PATHOLOGY OF EXPERIMENTAL PASTEURELLOSIS IN DUCKS

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Thesis submitted in partial fulfillment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2009

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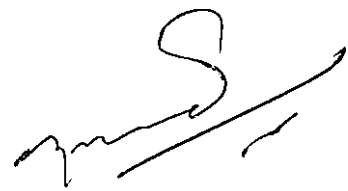
DECLARATION

I hereby declare that this thesis entitled **"PATHOLOGY OF EXPERIMENTAL PASTEURELLOSIS IN DUCKS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.



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ACKNOWLEDGEMENT

I express my deep sense of gratitude to ***Dr. N. Divakaran Nair,*** *Professor, Centre of Excellence in Pathology and Chairman of the Advisory Committee for the able guidance and constant encouragement that helped bring out the best in me, since he let me fly away with my own intuitions and provided all support in whatever things I ventured onto without any prejudices.*

I owe my deep gratitude to ***Dr. C. R. Lalithakunjamma,*** *Professor and Head, Centre of Excellence in Pathology for her help and constructive criticism throughout the study.*

I was lucky to have ***Dr. N. Vijayan,*** *Professor, Centre of Excellence in Pathology and* ***Dr. G. Krishnan Nair,*** *Professor and Head, Department of Veterinary Microbiology as members of the Advisory Committee. Their never ending support, enduring interest, valuable suggestions and critical criticisms helped me a lot in shaping this manuscript.*

I am indebted to ***Dr. Mamman J. Abraham,*** *Associate Professor, Centre of Excellence in Pathology and* ***Dr. Sreedharan Unni,*** *Associate Professor, Department of Veterinary Anatomy for the constant support and encouragement accorded to me throughout the study.*

I am extremely privileged to be associated with ***Dr. Usha Narayana Pillai,*** *Associate Professor, Department of Clinical Veterinary Medicine for her valuable guidance and expert suggestions in all matters concerned.*

I place on record my sincere thanks to ***Dr. Koshy John,*** *Associate Professor, Department of Veterinary Microbiology who always encouraged me with his subtle presence and suitable suggestions.*

I am indebted to ***Dr. V. Ramnath,*** *Associate Professor, Department of Veterinary Physiology for his eagerness to help at all times.*

The help rendered by ***Smt. Sujatha****, Assistant Professor, Department of Statistics, during the statistical analysis of the data is gratefully acknowledged.*

I express my sincere thanks to ***Mrs. Baby Saroja*** *and* ***Mrs. Rathi Devi*** *who helped me a lot in all the office matters concerned.*

I am grateful to the ***Dr. E. Nanu****, Dean, Faculty of Veterinary and Animal Sciences, for providing the facilities for the study.*

The ***faculty members*** *of the COVAS, Mannuthy had always been a great source of help and encouragement. I fondly remember their help and concern at every stage of my work.*

I place on record my sincere thanks to ***Dr. M. A. Nazar,*** *Senior Veterinary Surgeon, Veterinary Poly Clinic, Karunagappally, Kollam whom I am loving and respecting as my elder brother for his valuable suggestions and warm company offered.*

I owe my deep gratitude to ***Dr. P. P. Kanaran,*** *one of my PG colleagues for the support and encouragement accorded to me throughout the study period. He helped me a lot in shaping this manuscript.*

I place on record my sincere thanks to ***Dr. Unnikrishnan*** and ***Dr. Ayoob*** *for their affection, help and encouragement.*

My heartfelt gratitude to my best friends in P. G. Hostel, ***Mr. Arun Raveendran****, Research Associate, CASABG,* ***Mr. Mohammed Nisar V. A****, Research Associate, NBPGR,* ***Drs. Midhun M. V, Sreejith J. R, Anas*** *and* ***Mr. Suresh*** *who were always with me providing their warm friendship.*

The sincere companionship provided to me by ***Dr. Ambily V. R,*** *who was actually the reason why I am now in the vast arena of Pathology, I put on record a very special bouquet of thanks for her.*

I am thankful to ***Dr. Bibu*** and ***Mr. Subin Jose****, Research Associates, Department of Clinical Veterinary Medicine for their eagerness to help me at needy times.*

I am thankful to my junior ***Dr. T. Senthilkumar*** *who shed on me brotherly affection and supported me in all matters concerned.*

I fondly remember the timely help rendered by my friends ***Drs. Raja Gopal. R*** *and* ***Rafeeque*** *by fetching some key research articles for my work*

I would like to give special thanks to ***Dr. Rojan, Dr. Hiron*** *and* ***Dr. Binoj Chacko*** *who were always willing to help me during the last stages of my work even without asking them to do so. I thank for the help provided during the setting of pictures and statistical analysis of the data.*

The invaluable help rendered by ***Dr. Kishore Kumar,*** *who taught me the jugular venipuncture of ducks is duly acknowledged.*

I place on record my sincere thanks to my friends ***Drs. S. Senthilkumar*** and ***Balaji*** *for their moral support and encouragement.*

I gratefully remember the help and support rendered to me by my class mates ***Drs. Mahesh R, Subin Mohan, Arunkumar, Vikas, Mohammed, Seena*** and ***Sumi.***

I remember with gratitude the warm friendship and affectionate encouragement of P.G. hostel inmates ***Jyothish, Shaiby, Jinu, Kadar, Lijo*** and ***Jinesh.***

The P.G. Hostel employees ***Mr. Vishwanathan, Mr. Sathikumar*** and ***Mr. Reji*** *are duly acknowledged.*

The co-operation extended by college security guards ***Sri. Gopi, Narayanan kutty,*** and ***Bhaskaran*** *is greatly acknowledged.*

I am thankful to ***Ms. Soumya*** *of J&C stores for providing me necessary stationary articles during the work.*

I extend my sincere thanks to my colleagues ***Drs. Litty Mathew, Arya, Indu*** *and junior PG scholars* ***Drs. Dali, Praveena, Divya*** *for their kind support and valuable help during the entire period of study. I also thank my seniors* ***Drs. Thomas .K. Thomas*** and ***Remya R. Nair*** *for the support and valuable information on various practical aspects of research.*

Words possess no enough power to reflect my thankfulness for the invaluable help, moral support, affection and pleasure rendered by ***Dr. Ezhil Praveena,*** *Teaching Assistant, Centre of Excellence in Pathology.*

The help and co-operation extended by ***Sasiyettan, Jessychechi, Gangadharettan, Sumathychechi, Karthikeyan, Seema, Seena chechi*** *and other staffs of Centre of Excellence in Pathology is gratefully acknowledged.*

Above all, I extend my sincere thanks to my ***Pappa, Amma, Ammachamma, Hari, Valyammachy, Lallu, Jishnu*** and ***Kannan chettan*** *for their blessings and prayers.*

Above all I bow to God almighty for his grace and blessings bestowed on me for the successful completion of this great task.

S. PRAMOD

CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

1. INTRODUCTION

Poultry industry in India has made rapid strides during the last two decades. In spite of improved breeding, feeding and management, diseases pose a great threat to the survival of this industry. One important threat is fowl cholera, causing massive mortality within short time period and heavy economic losses.

Duck - the poor man's bird - inhabit both fresh and sea water and thrive on water plants, insects, aquatic flora and fauna. Being a water bird, ducks can withstand extremes of ecological and geoclimatic conditions. Duck rearing is a major source of income especially for those living in coastal and water shed areas. Considering the geographical peculiarities of Kerala, having a very long coastline, duck farming opens a good option for the farmers to get a consistent economic source without much effort and investment. The favourable environmental conditions and extensive water logged areas offer a good natural habitat for these calm water birds.

Ducks constitute about 10 per cent of the total poultry population in India, occupying second position to chicken in the production of table eggs. Harbouring about 24 million ducks (Anon, 1996), India ranks second in the world after Indonesia in duck population. Kerala has a massive population of ducks, around 11.87 lakhs (Anon, 1996). Duck rearing is well practiced in Alappuzha, especially in Kuttanad region, Thrissur, Kottayam and Pathanamthitta districts of Kerala.

Compared to chicken, ducks are less prone to diseases. The major disease conditions affecting the duck population are duck virus hepatitis, duck plague, Newcastle disease and duck pasteurellosis. Among these diseases duck pasteurellosis, commonly known as duck cholera, is a serious problem in ducks. This disease is mostly seen in young birds of four weeks of age and mortality reaching as high as 50 per cent.

Fowl cholera was first recognized in the late 1700s. It is a highly contagious disease characterized by acute septicaemia affecting both domestic and wild birds. The causative organism is an encapsulated Gram-negative, non-motile, non sporulating coccobacillary organism namely *Pasteurella multocida.* It is an important primary and opportunistic pathogen as well as a commensal of the upper respiratory tract of various domestic and wild animals. Outbreak of the disease is sporadic, but devastating. An important feature is that the disease is precipitated by stress. Due to immunosuppression caused by stress factors such as overcrowding, laying, transportation, increased ammonia levels in air and severe climatic changes, the commensal becomes highly pathogenic. Although adult ducks show some resistance to the disease, ducklings are very susceptible.

Usually in Kerala, ducks are reared in large flocks in harvested paddy fields. In traditional paddy cultivation, lots of chemicals are used as pesticides and weedicides. Ingestion of these pesticide residues in large amounts results in immunosupression and this is the major predisposing factor of duck cholera outbreaks. Mortality get even worsened when duck cholera occurs in association with another dreadful disease like duck plague and in such a situation it becomes impossible to differentiate the disease by gross examination of various organs during postmortem.

