or ecchymotic haemorrhages were observed throughout the surface in all the lobes of both the lungs (Fig.6). Microscopically, engorged blood vessels and capillaries and areas of haemorrhage in the alveolar spaces were observed (Fig.7).

Figure 7 Lung: Congestion and haemorrhage - Section showing engorged blood vessels and alveolar capillaries and haemorrhages within alveoli H&E x 100.

Figure 8 Edema: Note paleness of the lung.

Figure 9 Edema: Cut section of the lung showing frothy, edematous fluid oozing out from trachea and bronchi.

Figure 10 Lung: Edema - Section showing homogenous, eosinophilic fluid in the alveoli H&E x100.

Figure 11 Acute Suppurative bronchopneumonia: Note cranio-ventral consolidation of the apical and cardiac lobe of the lung.

Figure 12 Acute Suppurative bronchopneumonia: Note mucopurulent exudates in the bronchiole on cut section.

4.2.2.2 Pulmonary edema

Pulmonary edema was observed in 18 (9.63%) cases out of 187 sheep lungs examined. Macroscopically, the affected lungs were pale and heavy (Fig.8). Upon incision, foamy, edematous fluid oozed out from trachea, bronchi and bronchioles (Fig.9). Microscopically, eosinophilic, homogenous fluid was noticed in the alveoli and in the interalveolar septa (Fig.10).

4.2.3 INFLAMMATORY CONDITIONS

The incidence of the inflammatory conditions was 62.03% in the present study which constituted various types of pneumonias and pleuritis.

4.2.3.1 Pneumonia

The incidence and types of pneumonia observed in the present study were described.

4.2.3.1.1 Incidence of pneumonia

The incidence of pneumonia observed in the present study was 58.82% (110 cases) out of 187 lungs examined.

4.2.3.1.2 Types of pneumonia

Based on morphologic changes observed, the lesions of pneumonia were grouped into bronchopneumonia and interstitial pneumonia.

Figure 13 Lung: Acute Suppurative bronchopneumonia - Section showing exudates within lumen of the alveoli H&E x 100.

Figure 14 Lung: Acute Suppurative bronchopneumonia - Section showing exudates consisting of abundant neutrophils within lumen of the alveoli H&E x 400.

Figure 15 Lung: Acute Suppurative bronchopneumonia - Section showing exudates in the lumen of the bronchi H&E x 40.

Figure 16 Lung: Acute Suppurative bronchopneumonia - Section showing purulent exudates in the lumen of the alveoli, bronchi and bronchiole H&E x 40.

Figure 17 Chronic suppurative bronchopneumonia - Note multiple purulent foci in the lung.

Figure 18 Chronic suppurative bronchopneumonia - Note the yellowish cheesy exudate in the cut section of the purulent foci in the lung.

4.2.3.1.2.1 Bronchopneumonia

In the present study, 105 (56.15%) cases of bronchopneumonia were noticed that were further subdivided into suppurative bronchopneumonia (98 cases) and fibrinous bronchopneumonia (7 cases) based on the type of exudate present.

4.2.3.1.2.1.1 Suppurative bronchopneumonia

In the present study, suppurative bronchopneumonia was observed with an incidence of 52.41% of which 64 (34.22%) were of acute and 34 (18.18%) were of chronic nature. In acute suppurative bronchopneumonia firm, enlarged lungs with cranio-ventral consolidation (Fig.11) were seen grossly. Consolidation was seen mostly on the apical lobe of both the lungs while in a few cases, diaphragmatic lobe was also involved. Cut section revealed pale and dark areas with mucopurulent exudates in the airways (Fig.12). Microscopically, the lesions were characterized by dilated and severely congested alveolar capillaries and edema of alveoli. Inflammatory cellular exudates with abundant neutrophils, few macrophages and cellular debris were present within the lumen of the alveoli (Fig.13&14), bronchi (Fig.15) and bronchioles (Fig.16). The cellular exudates were necrotic in some of the cases. Few bronchi and bronchioles revealed desquamation of the lining epithelium. Acute bronchiolitis with purulent exudates plugging the lumen and infiltration with inflammatory cells in and around the bronchiolar wall was also observed.

Figure 19 Lung: Chronic suppurative bronchopneumonia - Section showing mononuclear cells and few neutrophils in the lumen of the bronchi H&E x 100.

Figure 20 Lung: Chronic suppurative bronchopneumonia - Section showing an abscess in the parenchyma of lung H&E x 40.

Figure 21 Lung: Chronic suppurative bronchopneumonia - Section showing proliferation of fibrous connective tissue around the abscess H&E x 40.

Figure 22 Lung: Chronic suppurative bronchopneumonia - Section showing proliferation of fibrous connective tissue around the abscess Van-Giesson's stain x 40.

Figure 23 Lung: Chronic suppurative bronchopneumonia - Section showing thickened interlobular septa with extensive fibrous connective tissue proliferation H&E x 40.

Figure 24 Fibrinous bronchopneumonia: Note the surface of lung covered with yellowish fibrinous layer.

In few cases, peribronchiolar lymphoid hyperplasia was noticed with adjacent areas of emphysema and partial collapse.

In chronic suppurative bronchopneumonia, the lungs showed cranio-ventral consolidation involving the apical lobes mostly and diaphragmatic lobes in some cases. Multiple peanut to walnut sized abscesses were noticed distributed in one or all the lobes of either one or both the lungs (Fig.17). Cut surface of the abscess revealed dirty gray to yellowish, cheesy and inspissated pus (Fig.18). The pleura was thickened in most of the cases. Microscopically, infiltration of polymorphs, a few mononuclear cells and macrophages in the lumen of alveoli, bronchi (Fig.19) and bronchioles was seen along with thickening of alveolar septa due to fibrosis. Multiple large sized abscesses that consisted of degenerated neutrophils mostly were noticed in few cases (Fig.20). Fibrous tissue proliferation around the abscess was also evident (Fig.21&22). Fibrosis and infiltration of inflammatory cells were observed in the interlobular septa (Fig. 23). Atelectasis and edema were also observed in adjacent areas.

In the present study *S. aureus, Pasteurella spp., Klebsiella spp., E. coli, Enterobacter spp.* and *P. aeruginosa* were isolated from the suppurative bronchopneumonia cases.

4.2.3.1.2.1.2 Fibrinous bronchopneumonia

Fibrinous bronchopneumonia was observed in 7 (3.74%) cases. Grossly, the lungs appeared to be enlarged, heavy and firm with cranio-ventral

Figure 25 Fibrinous bronchopneumonia: Note fibrinous adhesions to the thoracic wall and deposition of yellowish fibrinous material on the lung.

Figure 26 Lung: Fibrinous bronchopneumonia - Section showing large number of mononuclear cells and fibrin within the alveoli H&E x 100.