The rapid onset and spread of the disease make treatments practically impossible and ineffective. Moreover, continuous use of once effective antibiotics like sulpha drugs leads to drug resistance also. Reports of this disease amongst ducklings and ducks have been described in different parts of India (Mulbagal *et al.*, 1972; Panda *et al.*, 1981; Karim, 1987) and also abroad (Nakamine *et al.*, 1992; Rhoades *et al.*, 1992; Mariana and Hirst, 2000). In India, Bhowmik and Dutta (1995) reported the incidence of duck cholera as 13 per cent based on the autopsy findings, whereas Das *et al.* (1991) noticed 19.5 per cent mortality in organized duck farms.

During the early half of 1993, severe mortality among ducks was reported in Kuttanad region of Kerala (Pillai *et al.*, 1993). The history revealed from the affected areas indicated that the said disease was different from duck plague which was frequently encountered. The major problem was the massive loss by this disease, even before attempting any treatment. In the absence of proper prophylactic measures till now, control remains as a big problem for the farmers. The onset of an epizootic of pasteurellosis may eliminate years of qualitative breeding efforts.

Although the disease is causing alarming mortality, the exact pathological features have not been delineated. Perusal of literature also revealed only a few reports on the pathological aspects of pasteurellosis in ducks. Hence the present study was undertaken with the following objectives.

a) Evaluate the pathology of pasteurellosis in ducks using locally isolated strain.

b) Compare the route of administration and production of disease.

c) Study the haemato-biochemical changes associated with the infection.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PREVALENCE

2.1.1 Prevalence world wide

First report on the occurrence of fowl cholera in wild waterfowl in Japan was made by Fujihara *et al.* (1986).

In Canada, Wobeser and Leighton (1988) reported an acute outbreak of pasteurellosis in redhead ducks.

Dermal necrosis caused by *P. multocida* infection in turkeys was reported by Glass and Panigrahy (1990) from Texas, U.S.A.

Rhoades *et al.* (1992) reported outbreak of fowl cholera involving turkey flocks of several farms in Utah, United States.

Morishita *et al.* (1996) reported an outbreak of fowl cholera in psittacines in U.S.

From California, Gustafson *et al.* (1998) reported *P. multocida* infection in white leghorn chicken.

Samuel *et al.* (2003) cultured *P. multocida* isolates from wetland ecosystems in America and reported mortality in peckin ducks, when tested for virulence. Mortality was higher in male ducks than females.

In Thailand, Songserm *et al.* (2003) reported *P. multocida* - associated sinusitis in ducks.

2.1.2 Prevalence in India

In West Bengal, Bhadury (1948) reported a sub acute outbreak of duck cholera.

In India, the incidence of fowl cholera in ducks was reported as early as 1947. (Mulbagal *et al.*, 1972).

In Orissa, Panda *et al.* (1981) observed duck cholera in adult birds. The dying birds didn't show any symptoms.

Clinical pasteurellosis in ducks of Assam was investigated by Karim (1987).

Duck cholera outbreak in a group of ducklings of 20 to 60 days age was reported by Bhaumik and Dutta (1995) from Tripura.

An outbreak of duck cholera in Tripura caused by *Pasteurella multocida* A: 1 strain with a mortality percentage of 30 to 35% was reported by Murugkar and Ghosh (1995).

Hegde *et al.* (1996) reported a duck pasteurellosis outbreak in Central Duck Breeding farm, Bangalore, Karnataka.

Prasad *et al.* (1997) isolated *P. multocida* from specimens of birds suspected for pasteurellosis with characteristic signs and gross lesions.

Investigation on duck cholera outbreaks in Andhra Pradesh by Devi *et al.* (2000) revealed that most of the outbreaks occurred during early monsoon period and the age group affected was four to twelve months.

Bhattacharya (2005) reported mortality ranging from 11 to 20 per cent after investigating duck cholera outbreaks of Tripura. A high percentage (77.77%) of *P. multocida* isolates were recovered from ducklings as early as six weeks of age, where as only 22.22% of the isolates was from adult ducks.

123 avian strains of *P. multocida* isolated from cases of avian pasteurellosis belonging to different states of India were biochemically characterized by Shivachandra *et al.* (2006).

2.1.3 Prevalence in kerala

Pillai *et al.* (1993) investigated field reports of duck cholera outbreaks in Kuttanad region of Kerala.

Jayakumar (1998) isolated *P. multocida* from birds died of fowl cholera outbreak in Kerala.

Rajagopal (2007) reported pathological effects of *P. multocida* in experimentally infected young and adult ducks.

2.2 ISOLATION AND CHARACTERIZATION OF *P.multocida.*

In an outbreak of chicken pasteurellosis, Panda *et al.* (1981) noticed that in birds those died suddenly, blood smears and impression smears of visceral organs were negative for bacteria. Bacterial isolations done on blood agar and brucella agar from heart blood, liver and spleen samples were also negative for *Pasteurella*. In birds died later, the smears on staining revealed numerous bipolar stained *P. multocida* organisms.

Bipolar staining characteristic of *P. multocida* was evident with methylene blue, Wright's stain, Giemsa stain and Gram stain (Wobeser and Leighton, 1988).

Rhoades *et al.* (1992) isolated *P. multocida* strains from turkeys in a fowl cholera outbreak. They were antigenically characterized as A: 1, A: 3, and B:4 based on capsular serogrouping and somatic serotyping results.

In a duck pasteurellosis outbreak, Pillai *et al.* (1993) found that the presence of organisms in heart blood smears was less when compared to impression smears from heart, liver and lungs. Grams staining of smears revealed

the presence of gram negative coccobacilli. Bacterial isolations were done on blood agar and brucella agar from heart blood, liver and spleen samples. Discrete, minute, non haemolytic colonies of *P. multocida* were obtained.

In a duck cholera outbreak, Bhowmik and Dutta (1995) noted absence of haemolysis on blood agar plates and round flat colonies of sticky mucoid consistency on nutrient agar plates on cultural examination of *P. multocida.* No growth was recorded on Mac Conkey's bile salt agar. Morphologically, organisms were gram negative, short ovoid in shape and non motile.

Gustafson *et al.* (1998) cultured *P. multocida* from cranial air spaces, sinus, and brain of fowl cholera affected chicken on blood agar. The isolate was found to be of serotype 9.

In fowl cholera infected quails, Srilatha *et al.* (2003) observed bipolarity of *P. multocida* organisms in heart blood and liver impression smears.

Roy *et al.* (2004) reisolated and characterized *P. multocida* from liver and heart in an outbreak of pasteurellosis in quails. The organisms gave tiny colonies on blood agar but no growth was there on MacConkeys agar.

Shivachandra *et al.* (2006) biochemically characterized 123 *P. multocida* strains isolated from birds. All strains exhibited similar cultural, morphological and biochemical characteristics, however, they differed in their ability to utilize various sugar.

2.3 ROUTE OF INOCULATION AND VIRULENCE

Hunter and Wobeser (1980) reported that ducks of different age groups challenged intraperitonially with two isolates of *P. multocida* developed either acute or chronic lesions depending on the isolate of *P. multocida* and the inoculation dose.

The isolate, *P. multocida* A: 1, when injected intramuscularly was found highly virulent in chicken killing them within 24 hr of inoculation showing typical signs of fowl cholera (Sambyal *et al.*, 1988).

Prantner *et al.* (1990) noticed severe progressive bacteremia after experimental inoculation of *P. multocida* A: 3, 4 strain in turkeys by an occulo-nasal route.

Rhoades and Rimler (1990) observed that the turkey poults exposed by intra- airsac inoculation with relatively few organisms of the more virulent strains of *P. multocida* (capsular serotype D strains) had a high mortality rate. However intranasal exposure of poults with this strain didn't cause clinical disease or establish infections.

Pillai *et al.* (1993) assessed the pathogenicity of one of the isolates of *P. multocida* in ducks by administering the saline suspension of overnight growth in blood agar by different routes – oral, subcutaneous, and intra muscular. Time of mortality was recorded to be 36-48 hours, 20-24 hours and 12-14 hours respectively. So the time taken for the death was dependent on the route of infection. Non parental route of administration took more time for establishment of infection and death.

Rajiny *et al.* (1995) isolated *P. multocida* from ailing birds suspected for fowl cholera and tested for pathogenecity in mice. Majority of the isolates showed a Mean Death Time between 10-15 hours.

Following inoculations of the strains of *P. multocida* by an occulo-nasal route, endotoxaemia was detected for a longer period of time in birds challenged with the field strain (Christensen *et al.*, 1998).

Fisher *et al.* (1998) experimentally inoculated *P. multocida* into thoracic air sacs of broilers and observed an onset of acute septicaemia.

Pehlivanoglu *et al.* (1999) challenged one month old ducklings with *P. multocida* isolates by five different inoculation routes (intranasal, intraocular, intravenous, oral and subcutaneous) and reported that the intravenous route caused most mortality.

Twenty groups, each of ten chickens were inoculated with *P. multocida* at a standard dose of 10^5 cfu/ml by intramuscular, intravenous, intratracheal and conjunctival routes. The highest mortality occurred in groups dosed intramuscular and intravenous, followed by intratracheal inoculation (Wilkie *et al.*, 2000).

Chickens, turkeys, partridges and pheasants were infected intra-tracheally with a strain of *P. multocida* subsp. *multocida* (40605-1) isolated from outbreaks of fowl cholera in wild birds in Denmark. *P. multocida* subsp. *multocida* strain P-1059 was included as a reference strain. The outbreak strain was highly virulent for turkeys, partridges and pheasants, while chickens were more resistant (Petersen *et al.*, 2001).

Sarkozy *et al.* (2002) experimentally induced *P. multocida* infection in broilers and turkeys by inoculating intramuscularly at a dose rate of 80 cfu/ml/bird. Clinical signs were first seen within 24 h after inoculation. The most severe clinical signs were observed between 36 and 72 h post-infection.