Figure 27 Lung: Fibrinous bronchopneumonia - Section showing exudate within the lumen of the bronchiole and alveoli H&E x 100.

Figure 28 Lung: Fibrinous bronchopneumonia - Section showing abundant eosinophilic fibrin in the interlobular septa H&E x 100.

Figure 29 Lung: Fibrinous bronchopneumonia - Section showing thickened pleura with fibrinous exudates H&E x 40.

Figure 30 Interstitial pneumonia: Note lungs with rib impressions on the surface.

consolidation. A yellowish fibrinous layer covered the surface of the lungs (Fig.24) which on cut section revealed fibrinous exudation. In two cases, greyish white thickened pleura with fibrinous adhesions with the thoracic wall were noticed (Fig.25).

Microscopically, the lumen of alveoli was filled with fibrino-cellular exudates that consisted of predominantly polymorphonuclear cells, few mononuclear cells and fibrin along with severe congestion of blood vessels (Fig.26). Desquamation of epithelial lining of the bronchi and bronchioles was observed in few cases and the bronchial lumen contained cellular exudates composed of fibrin, few neutrophils and large number of mononuclear cells (Fig.27). The interlobular septa were dilated, edematous and filled with fibrin (Fig.28). Marked thickening of pleura was found with fibrinous exudates containing polymorphonuclear cells, fibrin and edema fluid along with proliferation of fibrous connective tissue (Fig.29).

In the present study, various types of bacteria isolated from fibrinous bronchopneumonic cases were *Pasteurella spp.*, *E. coli. Edwardsiella spp.* and *Serratia spp.*

4.2.3.1.2.2 Interstitial pneumonia

Interstitial pneumonia was noticed with an incidence of 2.67%. Grossly, the lungs were pale to red, heavy and firm, that failed to collapse when thorax was opened. The lungs showed rib impressions on the surface (Fig.30). Cut section revealed a slightly meaty appearance.

Microscopically, the alveoli were distorted in shape. The alveolar septae were congested and thickened with infiltration of mononuclear cells, and macrophages (Fig.31). Hyperplasia of bronchial and bronchiolar epithelium into the lumen was observed along with lymphoid hyperplasia in the peribronchiolar regions (Fig.32). At some places, the alveolar epithelium revealed foetalization.

Various types of bacteria isolated from the cases of interstitial pneumonia were *Edwardsiella spp.* and *Klebsiella spp.*

4.2.3.2 Pleuritis

Pleuritis was observed in 6 (3.21%) cases out of 187 sheep lungs examined. Grossly, thickened pleura with glistening surface was seen which was hard to cut (Fig.33). Microscopically, the pleura was edematous and revealed increased proliferation of fibrous connective tissue with infiltration of lymphocytes (Fig. 34&35).

4.2.4 PARASITIC CONDITIONS

In the present study, parasitic conditions were observed in 7 (3.74%) cases out of 187 lungs examined which included pulmonary hydatidosis and fasciolosis.

4.2.4.1 Pulmonary Hydatidosis

A single case (0.53%) of pulmonary hydatidosis was noticed out of 187 sheep lungs examined. Grossly, a single, large sized, unilocular cyst was

Figure 37 Cut section of hydatid cyst: Note white translucent membrane attached to the cyst wall with grape sized brood capsules.

Figure 38 Cut section of fertile hydatid cyst: Note multiple, cream colored brood capsules attached to the membrane lining the cyst.

Figure 39 Eosin stained hydatid cyst fluid: Note protoscolices in various stages of development x 100.

Figure 40 Lung: Hydatid cyst - Section showing eosinophilic laminated layer H&E x 40.

Figure 41 Lung: Hydatid cyst - Section showing fibrous connective tissue around the cyst. Van- Giesson's stain x 40.

Figure 42 Fasciolosis: Note multiple dark haemorrhagic areas in the lung.

observed in the diaphragmatic lobe that was embedded deeply in the lung parenchyma (Fig.36). The cyst was soft to touch and contained a clear watery fluid. On cut section, small to grape sized, cream colored brood capsules attached to the white translucent membrane and also free in the cyst fluid were noticed (Fig.37 & 38). Microscopic examination of the cyst fluid stained with eosin revealed protoscolices in various stages of development (Fig.39).

On microscopic examination, hydatid cyst consisted of slight eosinophilic fluid and an eosinophilic, acellular laminated layer (Fig.40) that was lined by degenerated germinal epithelium. An inflammatory zone comprising of lymphocytes and macrophages was seen enclosed by fibrous connective tissue (Fig.41). The adjacent lung parenchyma showed atelectasis and compensatory emphysema.

4.2.4.2 Pulmonary Fasciolosis

In the present investigation, pulmonary fasciolosis was observed in 6 (3.21%) cases out of 187 sheep lungs examined. On gross examination, lungs showed multiple dark red haemorrhagic areas which were slightly depressed below the surface (Fig.42). Focal areas of consolidation and emphysema were noticed in the surrounding lung tissue. Cut section revealed dark, haemorrhagic tracts with flukes embedded inside the parenchyma (Fig.43) and also in the bronchi. The parasites were identified as *Fasciola gigantica.*

Microscopically, sections of fluke surrounded by haemorrhagic exudates were observed within the bronchus (Fig.44) and in the lung

Figure 43 Fasciolosis: Cut section of the lung showing fluke in the haemorrhagic tract.

Figure 44 Lung: Fasciolosis - Cut section of the fluke in the bronchial lumen and haemorrhages in the alveoli H&E x 40.

Figure 45 Lung: Fasciolosis - Cut section of the fluke in the lung parenchyma with haemorrhages and cellular infiltration in the alveoli H&E x 40.

Figure 46 Lung: Fasciolosis - Section showing neutrophils, erythrocytes and mononuclear cells within the bronchiole H&E x 100.

Figure 47 Lung: Fasciolosis - Note polymorphs, erythrocytes and few mononuclear cells within the bronchiole H&E x 400.

Figure 48 Lung: Fasciolosis - Section showing haemorrhagic tract with atelectasis, edema, haemorrhages and compensatory emphysema of surrounding alveoli H&E x 40.

parenchyma (Fig.45). Large number of mononuclear cells, eosinophils, few neutrophils and macrophages laden with hemosiderin granules were present in the adjacent alveoli. The affected bronchi and bronchioles also revealed similar type of cellular infiltrations along with desquamation of epithelium into the lumen (Fig.46&47). Haemorrhages, atelectasis, edema and emphysema were also noticed in the surrounding lung parenchyma (Fig.48).

4.2.5 NEOPLASTIC CONDITIONS

4.2.5.1 Ovine Pulmonary Adenocarcinoma

In the present study, two cases (1.07%) of Jaagsiekte were noticed among the 187 lungs examined. Grossly, the lungs showed increase in size and weight and were grayish white in color (Fig.49). Cut section revealed meaty appearance with moist surface (Fig.50). Microscopically, there were papillary growths of tumor cells in to the affected alveoli (Fig.51). In addition, papillary and polypoid proliferation of bronchiolar epithelium that partially filled the lumen was also noticed in the bronchiole (Fig.52). The lumen of the alveoli contained macrophages, lymphocytes, plasma cells and neutrophils. There were variable amounts of dense fibrous connective tissue which surrounded and divided the neoplastic foci into lobules (Fig.53). In one case, there was an abscess surrounded by a fibrous capsule adjacent to the affected alveolar tissue (Fig.54)

4.2.6 MISCELLANEOUS CONDITIONS

In the present study, sheep pox (2 cases) and PPR (1 case) observed were included under miscellaneous conditions and the lung lesions were described.

4.2.6.1 Sheep pox

Sheep pox was observed with an incidence of 1.07%. In the affected animals, typical pock lesions with ulceration were observed on the face at the mandibular region (Fig. 55). On gross examination of the lungs, numerous circular, well demarcated, discrete to coalescing pock nodules of varying sizes were found distributed throughout the parenchyma (Fig.56). Cut section of the nodule was gray, firm and surrounded by edematous areas of lung (Fig.57). Microscopically, proliferative alveolitis (Fig.58) and bronchiolitis (Fig.59) giving the appearance of gland-like structure was observed in the parenchyma. Interalveolar septa was thickened and infiltrated with lymphocytes and macrophages. Severe congestion of blood vessels and focal areas of necrosis were noticed (Fig.60). Alveolar epithelium showed areas of foetalization (Fig.61). Eosinophilic intra-cytoplasmic inclusions were noticed in the pulmonary macrophages and in bronchial epithelial cells (Fig.62). Further, sheep pox was also confirmed in the samples by PCR examination (The lung samples were found positive by PCR conducted for sheep pox genome (237bp) at NIVEDI, Bangalore).

Figure 55 Sheep pox: Note typical pox nodules on the face with ulceration.

Figure 56 Sheep pox: Note numerous circular, well demarcated, discrete to coalescing pock nodules of varying sizes distributed throughout the lung parenchyma.

Figure 57 Sheep pox: Cut section of the nodules showing grey and firm appearance.

Figure 58 Lung: Sheep pox - Section showing proliferative alveolitis and infiltration of sheep pox cells, lymphocytes in alveolar spaces H&E x 100.

Figure 59 Lung: Sheep pox - Section showing proliferation of bronchial epithelium into bronchial lumen H&E x 40.

Figure 60 Lung: Sheep pox - Note an area of necrosis surrounded by areas of alveolar emphysema H&E x 40.

The ultrastructural changes noticed in the affected lung by, TEM included macrophages with irregularly shaped nucleus (Fig.63), distorted endoplasmic reticulum and cytoplasm containing immature viral particles seen as electron dense particles (Fig.64). Nuclear changes like karyorrhexis and margination of chromatin (Fig.65) were also seen in the affected macrophages.

4.2.6.2 Peste Des Petitis Ruminants (PPR)

During screening of field mortalities one animal (0.54%) exhibited lesions of PPR. In the affected animal, erosive and necrotic lesions on the tongue and gums were observed (Fig. 66). At necropsy, lungs revealed diffuse areas of congestion and consolidation in the apical and diaphragmatic lobes (Fig.67) and the trachea was filled with froth (Fig.68). Microscopically, severe congestion of blood vessels and alveolar capillaries with varying degrees of infiltration with mononuclear cells, polymorphs and macrophages in the lumen of alveoli and bronchi were noticed. Several distinct giant cells were observed in the alveolar spaces (Fig.69&70). Bronchial epithelium revealed mild hyperplasia with desquamation of epithelial cells, polymorphonuclear cells, mononuclear cells and giant cells in the bronchial lumen (Fig.71). Moderate peribronchial cuffing with lymphoid cells was seen at several places in the lung parenchyma. Further, PPR was also confirmed in this animal by VBRI, Hyderabad.

Figure 61 Lung: Sheep pox - Section showing foetalization of alveolar epithelium H&E x 400.

Figure 62 Lung: Sheep pox - Section showing eosinophilic intracytoplasmic inclusion bodies in bronchial epithelium H&E x 100.

Figure 63 Ultrastructural changes Sheep pox lung: Note irregular shaped nucleus and presence of immature viral particles in the cytoplasm of macrophages x 9650.

Figure 64 Ultrastructural changes Sheep pox lung: Note irregular shaped nucleus and presence of immature viral particles in the cytoplasm of macrophages x 23160.

Figure 65 Ultrastructural changes: Sheep pox lung- Note nuclear changes like karyorrhexis and margination of chromatin in the macrophage x 15440.

Figure 66 PPR: Note erosive and necrotic lesions on the tongue and gums.

Figure 67 PPR: Note diffuse congestion and consolidation of the lungs.

Figure 68 PPR: Cut section of the lung showing frothy edematous fluid in the trachea.

4.3 BACTERIOLOGICAL STUDIES

In the present study, 52 samples from pneumonic sheep lungs were subjected for bacterial isolation. Out of these, primary pathogens were isolated from 50 lung samples and from two samples no bacteria could be isolated. A total of eight isolates of different bacterial spp. were identified *viz; Staphylococcus aureus* in 13 (26%) followed by *Klebsiella spp.* in 9 (18%), *Escherichia coli* and *Enterobacter spp.* in 7(14%) each, *Edwardsiella spp.* and *P. aeruginosa* in 4 (8%) samples, *Pasteurella spp.* and *Serratia spp.* in 3 (6%) samples each.

In the present study, *Staphylococcus aureus* (Fig.72) was the major organism isolated from suppurative bronchopneumonia and also from OPA with abscesses. *E.coli* (Fig. 73), *Edwardsiella spp.*, *Pasteurella spp.* and *Serratia spp.* were isolated from cases of suppurative and fibrinous bronchopneumonia whereas *Klebsiella spp.* and *P.aeruginosa* were (Fig. 74) isolated from cases of suppurative and interstitial pneumonia.

CHAPTER - V

DISCUSSION

In India, sheep farming is one of the major contributors to meat production. The ovine respiratory system constituting the most extensive surface gets exposed directly to the environment leading to damage of lungs by various infectious and non-infectious agents that produce significant lesions. Spontaneously occurring lung lesions in sheep were studied previously by a few workers in India and abroad (Chattopadhyay *et al.,* 1986, Kamil and Parihar 1990, Kumar *et al.,* 2005, Dar *et al.,* 2013a, Priyadarshi *et al.,* 2013 and Regassa *et al.,* 2013). Though, the occurrence of pneumonia was well documented, reports on pathomorphological conditions of lungs in sheep particularly from the state of Andhra Pradesh are scanty. Hence, the present investigation was undertaken to study the incidence and to describe the gross and histopathological changes of various spontaneously occurring lesions in lungs of sheep. Besides, an attempt was also made for isolation of bacteria from possible cases.