Songserm *et al.* (2003) intranasally inoculated *P. multocida* in ducks and the birds showed sinusitis. Ducks occularly inoculated with *P. multocida* had mild conjunctivitis.

In an experimental study conducted by Shilpa *et al.* (2005), layer chicken were injected intravenously with LD_{50} of a local isolate of *P. multocida* and noticed onset of clinical symptoms within 12 hour post inoculation.

Shivachandra *et al.* (2005) inoculated chicken with 1.5 ml of 18 hour BHI broth culture of *P. multocida* A:1 containing approximately 1.35×10^9 cfu/ml, by

intranasal, subcutaneous and intramuscular routes and observed that the degree of pathogenicity varied between different routes of infection in terms of mortality.

In a study conducted by Mbuthia *et al.* (2008), birds (both ducks and chicken) were inoculated intratracheally with *P. multocida* culture in brain heart infusion broth and observed typical signs of an acute bacterial septicaemia.

2.4 SYMPTOMS

2.4.1 Symptoms in peracute and acute cases

In a fowl cholera outbreak, Bhadury (1948) observed pyrexia, extreme dullness, sitting on hocks, head drawn in towards the wings, dribbling of watery mucus from nostrils and mouth, dyspnoea with rattling sound, yellowish white or greenish diarrhoea, cyanotic comb and increased thirst.

In an outbreak of pasteurellosis in broilers, Curtis and Ollerhead (1978) described two main disease manifestations- an acute form associated with sudden death and a chronic form associated with lameness. In five cases, the acute form was first observed and signs of lameness followed a day or two later. However in two cases, lameness was the first and prominent sign of the disease.

Verma and Mohanty, (1983) stated that symptoms like loss of appetite, increased thirst, pyrexia and sudden death were shown by birds with *P. multocida* infection. There were mucoid white droppings followed by greenish diarrhoea. The birds became fatigued on walking due to paralysis of limbs.

Char *et al.* (1987) observed in an outbreak of fowl cholera that most of the ducklings died suddenly without showing any symptoms. A few showed symptoms like dullness, unthriftiness, incoordination and greenish diarrhoea. Some showed lacrimation, occlusion of eyelids and paralysis of legs.

Ducks affected with pasteurellosis showed lameness, white diarrhoea, which later became greenish and contained mucus. Some showed severe

dyspnoea. Mucopurulent discharge from nostrils and conjunctivitis were marked. (Karim, 1987; Chakraborthy *et al.*, 1989; and Roy *et al.*, 1993).

Ramasastry and Ramarao. (1989), Rao (1990) and Hegde *et al.* (1996) explained that in per acute and acute forms of fowl cholera in chicken, sudden death without showing any signs was noticed.

Kumar (1990), Devi *et al.* (2000) and Shivachandra *et al.* (2005) described signs of fowl cholera as fever, anorexia, ruffled feathers, mucus discharge from beak, dyspnoea, tracheal rales, greenish diarrhoea, increased respiratory rate, cyanosis of head, comb, wattles and torticollis.

Nakamine *et al.* (1992) observed lameness, crouching, depression and slight diarrhoea in *P. multocida* infected muscovy ducks. All the birds developed symptoms and 25% of them died of acute disease by 11 days after the onset.

Clinical signs consisted of ruffled feathers, mucous discharge from mouth and nostrils, white or greenish diarrhoea, dyspnoea and occasional torticollis in *p. multocida* infected ducks. The morbidity was noted to be around 40% with 20% mortality. Mortality was noted to be higher in birds above one month of age (Ramanath and Gopal, 1993).

Bhaumik and Dutta (1995) pointed out that symptoms of duck cholera included loss of appetite, increased thirst, diarrhoea and sudden death. The affected ducks showed evidence of pain and became fatigued on walking small distance. The birds also showed paralysis of limbs and arthritis.

Affected quails huddled together with signs of depression, greenish diarrhoea, prostration and death. Within seven days of onset, 50% mortality was noticed (Chandran *et al.*, 1995).

Murugkar and Ghosh (1995) noticed that there were no apparent clinical symptoms except that the birds were off-feed and had whitish diarrhoea in an outbreak of duck cholera.

Pyrexia, loss of appetite, depression, drowsiness, ruffled feathers, mucous discharge from mouth and nostrils, diarrhoea, increased respiratory rate, cyanosis of wattles and combs, difficult breathing were seen in acute fowl cholera (Reddy, 1996).

Outbreaks of a sub acute disease characterized by lameness, corneal turbidity, dystasia and depression occurred in Muscovy ducks due to *P. multocida* was reported by Takahashi *et al.* (1996).

Fisher *et al.* (1998) observed signs such as ruffled feathers, reluctance to move, diarrhoea, sternal recumbency with heads down and eyes closed, moist rales, and elevated body temperature in broilers experimentally inoculated with *P. multocida.*

Gustafson *et al.* (1998) reported neurologic and respiratory signs in *P. multocida* infection in chicken. Symptoms included depression, reluctance to move, torticollis, twisting of neck, swollen eyelids, conjunctivitis and sinusitis.

Dyspnoea and periorbital sinus swelling with mucoid discharge were noticed by Miguel *et al.* (1998) in a fowl cholera outbreak in quails.

Wilkie *et al.* (2000) observed severe depression and inappetence in experimentally infected chicken. The birds became lame and were reluctant to move.

Goto *et al.* (2001) reported that the quails challenged with lethal doses became dejected, were unable to move, had severe diarrhoea and died with in 20 hours. The LD_{50} of *P. multocida* in quails was determined as 4.3×10^4 cfu.

Nasal discharges, diarrhoea, lameness, weakness, drop in water and food consumption, recumbency and moribund state were noticed in an experimentally induced *p. multocida* infection in broilers and turkeys. (Sarkozy *et al.*, 2002).

Clinical signs of dullness and depression were noticed by Srilatha *et al.* (2003) in fowl cholera infected quails. About 34% of mortality was noticed within 15 days.

Sinusitis was observed by Songserm *et al.* (2003) in ducks intranasally inoculated with *P. multocida.* Ducks occularly inoculated with *P. multocida* showed mild conjunctivitis.

Bhattacharya (2005) reported clinical signs such as loss of appetite, ruffled feathers, whitish followed by greenish diarrhea in Khaki Campbell and Vigova Super-M ducks.

In an experimental study of fowl cholera in chicken by Shilpa *et al.*, (2005) the affected birds revealed anorexia, dullness, depression, ruffled feathers, congested eyes and cyanotic wattles. At 24 hr post inoculation, most of the birds were recumbent and not inclined to move. The degree of lameness increased with the progress of the disease. Water and feed intake was reduced considerably from day 5 till 15 post inoculation. Blindness was observed in a few birds at 7 and 10 days post inoculation.

Shilpa *et al.* (2005) noticed anorexia, dullness, depression, congested eyes, lameness and conjunctivitis with cheesy exudates in layer birds on *P. multocida* inoculation.

Clinical signs including general depression, diarrhoea, torticollis, decreased egg production and facial oedema were detected by Eigaard *et al.* (2006) in fowl cholera affected layers.

Depression, nervous signs, ruffled feathers, sneezing, ataxia, nasal discharges, dyspnoea, mouth discharges, diarrhoea, cyanosis, rales, fever, head scratching, coughing and eye discharges were observed by Mbuthia *et al.* (2008) in birds experimentally inoculated with *P. multocida.* The frequency of clinical signs varied significantly between age groups of chicken except for mouth

discharges, diarrhoea and cyanosis. Between age groups of ducks, signs varied except for diarrhoea and fever.

2.4.2 Symptoms in chronic cases

Bilateral bumble foot lesions due to *P. multocida* infection were reported by Joshi *et al.* (1987).

Chronic cases of fowl cholera were related to localized infections. Wattles, sinuses and leg joints were reported to have swelling leading to lameness (Kumar, 1990).

Swollen wattles filled with caseous exudates were noticed by Glisson (1994) in a fowl cholera infection.

Reddy (1996) observed depression, conjunctivitis, enlargement of joints, lameness, torticollis, swelling of wattles, tracheal rales, dyspnoea and emaciation in fowl cholera affected chicken.

Periorbital sinus swelling, droopiness and lameness due to joint infection were noted by Miguel *et al.* (1998) in a fowl cholera outbreak in quails.

Due to localized infection by *P. multocida*, lameness and swelling of hock joints and foot pads were observed in ducks (Verma and Mohanty, 1983; Rao, 1990; Devi *et al.*, 2000; Bhattacharya, 2005).

2.5 GROSS PATHOLOGY

2.5.1 Lesions in peracute and acute cases

2.5.1.1 Natural outbreaks

Liver lesions such as pin point, minute, grayish necrotic foci were clearly evident only in the later stages of a fowl cholera outbreak in chicken. Blood smear examination during initial stages was also negative. In later stages, lesions

of generalized septicaemia like petechiae on proventriculus and necrotic foci on liver were clearly evident (Bhadury *et al.*, 1948).

In an outbreak of fowl cholera in broilers, post mortem findings varied from a typical haemorragic septicaemic appearance in very acute cases to a less haemorrhagic appearance in others and suppurative lesions of the leg joints and bursae in lame birds (Curtis and Ollerhead, 1978).

Panda *et al.* (1981) noted lesions like petechial and echymotic haemorrhages in proventriculus, caecal tonsils, intestinal mucosa, breast and leg muscles and on subepicardial fat In an outbreak of pasteurellosis in chicken. Intestines in some birds had catarrhal to diphtheritic enteritis with few ulcerations. Liver had numerous pin head size grayish white necrotic foci. Hemorrhages and necrotic foci on spleen, ruptured follicles with few grayish necrotic foci in the ovary were the other lesions.

Haemorrhages over heart, peritoneum, air sacs, intestine and under the skin were the lesions observed by Verma and Mohanty (1983). Liver and spleen were enlarged with pin point haemorrhages.

Multiple petechiae over the epicardium, ventricle intima and serosal surface of liver and spleen were noticed by Fujihara *et al.* (1986). Light yellow thin fibrin covered on the surface of tunica serosa in liver. Petechiae were also observed on subcutaneous fatty tissue of the thigh and neck.

Karim (1987) found on postmortem, pin point haemorrhages in the lungs and epicardium in ducks died due to fowl cholera. Pericardial sac contained excessive amount of straw coloured fluid. Liver was congested and swollen with minute necrotic foci on surface. The carcasses were dehydrated. Petechiae were noticed along the coronary groove of heart and intestinal tract.

In an outbreak of fowl cholera, Char *et al.* (1987) reported that the liver was very friable and revealed lesions of necrotic hepatitis. All the

parenchymatous organs and respiratory tract were congested. Petechiae on pericardium, catarrhal enteritis, enlarged spleen and pneumonic lungs were also noted.

Windingstad *et al.* (1988) found on necropsy gross lesions of petechial and echymotic epicardial haemorrhages, serosal haemorrhages along the small intestine and swollen and friable livers with multifocal necrosis in an avian cholera epizootic in wild mallards. Distended small intestines contained thick mucoid material and occasional petechiae in subcutaneous fat.

The pathologic changes observed in a natural outbreak of duck pasteurellosis comprised of massive haemorrhages and congestion in all visceral organs with or without necrotic foci. There were also intravascular occlusion by emboli consisting of haemorrhagic exudates and blood debris. The ulcers in lungs were irregular in shape, varying in size with raised borders and contained haemorrhagic and necrotic debris. (Chakraborthy *et al.*, 1989).

In ducklings died due to fowl cholera, Ramasastry *et al.* (1989) noticed petechiae on subcutaneous tissues, congestion of lungs & enlarged and haemorrhagic liver with pin point necrotic foci on surface. Intestinal tract was uniformly haemorrhagic from proventriculus to cloaca. The faecal material was completely red tinted. Haemorrhages were evident on heart.

Enlarged heart, hydropericardium and hepatomegaly were the post mortem lesions observed by Roy *et al.* (1989) in *P. multocida* affected layers.

Glass and Panigrahy (1990) observed extensive dermal necrosis in fowl cholera affected turkeys. The liver was enlarged and dark and had a parboiled appearance. The lungs were consolidated and haemorrhagic. Haemorrhage, necrosis and sloughing of intestinal mucosa along with fibrinous peritonitis with adhesions of intestine to abdominal wall were also seen.

Gross lesions of enlarged spleen and multifocal areas of hepatic necrosis were present in mallard ducks with a history of sudden and massive mortality. Affected birds were found to be in good body condition. *P. multocida* were recovered in pure culture from these specimens (Lowes, 1990).

Rao (1990) observed no gross lesions in peracute and acute forms of fowl cholera affected chicken.

Pathological examination of *P. multocida* infected muscovy ducks showed that subepicardial haemorrhages and multiple petechiae in the swollen liver were the most prominent gross lesions in the dead birds (Nakamine *et al.*, 1992).

Rhoades *et al.* (1992) found lesions typical of fowl cholera such as generalized congestion, subserosal petechiae, increased pericardial and peritoneal fluids and mottled spleen and liver in turkey poults.

Severe cephalic swelling and facial cellulitis in *P. multocida* infected turkeys was reported by Jeffrey *et al.* (1993). Pockets of serous fluid, blood, and caseous exudate were seen in the dermis and subcutis. Mucoid to caseous sinusitis was also there. Birds with facial cellulitis had no internal lesions of fowl cholera.

In a duck pasteurellosis outbreak, Pillai *et al.* (1993) found the presence of blood containing fluid in oral cavity. Lungs were congested and liver had numerous pin head sized necrotic foci and petechial haemorrhages. Echymotic and pinpoint haemorrhages on subcutaneous tissues, on the serous surfaces, muscles of the thigh and pectoral region were also observed. Enlarged and congested spleen, petechiae on the heart and haemorrhagic enteritis were the other lesions observed.

Rammanath and Gopal (1993) observed characteristic septicaemic lesions of acute fowl cholera showing haemorrhagic respiratory, gastrointestinal and cardiac lesions in Khaki Campbell ducks.

In an outbreak of pasteurellosis in ducks, Roy *et al.* (1993) noticed congested kidney, liver, spleen and pin point haemorrhages in the intestinal tract. Chalky white deposits were noticed around heart. Caecum contained white chalky material.

Glisson, (1994) stated that lesions in *P. multocida* affected birds may be totally inapparent or may consist of enlarged liver and spleen, petechiae on internal organs, fibrinopurulent arthritis ,encephalitis, osteomyelitis and peritonitis.

Sahoo *et al.* (1994) described lesions of fowl cholera as sub epicardial haemorrhages, flaccid ovarian follicles, multiple necrotic foci in liver and hyperemia of duodenum.

Enlargement of heart with petechiae, accumulation of excess pericardial fluid and petechiae to ecchymoses on enlarged liver, haemorrhagic enteritis and peritonitis were observed by Bhaumik and Dutta, (1995) in Khaki Kampbell ducklings affected by fowl cholera.

Postmortem examination of dead quails revealed generalized hyperaemia, swollen liver with multiple focal areas of necrosis and haemorrhages in duodenal mucosa characteristic of fowl cholera (Chandran *et al.*, 1995).

Murugkar and Ghosh (1995) noticed petechial haemorrhages on heart, enlarged liver with necrotic spots and congestion of intestine, spleen and liver in a duck cholera outbreak.

Hegde *et al.* (1996) observed pin point haemorrhages on the epicardium, airsacs, inner aspect of the keel and the intestinal serosa, congested and enlarged liver and grayish white pin point necrotic spots on surface, severe congestion of

kidneys, enlarged and mottled spleen, congestion of meningeal vessels, haemorrhagic enteritis with blood mixed contents in the duodenum and jejunum in an outbreak of pasterellosis in ducks.

In a report of fowl cholera in psittacines, Morishita *et al.* (1996) noted lesions in digits characterized by extensive necrotic areas of the epidermis that were overlaid with a fibrinopurulent membrane. Haemrrhages in muscles, congested liver with white multifocal areas, airsacculitis and enlargement of spleen were also noted.

Lesions in acute form of duck pasteurellosis included marked congestion of the carcass, petechial and echymotic haemorrhages on myocardium, gizzard, proventriculus, peritoneum, coronary and abdominal fat. The duodenum showed inflammation and contained viscous mucous. Liver was often enlarged with multiple pin point grayish necrotic foci. Flaccidity and rupture of mature ovarian follicles and hyperemia of immature follicles were also observed (Reddy, 1996).

Morishita *et al.* (1997) came across lesions like polyserositis, fibrin deposits in airsacs, oesophageal abcesses, mild haemorrhagic enteritis in fowl cholera affected raptors. Liver showed fibrinous capsulitis and multifocal areas of necrosis. Edema, congestion, atelectiasis, pneumonic changes and necrosis were seen in lungs. Fibrinonecrotic pericarditis, valvular endocarditis, congested spleen with multiple randomly distributed foci of acute necrosis and congestion of kidneys was also evident.

Sinusitis with clear thick sinus exudate, conjunctivitis with watery eyes and crusty eyelids, hepatomegaly, spleenomegaly and multifocal pale pinpoint foci in liver were evident in chicken pasteurellosis (Gustafson *et al.*, 1998).

The necropsy findings in a subacute to chronic fowl cholera in quails were emaciation, generalized carcass congestion, mild hepatomegaly with green discolouration, congested intestinal mucosa, caseous purulent arthritis-

osteomyelitis of the hock joints and thickened crop epithelium (Miguel *et al.*, 1998).

In a duck cholera outbreak, Devi *et al.* (2000) observed petechiae on pericardium, congested and swollen liver with necrotic foci on surface, congestion of lungs, spleen and intestine along with pin point haemorrhages in the intestine.

Kwon and Kang (2003) observed lesions consistent with fowl cholera including multifocal necrotic foci in the liver with enlargement, petechial or ecchymotic hemorrhages on the heart and mucoid exudates in the duodenal mucosa in layers.

Quails infected with fowl cholera enlarged friable liver with mottled appearance and focal areas of necrosis, haemorrhagic and mottled appearance of spleen and swollen and mottled kidneys were seen in few birds. Lungs and intestinal mucosa were congested in all the cases (Srilatha *et al.*, 2003).

At necropsy, the fowl cholera affected ducks had swollen infraorbital sinuses consisting of serous discharge, mucopurulent exudate, and or cheese-like material. Little frothy fluid was present in air sacs in all affected birds. Some ducks had fibrinous polyserositis including perihepatitis, airsacculitis, and pericarditis. The fibrinous polyserositis was present in ducks that were older than two weeks. (Songserm *et al.*, 2003)

In an outbreak of pasteurellosis among Japanese quails spleen, intestine and kidneys were found congested on necropsy. Liver showed mottling in most of the birds and many had areas of necrosis. Lungs were congested and consolidated. Haemorrhages were noticed on epicardium and myocardium. (Roy *et al.*, 2004)

Bhattacharya (2005) observed congestion of Spleen, liver and lungs, haemorrhages on pericardium, proventriculus and intestine in an outbreak of duck pasteurellosis.