In the present investigation, a total of 988 sheep of either sex and of different age groups from slaughter houses located in and around Vijayawada and Tirupathi, from animals necropsied in the Department of Veterinary Pathology, Gannavaram and also from field mortalities were screened for the presence of various pathological lesions in the lungs. Of these, from 187 lungs that showed gross abnormalities, representative tissue samples were collected for further studies and swabs were collected for bacterial isolations aseptically from lungs that showed

gross pneumonic lesions. The parasites observed were collected in normal saline and were identified on the basis of their morphological characters. Few lung tissues were collected from suspected cases of sheep pox for ultrastructural studies by TEM.

In the present study, out of 988 lungs screened, 187 (18.93%) lungs revealed definite lesions on gross and microscopic examination that were broadly grouped into abnormalities of inflation 22 (11.76%), circulatory disturbances 37 (19.79%), inflammatory conditions 116 (62.03%), parasitic conditions 7 (3.74%), neoplastic conditions 2 (1.07%) and miscellaneous conditions 3 (1.61%).

In the present study, an overall incidence of 18.93% of lung lesions was observed in sheep that was in conformity with the earlier reports of Kumar *et al.,* (2005) and Priyadarshi *et al.,* (2013) with an incidence of 17.66% and 15.28% respectively. A higher incidence of lung lesions than the present study were noticed by Rahman and Iyer (1979), Chattopadhyay *et al.,* (1986), Belkhiri *et al.,* (2012), Dar *et al.,* (2013a) and Belkhiri *et al.,* (2014) whereas a lower incidence was reported by Kamil and Parihar (1990), Beytut *et al.,* (2002) and Mellau *et al.,* (2010) in sheep.

The percentage of lung lesions observed in the present study indicated that considerable number of sheep were affected by pulmonary lesions in the area under study. The variation in the incidence might be due to the influence of age, sex, breed, managemental practices and the difference in the environment in which the sheep were reared. Also, the present investigation included material from field

mortalities and post mortems apart from slaughter house samples whereas most of the earlier reports were based on slaughter house samples alone and that might have also influenced the incidence observed in the present study.

Abnormalities of inflation were observed in the present study with an incidence of 11.76% that included pulmonary emphysema and atelectasis.

Incidence of pulmonary emphysema (9.09%) noticed in the present investigation was lower than the previous reports by Sriraman and Rama rao (1980), Chattopadhyay *et al.,* (1986), Priyadarshi *et al.,* (2013) and Regassa *et al.,* (2013) and higher than that reported by Kamil and Parihar (1990), Kumar *et al.,* (2005), Dar *et al.,* (2013a) and Belkhiri *et al.,* (2014).

In the present study, emphysema of lungs revealed sharply defined foci of pale, enlarged areas projecting from surface involving one or more lobes of lungs grossly and distension and rupture of alveolar walls forming variably sized giant alveoli microscopically which were in accordance with the findings of Kumar *et al.,* (2005) and McGavin and Zachary, (2007). However, puffy lungs with rib imprints observed grossly by Priyadarshi *et al.,* (2013) were not evident in the present study.

Atelectasis was observed with an incidence of 2.67% in the present study. Perusal of the literature revealed a higher incidence of 5.14% (Kumar *et al.,* 2005) and a lower incidence of 1.0% and 2.55% by Sriraman and Rama rao (1980) and Belkhiri *et al.,* (2014) respectively in sheep.

Grossly, dark red to bluish areas depressed below the surface with firm texture were noticed in the present study that revealed slit-like, collapsed alveoli with narrow lumen microscopically. These findings were in accordance with the description of Kumar *et al.,* (2005). Obstructive atelectasis, also known as resorption atelectasis is common in animal species with poor collateral ventilation like sheep and is due to obstruction of air from reaching distal airways because of blockage by exudate (McGavin and Zachary, 2007).

In the present study circulatory disturbances were observed in 37 cases (19.79%) that included pulmonary congestion and haemorrhage and pulmonary edema.

Pulmonary congestion and haemorrhage was encountered with an incidence of 10.16% in the present study which was similar with the earlier report of 9.84% by Dar *et al.,* (2013a) in India. However, the incidence observed was lower compared to the findings of Sriraman and Rama rao (1980) and Priyadarshi *et al.,* (2013) and higher compared to that of Kumar *et al.,* (2005), Regassa *et al.,* (2013) and Belkhiri *et al.,* (2014).

In the present study, lesions of congestion and haemorrhage comprised of red, patchy areas and petechial and ecchymotic haemorrhages grossly that showed engorged blood vessels and erythrocytes in the lumen of the alveoli microscopically. The lesions observed were in accordance with the observations of Kumar *et al.,* (2005) and Priyadarshi *et al.,* (2013).

Pulmonary congestion is always a passive process resulting from defective venous return from the lungs. In congestion, the vascular endothelium undergoes degenerative changes due to inadequate amounts of oxygen and nutrients. The damaged vascular wall may rupture and cause haemorrhages too. Pulmonary haemorrhage might be due to trauma, congestive heart failure, infectious diseases, DIC, coagulopathies and septicemias etc. Pulmonary haemorrhages are always serious, which may fill and obstruct the airways, preventing the entry of air with resultant suffocation of the animal (Thomson, 1988).

In the present study, an incidence of 9.63% of pulmonary edema observed in sheep was similar to the earlier report of 10.90% by Priyadarshi *et al.*, (2013). However, Sriraman and Rama rao (1980), Kumar *et al.,* (2005), Dar *et al.*, (2013a) and Belkhiri *et al.,* (2014) reported a lower incidence of pulmonary edema in slaughtered sheep.

Grossly, pale and heavy lungs that revealed foamy, edematous fluid on section and presence of homogenous, eosinophilic fluid in the alveoli microscopically were observed in pulmonary edema cases. The gross and microscopic findings were in agreement with the earlier reports of Kumar *et al.*, (2005) and Priyadarshi *et al.*, (2013).

Alveolar edema always accompanies viral diseases, toxic pulmonary diseases, exposure to bacterial toxins and anaphylactic shock. Permeability edema may be due to damage in the endothelium and pneumocytes or due to release of

chemical mediators of inflammation or it might be because of exposure to bacterial toxins, DIC etc. (McGavin and Zachary, 2007).