2.5.1.2 Experimental cases

On experimental inoculation with *P. multocida* in Mallard ducks, lesions of an acute haemorrhagic septicaemia with petechiae over the epicardium and all serosal surfaces were observed by Hunter and Wobeser (1980). Spleenic and hepatic necrosis, intestinal haemorrhages and catarrhal enteropathy were also noted.

After intra- airsac inoculation with *P. multocida,* lesions in died turkey poults were vascular congestion and slightly increased amounts of peritoneal fluid, exudative airsacculitis and peritonitis. In the surviving exposed poults, lesions were limited to the site of injection. (Rhoades and Rimler, 1990)

Christensen and Bisgaard (1997) observed thickening of the air sac wall and pericarditis in an experimental *P. multocida* infection in turkeys.

Macroscopic lesions like petechiae on the coronary band on heart, atrophy of bursa and thymus, focal hepatic necrosis, peritonitis, and muscle wasting were observed by Fisher *et al.*, (1998) in broilers experimentally inoculated with *P. multocida.*

In chicken experimentally infected with *P. multocida,* gross lesions observed by

Wilkie *et al.* (2000) were multiple small serosal haemorrhages and focal necrotic foci in the liver. Less acute lesions such as fibrinous pericarditis, airsacculitis and arthritis were also observed.

In an experimentally induced *P. multocida* infection in broilers and turkeys, Sarkozy *et al.* (2002) reported subepicardial petechiae, swollen liver with

multiple necrotized foci, acute haemorrhagic catarrhal enteritis, necrotic enteritis, fibrinous exudates in the peritoneum, fibrinous pleuritis, pneumonia, arthritis and leptomeningitis.

Shilpa *et al.* (2005) observed petechiae on heart and thighs, congestion of lungs, spleen, liver and kidneys in *P. multocida* inoculated layers. Pericarditis, mottling of spleen, large discrete necrotic foci on liver and cysts on bursa were also noticed.

Shivachandra *et al.* (2005) experimentally inoculated chicken with *P. multocida* and observed lesions such as swollen liver with necrotic foci and petechiae, haemorrhages on subepicardial and subserosal areas. On embryo inoculation, the lesions were severe congestion of the entire embryo, haemorrhages in the feather tracts, oedema of the head region, mottled liver, petechiae on heart and lungs. CAM showed thickening with presence of petechial and echymotic haemorrhages. Yolk sac was also congested with thickening whereas allantioc and amniotic fluids were blood stained.

In ducks experimentally induced with *P. multocida,* major lesions noticed were airsacculitis, pericarditis and perihepatitis. Necrotic spots were seen on the spleen and liver (Mbuthia *et al.*, 2008).

2.5.2 Lesions in chronic cases

Fibrinous perihepatitis, pericarditis, pleurtis, diffuse fibrinous air sac lesions, ocular and joint lesions were recorded by Hunter and Wobeser (1980) in fowl cholera infected Mallard ducks.

Rao (1990) and Sahoo et al. (1994) noticed swollen wattles and joints containing caseous exudate in localized form of fowl cholera.

Caseous arthritis of the hock and foot joints, swelling with induration of one or both wattles, pneumonia, conjunctivitis, emaciation, debilitation and

caseous exudates in peritoneal cavity, oviduct, middle ear, sinuses and base of brain were observed by Reddy (1996) in fowl cholera affected chicken.

2.6 HISTOPATHOLOGY

2.6.1 Natural cases

Congestion and many areas of focal necrosis in liver was noticed by Fujihara *et al.* (1986) in an outbreak of pasteurellosis in ducks. The necrotic areas and the sinusoids contained a large number of bacteria. Necrotic areas in spleen and epicardial haemorrhages were seen. Haemorrhagic infarcts and bacteria were also present in tunica intima of ventricles. Congestion and *thrombosis* were observed in lungs. Tunica serosa was haemorrhagic. Haemorrhage, thrombi and bacteria were found in central nervous system.

Chakraborthy *et al.* (1989) observed thickening of interlobular septae and presence of serofibrinous exudates in lungs in a duck pasteurella outbreak. The ulcers observed in lungs were made of dead and degenerating heterophil polymorphs, surrounded by diphtheritic membranes outside with infiltration of mononuclears. In addition, the alveolar epithelium showed proliferation, the cells being rounded with foamy cytoplasm occupying the alveolar lumen.

Multiple small focal areas of coagulative necrosis and heterophilic infiltration, large number of bacteria in the necrotic areas and in the hyperemic vessels were the lesions observed in the liver of *P. multocida* infected muscovy ducks (Nakamine *et al.*, 1992). A decrease in lymphocytes and a marked increase in reticulocytes were the main changes in spleen. Edema was observed in lungs.

In *P. multocida* infected turkeys, the histological picture showed acute necrosis of collagen and loose connective tissue and infiltration of a large number of heterophils mixed with fibrin and bacterial colonies in the subcutis. There was degeneration and necrosis of underlying muscle fibres with infiltration of few heterophils. Multifocal areas of pyogranulomatous inflammation in the deep

dermis revealed infiltration of heterophils and multinucleated giant cells. Severe fibrinous pneumonia and acute multifocal hepatocellular necrosis with thrombosis of vessels were also encountered (Jeffrey *et al.*, 1993).

Coagulative necrosis and heterophilic infiltration in liver, fibrinosuppurative meningitis were the major lesions observed in chicken died of fowl cholera (Sahoo *et al.*, 1994).

In an outbreak of pasterellosis in ducks, liver showed multifocal haemorrhage, necrotic foci, lymphoid cell infiltration in the portal triad, interlobular connective tissue proliferation and biliary hyperplasia. Areas of lymphoid cell infiltration in the myocardium, massive necrosis in the lymphoid follicles of spleen, pneumonia and congested meningeal and cerebral vessels, haemorrhagic enteritis, degeneration and desquamation of villi epithelium and infiltration of mononuclear cells in lamina propria were also observed (Hegde *et al.*, 1996).

Morishita *et al.* (1996) described lesions such as degeneration of the koilin layer of gizzard, septic thrombi in pulmonary arteries, and myocarditis in fowl cholera affected psittacines. Liver lesions were areas of coagulation necrosis with aggregations of mononuclear lymphocytic inflammatory cells and periportal mononuclear hepatitis. In spleen multiple randomly distributed foci of coagulation necrosis containing degenerating mononuclears and numerous clusters of bacteria, lymphoid hyperplasia and plasmacytosis were evident. Inflammatory cell infiltrates were seen in renal parenchyma.

Morishita *et al.* (1997) inspected fowl cholera affected raptors and noticed haemorrhages, myofibril degeneration, necrosis with mineralization and heterophil aggregation in muscles and haemorrhagic enteritis with increased number of heterophils in lamina propria. Lymphatic dilatation was there in proventriculus. Liver showed periportal hepatitis with multiple foci of coagulation necrosis containing inflammatory cells. Haemosiderin laden

macrophages scattered throughout liver parenchyma. Edema, haemorrhages, areas of necrosis and foci of pneumonia were evident in lungs. Inflammation of the parabronchi was characterized by degenerating heterophils and lymphocytes.. Fibrinonecrotic pericarditis was also seen. In spleen and kidneys, multiple foci of acute necrosis containing degenerated heterophils and clusters of bacteria surrounded by multinucleated giant cells were observed.

Heterophilic infiltration and fibrinopurulent exudates filling the air spaces of cranial bones, heterophilic and lymphoplasmacytic infiltration with fibrinopurulent exudates in sub arachnoid space were reported by Gustafson *et al.* (1998) in chicken pasteurellosis. There was localized meningitis with lymphocytic infiltration of pia matter. Heamorrhages in dura and demyelination in medulla were the other brain lesions. Hyperplasia and lymphocytic infiltration of the lamina propria were evident in trachea. Periportal lymphocytic and heterophilic infiltration was found in liver.

Histopathological examination in a case of sub acute to chronic fowl cholera in quails revealed mild hepatic amyloidosis, proliferative parabronchitis, spleenic reticular cell hyperplasia, thymic cortical atrophy, subacute bacterial osteomyelitis, periarthritis and crop mycosis (Miguel *et al.*, 1998).

Hepatocytic necrosis with bacterial colonization, hemorrhage and necrosis in the myocardium, and hemorrhagic enteritis were reported by Kwon and Kang (2003) in fowl cholera affected chicken.

Songserm *et al.* (2003) observed degeneration and necrosis of nasal epithelium in *P. multocida* affected ducks. Granulocytic, macrophage, and lymphoid infiltrations were present in the nasal wall. Hyperplasia of nasal epithelium and gland was present in some affected ducks. The inflammatory reaction was found to be present extensively in all parts of the nasal cavity.

Roy *et al.* (2004) found histological changes of sinusoidal and central venous congestion of liver with focal areas of necrosis in an outbreak of fowl

cholera in quails. Heart revealed evidence of vascular disturbances characterized by congestion, haemorrhage and degeneration of myocardial fibres. Kidney showed vascular changes with congestion. Lungs showed congestion and pneumonia.

2.6.2 Experimental cases

In an experimental study of fowl cholera in Mallard ducks, multifocal necrotizing pneumonia, congestion and edema of lungs, acute fibrinonecrotic pericarditis serous myocarditis with vasculitis, necrosis of lymphoid follicles of spleen, haemorrhages and necrotizing fibrinous meningitis, edema and necrosis of lymphoid aggregations throughout alimentary canal were observed by Hunter and Wobeser (1980). Focal areas of acute coagulation necrosis in liver, sinusoidal dilatation with edema, fibrin and cellular debris were also seen.

Prantner *et al.* (1990) noted fibrinopurulent bronchopneumonia with marked heterophil infiltration followed by thickening of interlobular septae, severe pulmonary necrosis, pleuritis and vasculitis in lungs after experimental inoculation of *P. multocida* A: 3,4 strain in turkeys. Hepatic lesions included focal heterophil aggregates and hepatic necrosis. There was degeneration of periarteriolar reticular cells in spleen; those cells progressed to coalescing coagulative spleenic necrosis with extracellular bacterial colonies.

Severe swelling of epithelial and mesothelial cells, thickening by proteinaceous fluid and infiltration of heterophils were the lesions observed in air sacs in an experimental *P. multocida* infection in turkeys (Christensen *et al.*, 1998).

Fisher *et al.* (1998) noted microscopic lesions of airsacculitis, tracheitis, pneumonia, hepatitis and splenitis in broilers experimentally inoculated with *P. multocida.* Inflammatory exudates on serosal surfaces consisted of heterophils, macrophages and bacterial colonies enmeshed in fibrin and edema. Lymphocytic infiltration was seen in hepatic portal triads and fibrin thrombi were present

throughout the spleenic white pulp. Multinucleated giant cells and fibroblasts were observed bordering necrotic exudates. Inflammation of the pancreas, duodenum, jejunum and caeca occurred sporadically as localized extensions of peritonitis. Mild lymphoid depletion and foci of necrosis were present in bursa and thymus.

Wilkie *et al.* (2000) noticed extensive necrosis and cellular infiltration of both superficial and deep pectoral muscle around the injection site in chicken experimentally infected with *P. multocida.*

Shilpa *et al.* (2005) reported microscopic lesions like congestion, haemorrhages, presence of bacterial colonies, vasculitis and necrosis of liver and kidneys in *P. multocida* challenged layers. Spleenic changes were lymphoid depletion, RE hyperplasia and secondary lymphoid nodules. In kidneys, diffuse interstitial nephritis was observed with infiltration of intertubular area by mononuclears. Degeneration of tubular epithelium, focal areas of necrosis and casts were present in renal tubules. Lesions in liver were observed as large areas of suppuration and focal areas of coagulative necrosis involving parenchyma and mononuclear cell infiltration. Myocardium was congested and revealed fibrinous thickening, coagulative necrosis with varying degree of heterophilic infiltration.

Shivachandra *et al.* (2005) noticed congestion of blood vessels, haemorrhages and edema in the intermycium of heart in dead layers experimentally inoculated with *P. multocida.* Congestion of blood vessels and sinusoids, degenerative changes in hepatocytes, multifocal coagulative necrosis and heterophilic infiltration were evident in the liver. Congestion and serofibrinous exudation was noticed in lungs. Tubular degeneration, congestion and heterophilic infiltrations were noticed in kidneys. Similar lesions were observed on embryo inoculation studies also.

2.7 HAEMATOLOGICAL ALTERATIONS

Hunter and Wobeser (1980) noticed that blood samples taken from ducks 12 hr post inoculation had large numbers of circulating heterophils and phagocytic mononuclear cells containing bipolar bacteria. Few free bacteria were present in the 12 hr post inoculation blood smears. Samples drawn at 24 hr post inoculation from ducks that proceeded to death had a massive bacteremia with very few degenerating phagocytes.

Latimer *et al.* (1988) observed that during acute inflammation in chicken, significant leukocytosis and heterophilia developed by six hours and persisted through seven days. The peak mean heterophil and leucocyte counts occurred at 12 hours and three days respectively. Heterophil nuclear scores documented nuclear hyposegmentation (left shift) during early inflammation and nuclear hypersegmentation (right shift) during convalescence. Mean monocyte and lymphocyte counts peaked at second and third days, respectively. Toxic changes of heterophils were cell swelling, degranulation, cytoplasmic vacuolation and cytoplasmic basophilia. Ultra structurally, toxic heterophils had intracellular edema, dissolution of granules, retention of ribosomes, nuclear membrane blebs and decreased heterochromatin density.

Rose *et al.* (1988) inoculated *Pasteurella multocida* (P-1059) intravenously into turkeys.The leukocyte count of inoculated birds remained unchanged from that of the control. However the differential leukocyte count shifted in favor of significant increases in heterophils and decreases in lymphocytes and monocytes at 6 and 12 hr after inoculation.

Toth *et al.* (1988) found that intratracheal administration of a live, apathogenic *P. multocida* vaccine increased the number of avian respiratory phagocytes.

Prantner *et al.* (1990) observed marked increase in heterophil counts after experimental inoculation of *P. multocida* A:3,4 strain in turkeys.

Bojesen *et al.* (2004) pointed out that, recruitment of heterophils into the respiratory tract during *P. multocida* infection was found to contribute considerably to the lung lesions in chickens and was found to mediate tissue damage, possibly allowing a more rapid systemic spread of *P. multocida.* Heterophil activation plays a dual role for the outcome of infection, where it initially seems to promote invasion and systemic spread but subsequently helps limit the infection by giant cell formation and bacterial clearance.

In an experimental study of fowl cholera in chicken, at 5 day post inoculation there was a significant increase in haemoglobin concentration in the infected group. Average PCV did not vary significantly. In total erythrocyte count, a significant increase was observed initially in the infected group at 24 hour post inoculation. The mean TLC values in the infected group were higher at all intervals and significantly increased between 48 hour and 7 day post inoculation. In DLC, the heterophil count increased significantly from 24 hour to 15 day post inoculation. Consequent decrease was there in lymphocyte count. No difference in monocyte count, except at 10 and 15 hour post inoculation these values were lower in the infected group. At five day post inoculation eosinophils count increased significantly in infected group as compared to their counterparts (Shilpa *et al.*, 2005).

Latif and Dessouky (2006) studied about respiratory infections due to *P. multocida* in lambs. Haematological analysis revealed high significant decrease in the total erythrocyte count, haemoglobin concentration, packed cell volume and blood lymphocyte (per mm^3 blood) in diseased animals when compared with clinically healthy animals. Significant increase in total leukocyte count, neutrophil, eosinophil and monocyte cells were also recorded in diseased cases.

Haematological analysis of rabbits experimentally inoculated with *P. multocida* serotype A: 3 revealed significant decrease in values of Hb, PCV and TEC on 7^{th} and 14^{th} DPI and rising trend in values of TLC, heterophils, monocytes and corresponding lymphocytopenia (Rameshkumar *et al.*, 2006).

Praveena *et al.* (2007) studied haematological changes in mice, which were experimentally infected with *Pasteurella multocida* serotype A: 1 and sacrificed at different hours post-infection (HPI). A transient leukocytopenia at early HPI and progressive leukocytosis at later HPI were observed in all the infected animals. An increase in thrombocyte and decrease in total erythrocyte counts were observed, but the changes were not statistically significant between the infected and control group of animals. In differential leukocyte counts, a progressive neutrophilia and corresponding lymphocytopenia were observed throughout the course of infection. Monocytosis was observed in animals of all infected groups, while there were no changes in basophil and eosinophil counts. Among different erythrocytic indices, the changes were observed only in PCV, Hb, MCHC and MCH values.

2.8 SEROLOGICAL ALTERATIONS

In a study about respiratory infections due to *P. multocida* in lambs, blood biochemical analysis showed significant increase in the values of AST, ALT, GGT, creatinine and blood urea and significant decrease in the level of albumin, total protein, sodium chloride and calcium levels of diseased animals (Latif and Dessouky, 2006).

Decreases in serum total protein and calcium levels and increase in cholesterol were noticed in rabbits experimentally inoculated with *P. multocida* serotype A: 3 (Rameshkumar *et al.*, 2006).

Praveena *et al.* (2007) studied biochemical changes in mice, which were experimentally infected by *Pasteurella multocida* serotype A: 1. An increased total protein and albumin: globulin ratio was observed whereas the level of plasma fibrinogen and other serum biochemical values remained normal in animals of infected groups.

Materials and Methods

3. MATERIALS AND METHODS

3.1 *Pasteurella multocida* – THE PATHOGEN

The *Pasteurella multocida* A: 1 strain (DP 1) isolated from Niranam duck farm (Pathanamthitta district, Kerala), serotyped at IVRI, Izatnagar and maintained in the Department of Veterinary Microbiology, College of Veterinary & Animal sciences, Mannuthy was utilized for the present study.

Purity of the isolate was checked based on morphology, cultural and biochemical characteristics as described by Barrow and Feltham (1993).

3.2 PATHOGENECITY TESTING OF DP 1

3.2.1 Laboratory Animals

Swiss albino female mice, six to eight weeks of age (weighing approximately 20g) were procured from the Small Animal Breeding Station (SABS), College of Veterinary & Animal sciences, Mannuthy.

3.2.2 Lab animal inoculation

An 18 h broth culture of *Pasteurella multocida* containing approximately 3×10^9organisms/ml was inoculated (0.1millilitre) intraperitonially and subcutaneously to four mice each. Four mice were kept as controls which were mock inoculated with sterile PBS (pH 7.4). All the mice were observed for seven days post inoculation. Blood smears were prepared from the dead mice and stained with Leishman's stain.

3.2.3 Reisolation in pure culture

Reisolation of *Pasteurella multocida* from heart blood, lung, liver and spleen of the dead mice was carried out on Tryptone Soya Agar (TSA) by incubation at 37^0C under five per cent carbon dioxide tension.

3.3 EXPERIMENTAL BIRDS.

One month old unvaccinated ducklings, weighing approximately 350g, procured from a private breeder at Alappuzha, were used. Ducklings were maintained on standard feeding and management practices in laboratory for one week before commencement of studies.

3.4 PREPARATION OF LETHAL DOSE 50 (LD_{50}) DILUTION

Lethal dose 50 of *Pasteurella multocida* in one month old ducklings was determined as described by Jayakumar (1998) and Rajagopal (2007).