In the present study, inflammatory conditions were noticed with an incidence of 62.03% that included pneumonia and pleuritis.

The incidence of pneumonia observed was the highest among the lung lesions in the present study accounting for 58.82%. This finding was in accordance with that of Priyadarshi *et al.,* (2013) who recorded 59.39% pneumonia and also opined that pneumonia as the main pulmonary lesion in sheep was pneumonia. However, the incidence observed in the present study was higher than 30.09% and 48.84% reported by Bhagwan and Singh (1972) and Sriraman and Rama rao (1980) from India and 5.8%, 2.54% and 12.9% by Beytut *et al.*, (2002), Ezzi *et al.*, (2007) and Regassa *et al.*, (2013) respectively from abroad.

In the present study, the types of pneumonia observed were bronchopneumonia and interstitial pneumonia based on pathomorphological features of lungs. Usually, pneumonias in animals have been classified or named based on the course, type of exudation, morphologic features, distribution of lesions, epidemiologic attributes, geographical regions and miscellaneous attributes (McGavin and Zachary, 2007 and Jubb *et al.*, 2007).

The overall incidence of bronchopneumonia in the present study was 56.15% and it was in agreement with the reports of Priyadarshi *et al.*, (2013) who recorded 53.94% of bronchopneumonia in sheep. Incidences of 90.27%, 67.42% and 32.02% of bronchopneumonia were also reported previously by Sriraman and

Rama rao (1980), Kumar *et al.,* (2005) and Mellau *et al.,* (2010) respectively. Bronchopneumonia was stated the most common type of pneumonia seen in domestic animals by McGavin and Zachary (2007).

Based on the predominance of exudates, bronchopneumonia in the present study has been classified further into suppurative and fibrinous types.

In the present study, suppurative bronchopneumonia was observed with an incidence of 52.41% which was similar to the earlier report of 45.24% by Azizi *et al.,* (2013). In contrast to the present observation, a lower incidence was reported by Sriraman and Rama rao (1980), Kumar *et al.,* (2005), Yesuf *et al.,* (2012) and Priyadarshi *et al.,* (2013).

Suppurative bronchopneumonia observed in the present study included acute and chronic types based on the cellular component of the exudate.

Acute suppurative bronchopneumonia (34.22%) revealed firm and enlarged lungs with cranio-ventral consolidation grossly. On cut section mucopurulent exudates in the airways was noticed. Microscopically, dilated and severely congested alveolar capillaries and edematous changes within the alveoli were seen. Inflammatory cellular exudates consisting of abundant neutrophils, few macrophages and cellular debris within the lumen of the alveoli, bronchi and bronchioles were present. Acute bronchiolitis with purulent exudates in the lumen and infiltration with inflammatory cells in and around the bronchiolar wall was observed.

The gross and microscopic findings observed in the present study were in agreement with the findings of Bhagwan and Singh (1972), Kumar *et al.,* (2005), Oruc (2006), Azizi *et al.,* (2013), Dar *et al.,* (2013b) and Priyadarshi *et al.,* (2013).

Chronic suppurative bronchopneumonia was observed with an incidence of 18.18%. Multiple purulent foci with yellowish purulent material on cut section were noticed. Microscopically, focal areas of suppuration surrounded by pyogenic membrane with polymorphonuclear cell infiltration and fibrous tissue encapsulation around the abscess were observed.

The gross and microscopic findings observed in acute and chronic suppurative bronchopneumonia were in agreement with the reports of Dar *et al.,* (2012b), Azizi *et al.,* (2013) and Dar *et al.,* (2013b).

Leucocyte recruitment may be because of release of chemotactic factors in response to alveolar injury or chemotactic effect of bacterial toxins. The purulent or mucopurulent exudate may obliterate completely the lumen of bronchi and alveoli resulting in loss of air spaces (McGavin and Zachary, 2007).

In the present study, from 32 cases of suppurative bronchopneumonia, *S.aureus* was isolated majorly followed by *Klebsiella spp., E.coli, Enterobacter spp., P. aeruginosa* and *Pasteurella spp.* Beytut *et al.,* (2002) and Yesuf *et al.,* (2012) also found *S. aureus* as the most frequent organism isolated from pneumonic sheep lungs.

In the present study, fibrinous bronchopneumonia was observed with an incidence of 3.74%. A lower incidence of 0.55% and 0.61% were recorded earlier

by Sriraman and Rama rao (1980) and Priyadarshi *et al.,* (2013) respectively. However, a higher incidence compared to the present study were reported by Kumar *et al.,* (2005), Oruc (2006), Yesuf *et al.,* (2012) with an incidences of 13.71, 26.72 and 39.02 respectively.

Grossly, the lungs were enlarged, heavy and firm with cranio-ventral distribution. A fibrinous layer covered the surface of the lungs which on cut section revealed fibrinous exudation. In two cases, greyish white, thickened pleura with fibrinous adhesions with the thoracic wall were noticed. Microscopically, the lumen of alveoli, bronchi and bronchioles were filled with fibrino-cellular exudate with desquamation of epithelial lining. The interlobular septa were dilated, edematous and filled with fibrin. Marked thickening of pleura due to fibrinous exudates containing polymorphonuclear cells, fibrin and edema fluid along with proliferation of fibrous connective tissue was also recorded.

The gross and microscopic lesions observed in the present study was akin to the previous reports by Kumar *et al.,* (2005), Oruc (2006), Dar *et al.,* (2012b), Azizi *et al.,* (2013) and Priyadarshi *et al.,* (2013). Greyish white, thickened pleura with fibrinous adhesions were described earlier by Kumar *et al.,* (2005) and Dar *et al.,* (2012b).

A more severe lung injury usually results in fibrinous bronchopneumonia and therefore is more life threatening. Fibrin is chemotactic for neutrophils and hence neutrophils are always evident in areas undergoing fibrinous inflammation (McGavin and Zachary, 2007).

In the present study, *Pasteurella spp.* was the major organism isolated followed by *E.coli, Edwardsiella spp.* and *Serratia spp.* from lesions of fibrinous bronchopneumonia. Kumar *et al.,* (2005), Odugbo *et al.,* (2006), Ezzi *et al.,* (2007) and Ozyildiz *et al.,* (2013) also reported isolation of *Pasteurella spp.* from the sheep lungs with fibrinous bronchopneumonia.

In the present investigation, interstitial pneumonia was observed with an incidence of 2.67%. The occurrence in the present study was low when compared to the earlier reports of Bhagwan and Singh (1972), Oruc (2006), Yesuf *et al.*, (2012), Azizi *et al.,* (2013), Dar *et al.,* (2013a), Dar *et al.,* (2013b), Priyadarshi *et al.,* (2013) and Belkhiri *et al.*, 2014) and high when compared to the previous reports of Sriraman and Rama rao (1980) and Chattopadhyay *et al.*, (1986).