Pasteurella multocida A: 1 strain was passaged in mice to get a fully encapsulated virulent form. The virulent organisms isolated from mice were grown on Tryptose Soya Agar (TSA) at 37^0C for 24 h. The growth on TSA was harvested, washed thrice in PBS by centrifugation at 3000 rpm for 15 min and resuspended in the same buffer to contain 3×10^9 cells/ml. Then serial ten fold dilutions were made upto 3×10^0 cells/ml. Respective plate counts were done to assess the number of bacteria present in each dilution.

The LD_{50} was determined by the method described by Reed and Muench (1938). Four week old ducklings were randomly assigned to twelve groups of six ducklings each and the first seven groups were separately inoculated with the different dilutions of the bacteria with a dose of 0.1 millilitre per bird intramuscularly and the twelfth group served as controls which were sham inoculated with 0.1 millilitre of sterile PBS (pH 7.4). Mortality was recorded up to one week post inoculation. All the dead ducklings were examined for specific gross lesions caused by *P. multocida* and attempted re-isolation of the organism on blood agar from heart blood, liver and spleen.

3.5 EXPERIMENTAL DESIGN

Thirty six one month old ducklings were maintained under identical feeding and managemental conditions in the laboratory and were randomly divided into three groups of twelve each and designated as group I to group III.

3.5.1 Group I

Maintained as healthy control, under standard feeding and managemental conditions.

3.5.2 Group II

Pasteurella multocida LD_{50} dilution was injected subcutaneously at the dorsal neck region @ 0.1 ml/bird.

3.5.3 Group III

Pasteurella multocida LD_{50} dilution was administered by intra-nasal inoculation @ 0.1 ml/bird.

All the dead ducklings were examined for specific gross lesions caused by *P. multocida* and attempted re-isolation of the organism on blood agar from heart blood, liver and spleen.

3.6 PARAMETERS

3.6.1 Body weight

The body weight of individual birds was recorded before the experiment (day zero) and at weekly intervals, depending on the survivability of the birds. From this data, mean body weight was calculated.

3.6.2 Mortality pattern

Mortality of birds in each group after the administration of *P. multocida* inoculum was recorded till 30th day of experiment. The birds were routinely observed for clinical signs exhibited.

3.6.3 Blood collection

Blood samples were collected from all the birds before the start of experimental trial and at weekly intervals by jugular venipuncture. Blood was collected with appropriate anticoagulant for estimation of haematological parameters. Blood sample without anticoagulant was also collected for serum biochemical examination. The collected blood was allowed to clot and incubated at 37°C for 30 min. Serum was separated following overnight incubation at 4°C and stored at -20°C until use. Ethylene diamene tetra acetic acid (EDTA) was used as anticoagulant @ 2 mg/ml.

3.6.4 Haematological parameters

The haemoglobin (Hb) level of blood was estimated by acid haematin method (Feldman *et al.*, 2000). The method described by Natt and Herrick (1952) was followed for Total Leucocyte Count (TLC) and Total Erythrocyte Count (TEC). Packed cell volume (PCV) and Differential Leucocyte count (DLC) were estimated by the method suggested by Thrall *et al.* (2004). All parameters mentioned above were checked on day zero and at weekly intervals.

Blood smears were prepared on clean grease free glass slides using fresh blood at the time of blood collection. Air dried smears were stained with Leishman Giemsa stain solution.

3.6.4.1 Leishman Giemsa staining

3.6.4.1.1 Preparation of the stain

Ground 0.15 g of Leishman stain powder and 30 mg Giemsa powder with small amounts of acetone free methyl alcohol until an even suspension of stain was obtained. A total of 100 ml acetone free methyl alcohol was added to produce a complete solution.

Filtered stain solution was poured into a dark bottle and aged for three weeks prior to use.

3.6.4.1.2 Method of staining

The air dried blood film was flooded with filtered the stain solution made from undiluted stock solution of Leishman-Giemsa stain and kept for 45 sec to fix. The stain on the smear was diluted with double the volume of buffered distilled water, mixed by gentle blowing and stained for three minutes. The slides were washed in distilled water. The back of the slides were wiped to remove excess stain and air dried in an upright position.

The stained smears were checked for the presence of bipolar *P. multocida* organisms under oil immersion objective of the microscope. Blood cells were counted and their percentages were calculated.

3.6.5 Biochemical studies

Serum protein estimation was done at day zero and at weekly intervals. Blood collected in fresh vials without anticoagulant was kept at room temperature for one hour. Then it was centrifuged at 2000 rpm for 20 min. The serum was aspirated into another vial and used for the estimation of total protein and albumin.

Total serum protein was estimated by Biuret method (Henry *et al.*,1957). Albumin was estimated by Douma's method (Doumas *et al.*, 1971), using kit supplied by Agappe Diagnostics pvt Ltd, Ernakulam, Kerala.

3.7 OBSERVATIONS

3.7.1 Pathoanatomical studies.

Detailed postmortem examination was conducted on the birds which died after the inoculation of *Pasteurella multocida*. At the end of experiment, the remaining birds were sacrificed. Postmortem was conducted and gross lesions were recorded. Liver, heart, spleen, kidney, brain, lungs, intestine, caecal tonsils and bursa were collected for histopathology. Brain was fixed in neutral buffered formalin and other tissues were fixed in 10 per cent formalin. They were then processed and paraffin embedded as described by Sheehan and Hrapchak (1980). Sections were cut at four micron thickness and stained with routine Haematoxylin and Eosin stain (Bancroft and Cook, 1984). The sections were examined in detail under light microscope.

3.7.2 Reisolation from dead birds

Attempts were made to reisolate *P. multocida* from heart, liver and spleen of the dead birds in Tryptone Soya Agar plates. Characterization and identification of the isolate was done based on the morphology, cultural and biochemical characteristics. The organisms in the heart and organ impression smears were demonstrated using Leishman's staining.

3.8 STATISTICAL ANALYSIS

Data collected from various parameters were analysed as per the method of Snedecor and Cochran (1994) by using one way analysis of variance (ANOVA), followed by Duncan's multiple range test for grouping means having significance.

Results

4. RESULTS

Results obtained from experimental study of *Pasteurella multocida* inoculation in ducks are tabulated and presented in tables and graphs/diagrams.

4.1 PURITY CHECKING OF THE ISOLATE OF *P. multocida*

Purity checking of the isolate was done based on the assessment of morphology, cultural characteristics and biochemical characterization.

4.1.2 Purity Checking and Characterization

The *Pasteurella multocida* A: I strain (DP I) produced typical colonies of *P multocida* on Tryptone Soya Agar after incubation at 37°C for 24 h and were smooth, convex, translucent, and butyraceous. Gram staining revealed Gram negative cocco-bacillary organism arranged singly or in pairs.

The biochemical reactions used for characterization of *P. multocida* (Barrow and Feltham, 1993) gave the expected results, confirming the identity of the organism. No growth on Mac Conkey's agar, negative for haemolysis on blood agar and urease activity were included as primary characters for *P. multocida.* All these reactions were the same as above for DP1. It was catalase and oxidase positive and fermentative. Heddleston (1976) reported that all the cultures of *P. multocida*, fermented glucose and sucrose and also reduced nitrate, while none of the cultures fermented salicin or produced haemolysin. The reactions given by DP1 were in accordance with these findings.

Mutters *et al.* (1985) based on fermentation patterns of dulcitol and sorbitol categorized those positive for sorbitol but negative for dulcitol as *P. multocida* subsp. *multocida.* The reactions given by DP1 for these sugars were the same as described by them.

The isolate in this study gave a positive reaction for both indole and ornithine decarboxylase.

4.2 PATHOGENICITY TESTING IN MICE

Pasteurella multocida serotype A: 1 killed the mice inoculated with 0.3 × 10^8 organisms/0.1 ml intraperitoneally within eight hours and within 24 h when injected by subcutaneous route. The control mice were alive even after an observation period of seven days.

Similar lesions were observed in the inoculated mice irrespective of the route of inoculation. The gross lesions observed in the inoculated mice were petechial haemorrhages in the epicardium and general congestion of all the visceral organs, particularly of lung, liver and spleen. Fluid accumulation was also noticed in the peritoneal cavity of mice inoculated intraperitoneally. Blood smear and impression smears from spleen and liver following Leishman's staining revealed large number of bipolar stained organisms. Re-isolation of *P. multocida* in pure culture was done from the heart blood, lungs, liver and spleen on bovine blood agar at 37°C under five per cent carbon dioxide tension.

4.3 LETHAL DOSE 50

4.3.1 Lethal Dose 50 in Duckling

Median lethal dose was determined in ducklings after the *in vivo* passaging in mice to increase the virulence of the organism. The LD_{50} was calculated as 31.7 x$10^8$$LD_{50}$/ml. The methods followed for LD_{50} calculation are furnished in Table 1.

Table. 1. Different dilution of bacteria inoculated i/m into one month old ducklings to estimate LD_{50} value.