Grossly, the lungs were pale, heavy and firm, failed to collapse when thorax was opened and showed rib impressions on the surface and appeared meaty on cut section. Microscopically, distortion of alveoli, congestion and thickening of septa with infiltration of mononuclear cells and lymphoid hyperplasia in the peribronchiolar regions were observed along with bronchial and bronchiolar epithelial hyperplasia and alveolar epitheliolization.

The gross and microscopic findings in the present study were in accordance with Bhagwan and Singh (1972), Dar *et al.,* (2013b), Priyadarshi *et al.,* (2013) from India and Oruc (2006) and Azizi *et al.*, (2013) from abroad.

Usually interstitial pneumonia can result from aerogenous injury to the alveolar epithelium or from hematogenous injury to the alveolar capillary

endothelium or alveolar basement membrane. Elastic nature or meaty appearance of the lungs observed in interstitial pneumonia can be attributed to the thickening of alveolar walls resulted in differentiation and replacement of destroyed type-I pneumocytes by the progenitors *viz.* type II pneumocytes. (McGavin and Zachary, 2007).

In the present study, from 2 cases of interstitial pneumonia *Klebsiella spp.*, and *Pseudomonas spp.* were isolated similar to that reported by Azizi *et al.*, (2013).

In the present study, 6 (3.21%) cases manifested pleuritis which was higher when compared with the reports of Chattopadhyay *et al.*, (1986) and Azizi *et al.*, (2013).

In this study, the affected areas revealed excessive pleural thickening with glistening surface grossly and edema, infiltration with numerous lymphocytes and proliferation of fibrous connective tissue microscopically. The gross and histological picture in the present study was in agreement with the findings of Dar *et al.*, (2012d).

Usually, pleuritis may be due to trauma or infectious agent as that of pneumonia, but most possibly it is of bacterial cause, through hematogenous route or blood borne seeding (McGavin and Zachary, 2007).

In the present study, an overall incidence of parasitic conditions noticed was 3.74% which included pulmonary hydatidosis and fasciolosis.

A solitary case of pulmonary hydatidosis (0.53%) was observed in the present study. Perusal of literature revealed that pulmonary hydatidosis was frequently reported with varying incidence rates and was most common in the lungs of sheep (Sriraman and Rama rao 1980, Chattopadhyay *et al.,* 1986, Kamil and Parihar 1990, Das *et al.,* 1998, Dalimi *et al.,* 2002, Raman and John 2003, Ahmed *et al.,* 2006, Azlaf and Dakkak 2006, Scala *et al.,* 2006, Sangaran and John 2009, Belkhiri *et al.,* 2012, Daniel Getachew *et al.,* 2012, Fikire *et al.,* 2012, Helina Getachew *et al.,* 2012, Iqbal *et al.,* 2012, Jarjees and Al-Bakri 2012, Kouidri *et al.,* 2012, Priyadarshi *et al.,* 2013, Regassa *et al.,* 2013, Valiyeva *et al.,* 2013 and Belkhiri *et al.,* 2014).

The variation in the incidence rate might be attributed to several factors such as age, different managemental practices, relationship with dogs and unhygienic conditions of the environment (McGavin and Zachary, 2007).

Grossly, a single large sized unilocular cyst embedded deeply in the lung parenchyma of diaphragmatic lobe was observed in the present study. The cyst was soft to touch and contained a clear watery fluid and on section, small, grape sized brood capsules escaped. Microscopically, the hydatid cyst consisted of slight eosinophilic fluid and an eosinophilic, acellular laminated layer lined by degenerated germinal epithelium and an inflammatory zone comprising of lymphocytes, eosinophils, macrophages enclosed by fibrous connective tissue. Adjacent areas showed atelectasis and compensatory emphysema. Similar gross and histopathological changes were noticed by Kamil and Parihar (1990), Valiyeva *et al.,* (2013) and Priyadarshi *et al.,* (2013).

The incidence of pulmonary fasciolosis was found to be 3.21% in the present investigation that was akin to the observations of Maraqa *et al.,* (2005). A lower incidence of 0.29% by Kamil and Parihar (1990) and a higher incidence of 5.29% by Soundararajan and Iyue (2005) were also recorded previously. The aberrant location of *F. gigantica* in the lung was also noticed by Kamil and Parihar (1990), Pandit *et al.,* (1991), Rao and Madhubala (1998), Yoshihara *et al.,* (1998), Maraqa (2005) and Soundararajan and Iyue (2005).

In the present study, lungs affected with *F.gigantica* revealed dark red haemorrhagic areas grossly and haemorrhagic tracts with flukes embedded inside were noticed on cut section. Section of lung revealed, cut section of the fluke at the end of the bronchus along with haemorrhagic exudate and macrophages laden with haemosiderin pigment microscopically. Infiltration with mononuclear cells, eosinophils and neutrophils were also observed in the alveoli, bronchi and bronchiole.

Rao and Madhubala (1998) and Soundararajan and Iyue (2005) described gross changes that were akin to the findings of present investigation. The gross and microscopic observations were similar to Kamil and Parihar (1990) and Yoshihara *et al.,* (1998) except that mixed thrombus noticed by Yoshihara *et al.,* (1998) microscopically was not seen in the present study.

The incidence of OPA was found to be 1.07% in the present investigation and supported the observations of Rahman and Iyer (1979), Sriraman and Rama rao (1980), Chattopadhyay *et al.,* (1986), Kumar *et al.,* (2005), Khodakaram-Tafti

and Razavi (2010) who reported an incidences of 4.44, 2.66, 2.38, 7.43 and 0.22 respectively. In contrast, a significantly higher incidence of up to 20.3 also had been reported by Amini and Tehrani (2013) in a survey conducted for five years on OPA in mixed-breed sheep flocks.

Grossly, the lungs were increased in size and weight and were grayish white in color that revealed meaty appearance with moist surface on cut section. Microscopically, there were papillary growths of tumor cells in the affected alveoli sometimes filled the lumen. In addition, papillary and polypoid proliferation of bronchiolar epithelium that filled the lumen was also noticed in the bronchiole. The lumen of the alveoli contained macrophages, lymphocytes, plasma cells and neutrophils. There were variable amounts of dense fibrous connective tissue (desmoplasia) which surrounded and divided the neoplastic nodules into lobules. In one case, there was an abscess surrounded by a fibrous capsule adjacent to the affected alveolar tissue. The gross and histopathologic findings in the present study were in accordance with the earlier reports (Rahman and Iyer 1979, Rama Devi *et al.,* 2001, Ramesh Kumar *et al.,* 2005, Beytut *et al.,* 2009 and Khodakaram-Tafti and Razavi 2010). In the present study based on gross and histopathology, the lesions were suggestive solely of classical OPA as described previously (Beytut *et al.,* 2009 and Khodakaram-Tafti and Razavi 2010).

In the present study, the lung lesions described in two cases of sheep pox and one case of PPR were grouped under miscellaneous conditions.

Though two cases of sheep pox were found in the present study, there were reports of higher incidences by various authors (Chattopadhyay *et al.,* 1986, Tarello and Kinne 2007, Chanie *et al.,* 2011 and Zangana and Abdullah 2013). This might be due to their studies based exclusively on sheep pox outbreaks and hence had higher incidence rates. Due to systemic nature of sheep pox, it causes significant mortality especially in lambs and it can be one of the leading causes for recording higher incidences in earlier reports.

Postmortem examination of infected animals showed typical nodular pock lesions on the face at the mandibular region and on other parts of the body. At necropsy, gross lesions in the present study included numerous circular, well demarcated, discrete to coalescing pock nodules of varying sizes distributed throughout the parenchyma and on cut section the nodule was grey, firm and the surrounding area was edematous. Microscopically, proliferative alveolitis and bronchiolitis giving the appearance of gland-like structure, areas of necrosis and eosinophilic intra-cytoplasmic inclusions in the pulmonary macrophages and bronchial epithelial cells were noticed. Interalveolar septa is thickened and infiltrated with lymphocytes and macrophages. Severe congestion, edema, presence of sheep pox cells and infiltration of lymphocytes were observed.

The gross and microscopic lesions observed in the present study were in agreement with that of the earlier reports by Afshar *et al.,* (1986), Bhanuprakash *et al.,* (2006), Gulbahar *et al.,* (2006), Beytut (2010), Chanie (2011), Dar *et al.,* (2012a) and Sharma *et al.,* (2013).

Ultrastructurally, electron dense immature virus-like particles present in the cytoplasm of alveolar macrophages in affected lungs in the present study were previously noticed by Afshar *et al.,* (1986) and Bhanuprakash *et al.,* (2010) in sheep pox affected lungs.

PPR was noticed with an incidence of 0.54% in the present study. Grossly, congestion and consolidation in the apical and diaphragmatic lobes of the lungs and froth filled trachea were noticed. Microscopically, presence of several giant cells in the alveolar spaces and in the bronchial lumen, severe congestion of blood vessels and capillaries with varying infiltration of mononuclear cells and polymorphs in the lumen of alveoli and bronchi were seen. Moderate peribronchial cuffing with lymphoid cells was also noticed in the lung parenchyma.

The gross findings in the present study were in agreement with Aktas *et al.*, (2011), Muse *et al.,* (2012) and Madboli and Ali (2012) whereas microscopic lesions observed were akin to Bundza *et al.,* (1988), Ettorre *et al.,* (2007) and Goodnight *et al.,* (2013).

The appearance of giant cells might be an evasive response of the host immune system to overcome the inflammatory changes induced by the etiological agents, especially viruses like PPR, Respiratory Syncytial Virus etc. (Ettorre *et al.*, 2007)

In the present study, 52 samples from pneumonic lungs were subjected to bacterial isolation. Various primary pathogens were isolated from 50 pneumonic lung samples. A total of 8 isolates were obtained that included *Coagulase positive*

Staphylococcus spp. in 13 (26%) followed by *Klebsiella spp.* in 9 (18%), *E.coli* and *Enterobacter spp.* in 7(14%) each, *Edwardsiella spp.* and *Pseudomonas aeruginosa* 4 (8%) each, *Pasteurella spp.* and *Serratia spp.* 3 (6%) samples each. A single pathogenic bacterium was isolated from all the samples and there were no mixed infections.

In the present study, *Staphylococcus aureus* (26%) was observed as the major organism isolated that was similar to the findings of Beytut *et al.,* (2002) who opined *S. aureus* as the most frequent organism causing pneumonia in sheep. Previously the incidence of *S. aureus* was reported by Rajiv Kumar *et al.,* (2000) and Srinivasan *et al.,* (2003) from India and Bakr *et al.,* (1980), Al-Sultan and Zubaidy (1978), Yesuf *et al.,* (2012) and Azizi *et al.,* (2013) from abroad.

Staphylococcus aureus is the main inhabitant of upper respiratory mucosa playing a pathogenic role in immune-compromised hosts with zoonotic implications, when natural barriers are compromised (Ajuwape and Aregbesola, 2002). Most of these isolates are secondary invaders and gets colonized and establish the infection only when the pulmonary defense mechanism gets impaired due to some virulent factors or noxious agents (McGavin and Zachary, 2007).

In the present study, *E. coli, Enterobacter spp.,* and *Pasteurella spp.* were isolated from cases of suppurative and fibrinous bronchopneumonia. Previously, *E. coli* was isolated from pneumonic lungs by Al-Sultan and Zubaidy (1978), Rhamyas *et al.,* (2001) and Srinivasan *et al.,* (2003).

Pasteurella spp. was the major organism isolated from fibrinous bronchopneumonia cases in the present study. Similarly Rajiv Kumar *et al.,* (2000), Rhamyas *et al.,* (2001), Srinivasan *et al.,* (2003) and Kumar *et al.,* (2005) in India and Obasi *et al.,* (2001), Oruc (2006), Odugbo *et al.,* (2006), Ezzi *et al.,* (2007), Yesuf *et al.,* (2012), Ozyildiz *et al.,* (2013) and Azizi *et al.,* (2013) in abroad also isolated *Pasteurella spp.* from sheep lungs.

Pasteurella spp. may be present in the respiratory tract as a commensal and may not be able to produce the disease alone, unless predisposed by factors like fatigue, transportation, over exertion, close confinement to damp and humid atmosphere that may impair the host defense mechanisms and help the organism to colonize, assume virulent role and set up the disease process. The organism releases an exotoxin (Leucotoxin) that lyses the macrophages and neutrophils making the animal immunosuppressive and prone for various other infections resulting in heavy mortalities (McGavin and Zachary 2007).

CHAPTER - VI

SUMMARY

Pathomorphological studies on spontaneous lung lesions in slaughtered sheep were undertaken to know the incidence, to describe the lesions observed in various pathological conditions affecting the lungs and to isolate the bacterial agents responsible in possible cases.

A total of 988 sheep from various slaughter houses, necropsies and field mortalities were screened, of which 187 (18.93%) lungs revealed definite lesions on gross and microscopic examination. The lesions were broadly grouped into abnormalities of inflation 22 (11.76%), circulatory disturbances 37 (19.79%), inflammatory conditions 116 (62.03%), parasitic conditions 7 (3.74%), neoplastic conditions 2 (1.07%) and miscellaneous conditions 3 (1.61%) out of 187 lungs examined.

Abnormalities of inflation included pulmonary emphysema (9.09%) and atelectasis (2.67%). In emphysema lungs appeared pale with enlarged areas projecting from the surface grossly and distended ruptured alveoli forming giant alveoli microscopically. In atelectasis, dark, depressed areas below the surface were seen grossly and slit-like, collapsed alveoli were found microscopically.

The circulatory disturbances observed in the present study consisted of pulmonary congestion and haemorrhage (10.16%) and edema (9.63%). Pulmonary congestion and haemorrhages were evidenced by red, patchy areas and petechial

and ecchymotic haemorrhages grossly that showed engorged blood vessels and erythrocytes in the lumen of the alveoli microscopically. Pulmonary edema was characterized grossly by pale, puffy and heavy lungs that pitted on pressure. Upon incision, foamy, edematous fluid oozed out from trachea and airways. Microscopically, a homogenous, eosinophilic fluid was noticed in the alveoli and in the interalveolar septa.

The incidence of the inflammatory conditions was 62.03% in the present study which constituted various types of pneumonias and pleuritis.

Pneumonic changes were noticed with an incidence of 58.82% that were categorized into bronchopneumonia and interstitial pneumonia based on the morphologic changes.

The incidence of bronchopneumonia was 56.15% in the present study, which was the highest among other pathological conditions observed. This was subdivided into suppurative bronchopneumonia (52.41%) and fibrinous bronchopneumonia (3.74%). Suppurative bronchopneumonia was characterized grossly by cranio-ventral consolidation which on cut section revealed mucopurulent exudates. Microscopically, the alveoli, bronchi and bronchioles were filled with exudates consisting mainly of polymorphs along with desquamation of epithelium. In chronic cases, abscess formation along with fibrous tissue encapsulation was noticed in the lung parenchyma. Fibrinous bronchopneumonia was characterized by widened interlobular septa with fibrinous adhesions to the thoracic wall in two cases. Microscopically, the alveoli and bronchi were filled with fibrin, neutrophils

and mononuclear cells. Pleura were thickened due to fibrinous exudates and proliferation of fibrous connective tissue.

Interstitial pneumonia was seen in 5 (2.67%) cases characterized grossly by pale, heavy lungs with rib impressions on the surface that failed to collapse when thorax was opened. Thickened alveolar septa infiltrated with mononuclear cells and epitheliolization of alveoli were seen microscopically.

Pleuritis was observed in 6 (3.21%) cases characterized grossly by excessive thickening of pleural membrane and microscopically by proliferation of fibrous connective tissue and infiltration of lymphocytes.

The overall incidence of parasitic conditions observed in lungs was 3.74% which included pulmonary hydatidosis and fasciolosis.

A solitary case (0.53%) of pulmonary hydatidosis was observed in this study that revealed a single large sized unilocular cyst embedded deeply in the lung parenchyma of diaphragmatic lobe. On cut section, small to grape sized, cream colored brood capsules attached to the white translucent membrane and also free in the cyst fluid were noticed. Microscopically, hydatid cyst consisted of slight eosinophilic fluid and an eosinophilic, acellular laminated layer that was lined by degenerated germinal epithelium. An inflammatory zone comprising of lymphocytes and macrophages was seen enclosed by fibrous connective tissue.

In the present study, fasciolosis (3.21%) was noticed due to aberrant location of *Fasciola gigantica* in the lung parenchyma. Grossly, dark red haemorrhagic tracts were seen that revealed flukes on cut section. Microscopically,

sections of flukes with haemorrhagic exudates bordered by fibrous connective tissue were seen in the haemorrhagic tracts along with infiltration of mononuclear cells, eosinophils and macrophages laden with hemosiderin in the alveoli.

Ovine Pulmonary Adenocarcinoma was noticed in two cases (1.07%) characterized by an increase in size and weight of the lungs that were grayish white in color and meaty in appearance with moist surface on cut section. Microscopically, there were papillary growths of tumor cells in the alveoli and bronchiole. The lumen of the alveoli contained macrophages, lymphocytes, plasma cells and neutrophils and dense fibrous connective tissue surrounded and divided the neoplastic nodules into lobules. In one case, there was an abscess enclosed by a fibrous capsule adjacent to the affected alveoli.

In miscellaneous conditions, lung lesions noticed in few cases of sheep pox and PPR were described.

Sheep pox was observed in 2 (1.07%) cases that showed typical nodular pock lesions on the skin and numerous circular, well demarcated, discrete to coalescing pock nodules of varying sizes were found distributed throughout the lung parenchyma. Microscopically, severe congestion, focal areas of necrosis, proliferative alveolitis and bronchiolitis and thickened interalveolar septa infiltrated with lymphocytes and macrophages were seen. Eosinophilic intra-cytoplasmic inclusions were noticed in the pulmonary macrophages and bronchial epithelial cells. The ultrastructural changes observed in the macrophages were karyorrhexis and margination of chromatin with irregularly shaped or indented

nucleus and presence of immature virus-like particles seen as electron dense particles in the cytoplasm.

The lung lesions in a case (0.54%) of PPR were recorded in the present study and the animal showed purulent nasal discharges, erosive and necrotic stomatitis with ulceration. Grossly, severely congested and consolidated lungs with froth-filled trachea were observed. Microscopically, inflammatory cellular exudate infiltrated with abundant polymorphs, severe congestion and several distinct giant cells in the alveolar and bronchial lumen were present.

Bacteriological studies on 52 samples from sheep pneumonic lungs resulted in isolation of pathogenic bacteria from 50 samples, whereas in 2 samples no bacteria could be isolated. In total, 8 isolates of bacteria were obtained which included *Staphylococcus aureus* in 13 (26%) followed by *Klebsiella spp.* in 9 (18%), *Escherichia coli* and *Enterobacter spp.* in 7(14%) each, *Edwardsiella spp.* and *Pseudomonas aeruginosa* in 4 (8%) each, *Pasteurella spp.* and *Serratia spp.* each in 3 (6%) samples respectively. *S. aureus* was the major pathogen isolated in this study and was found from cases of suppurative bronchopneumonia. *E.coli, Enterobacter spp., Edwardsiella spp., Pasteurella spp.* and *Serratia spp.* were isolated from both suppurative bronchopneumonia and fibrinous bronchopneumonia cases whereas *Klebsiella spp.* and *Pseudomonas spp.* were isolated from suppurative bronchopneumonia and interstitial pneumonia cases.

The results observed in the present study revealed the spectrum of spontaneous lung lesions noticed in sheep of which the incidence of

bronchopneumonia was found to be the highest. *S. aureus* was the major bacterial pathogen causing pneumonia in sheep in the area under study.

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