Dilution	Organisms present in the inoculum	No: of birds inoculated	No: dead	No: alive	Cumulative value		Ratio +ve	per cent of +ve
					+ve	-ve		
10^{-1}	3 x 10^7in 0.1 ml	6	6	0	60	0	60/60	100
10^{-2}	3 x 10^6 in 01.ml	6	6	0	54	0	54/54	100
10^{-3}	3 x 10^5 in 0.1 ml	6	6	0	48	0	48/48	100
10^{-4}	3 x 10^4 in 0.1ml	6	6	0	42	0	42/42	100
10^{-5}	3 x 10^3 in 0.1ml	6	6	0	36	0	36/36	100
10^{-6}	3 x 10^2 in 0.1ml	6	6	0	30	0	30/30	100
10^{-7}	3 x 10^1 in 0.1ml	6	6	0	24	0	24/24	100
10^{-8}	3 x 10^0 in 0.2ml	6	6	0	18	0	18/18	100
	3 x 10^0 in0.15ml	6	6	0	12	0	12/12	100
	3 x 10^0 in 0.1ml	6	5	1	6	1	6/7	85.71
	3 x 10^0 in0.05ml	6	1	5	1	6	1/7	14.29

Calculation of LD_{50}

The results of LD_{50} are furnished in Table 1.

$$\text{Proportionate distance} = \frac{85.71 - 50}{85.71 - 14.29} = \frac{35.79}{71.42} = 0.501$$

So titre of original suspension = $10^{8.5}$/0.1 ml

So titre of the undiluted sample contain = $1/0.1 \times 10^{8.5} LD_{50}$

$= 10 \times 10^8 \times$ antilog of 0.501

$= 10 \times 10^8 \times 3.17$

$= 31.7 \times 10^8 LD_{50}$/ ml

4.4 PHYSIOLOGICAL PARAMETERS

4.4.1 Body Weight

The individual and mean body weight of ducks of Group I, II and III were recorded on 0, 7, 14, 21 and 28th days of the experiment and are presented in the Table 2 and Figure 1. Body weight of ducks of all group showed a gradual increase throughout the experimental period. No significant differences were observed between all the three groups. However, the birds in treatment groups did not gain weight as much as the control group although it was not statistically significant.

4.5 HAEMATOLOGICAL PARAMETERS

4.5.1 Packed Cell Volume (PCV)

The average packed cell volume of each group at weekly intervals are shown in Table 3 and Figure 2. A significant increase ($p<0.05$) in the levels was observed in both treatment groups on day seven when compared to the control group. Significant difference was observed between treatment groups also. Mean PCV of control group was 29.67 ± 0.71 per cent and that of groups II and III were 39.00 ± 0.89 per cent and 33.27 ± 0.74 per cent respectively on that day. On 14th

and 21st days, there was significant increase in the levels in group III compared to control. Mean values were 30.25 ± 0.80 per cent and 29.25 ± 0.52 per cent for control group and 35.45 ± 1.32 per cent and 35.73 ± 1.05 per cent for group III on 14th and 21st day respectively.

4.5.2 Haemoglobin

The mean values of haemoglobin of each group at weekly intervals are shown in Table 4 and Figure 3. A significant increase ($p< 0.05$) in the haemoglobin levels was observed in both treatment groups on seventh day, when compared to the control group. Significant difference was there between treatment groups also. On day 7, mean haemoglobin concentration of control group was 11.90 ± 0.12 g/dl and that of groups II and III were 13.33 ± 0.18 g/dl and 12.58 ± 0.13 g/dl respectively. On 14th and 21st days, group III showed significant increase ($p<0.05$) in the levels compared to control. Mean values were 11.71 ± 0.18 g/dl and 11.65 ± 0.12 g/dl for control group and 12.23 ± 0.22 g/dl and 12.22 ± 0.20 g/dl for group III on 14th and 21st days respectively.

4.5.3 Total erythrocyte Count (TEC)

The mean erythrocyte count of group I, II and III at weekly intervals are shown in Table 5 and Figure 4. Significant increase ($p< 0.05$) in the erythrocyte count was observed in both treatment groups on day 7, when compared to the control group. Significant difference was also observed between treatment groups. On day 7, mean erythrocyte count of control group was 2.10 ± 0.10 $\times 10^6/\mu l$ and that of groups II and III were 4.02 ± 0.18 $\times 10^6/\mu l$ and 3.15 ± 0.16 $x10^6/\mu l$ respectively. On 14th day, group II and III showed significant increase ($p<0.05$) in the levels compared to control. Mean TEC of control group was 1.82 ± 0.16 $\times 10^6/\mu l$ and that of groups II and III were 3.35 ± 0.18 $\times 10^6/\mu l$ and 2.98 ± 0.19 $\times 10^6/\mu l$ respectively on that day. Group III showed significant increase (3.18 ± 0.16 $\times 10^6/\mu l$) on 21st day compared to control (1.86 ± 0.12 $\times 10^6/\mu l$).

4.5.4 Total leucocyte Count (TLC)

The mean leucocyte count of group I, II and III at weekly intervals are shown in Table 6 and Figure 5. Except on day zero, a significant increase ($p< 0.05$) in the levels was observed in both treatment groups on all periods, when compared to the control group. Significant difference was also observed between treatment groups. The mean values on day 7 were ($53000.00 \pm 4024.92/mm^3$ for group II, $35090.91 \pm 1855.97/mm^3$ for group III, and $21166.67 \pm 1445.12/mm^3$ for control). On day 14, the values were ($43000.00 \pm 2236.07/mm^3$ for group II, $33454.55 \pm 1770.28/mm^3$ for group III and $19333.33 \pm 864.57/mm^3$ for control). On day 21, the values were ($28000.00 \pm 2529.82/mm^3$ for group II, $34727.27 \pm 1854.19/mm^3$ for group III and $19500.00 \pm 1671.96/mm^3$ for control). On 28th day, the observed values were $26333.33 \pm 2703.91/mm^3$ for group II and $19000.00 \pm 1604.92/mm^3$ for control group.

4.5.5 Differential Leukocyte Count- Lymphocytes (percentage)

The mean lymphocyte count of group I, II and III at weekly intervals are shown in Table 7 and Figure 6. Except on day zero, both treatment groups showed a significant decrease ($p< 0.05$) in the levels on all periods, when compared to the control group. The mean values on day 7 were (40.17 ± 1.35 per cent for group II, 41.91 ± 0.90 per cent for group III, and 64.67 ± 1.18 per cent for control). On day 14, the values were (41.50 ± 1.38 per cent for group II, 39.64 ± 0.83 per cent for group III and 63.67 ± 1.01 per cent for control) and on day 21 (42.00 ± 0.86 per cent for group II, 40.55 ± 0.99 per cent for group III and 66.75 ± 2.11 per cent for control). On day 28, the values were (53.67 ± 1.99 per cent for group II and 64.33 ± 1.19 per cent for control).

4.5.6 Differential Leukocyte Count- Heterophils (percentage)

The mean heterophil count of group I, II and III at weekly intervals are shown in Table 8 and Figure 7. Except on day zero, both treatment groups showed a significant increase ($p< 0.05$) in the levels on all periods, when

compared to the control group. The mean values were, on day 7 (55.33 ± 1.43 per cent for group II, 53.18 ± 0.89 per cent for group III, and 29.58 ± 2.34 per cent for control), on day 14 (52.00 ± 1.06 per cent for group II, 51.91 ± 0.96 per cent for group III and 28.08 ± 1.17 per cent for control), on day 21 (49.83 ± 1.14 per cent for group II, 51.73 ± 0.81 per cent for group III and 28.83 ± 1.24 per cent for control) and on day 28, (39.83 ± 1.49 per cent for group II and 27.75 ± 1.23 per cent for control).

4.5.7 Differential Leukocyte Count- Monocytes (percentage)

The mean monocyte count of group I, II and III at weekly intervals are shown in Table 9 and Figure 8. Significant decrease ($p< 0.05$) in the levels was observed in both treatment groups on day seven, when compared to the control group. On day seven, mean monocyte count of control group was 6.08 ± 0.40 per cent and that of groups II and III were3.00 ± 0.45 per cent and 3.36 ± 0.34 per cent respectively. On 14th day, only group II (3.83 ± 0.31 per cent) showed significant decrease in the levels compared to control (6.58 ± 0.51 per cent).

4.5.8 Differential Leukocyte Count- Eosinophils (percentage)

The mean eosinophil count of group I, II and III at weekly intervals are shown in Table 10 and Figure 9. Significant increase ($p< 0.05$) in the levels was observed in group II and III (values were 2.33 ± 0.42 per cent and 1.64 ± 0.24 per cent respectively) on day 14, when compared to the control group (value 0.92 ± 0.23 per cent).

4.5.9 Differential Leukocyte Count- Basophils (percentage)

The mean basophil count of group I, II and III at weekly intervals are shown in Table 11. Results indicated no significant differences in basophil counts between all the groups.

4.6 BIOCHEMICAL STUDIES

4.6.1 Total protein (g/dl)

The mean total protein values of group I, II and III at weekly intervals are shown in Table 12 and Figure 10. Significant decrease ($p<0.05$) was observed in both the treatment groups on day 21 compared to control. Significant difference was also noticed between treatment groups. On day 21, mean total protein of control group was 4.80 ± 0.25 g/dl and that of groups II and III were 4.55 ± 0.21 g/dl and 4.13 ± 0.17 g/dl respectively. Group II and III showed significant decrease (4.26 ± 0.20 g/dl and 4.18 ± 0.22 g/dl respectively) on 14th day compared to control (4.62 ± 0.15 g/dl).

4.6.2 Albumin (g/dl)

The mean albumin values of group I, II and III at weekly intervals are shown in Table 13 and Figure 11. Significant increase ($p<0.05$) was seen in both the treatment groups on seventh day compared to control. Between treatment groups also, there was significant difference. Mean albumin values of control group was 2.85 ± 0.10 g/dl and that of groups II and III were 3.23 ± 0.15 g/dl and 3.75 ± 0.11 g/dl respectively on that day. On 21st day, group II and III showed significant decrease in the levels (2.88 ± 0.10 g/dl and 2.78 ±0.12 g/dl respectively), compared to control (3.12 ± 0.12 g/dl).

4.6.3 Globulin (g/dl)

The mean globulin values of group I, II and III at weekly intervals are shown in Table 14 and Figure 12. Significant decrease ($p<0.05$) was observed in both the treatment groups on day 14 when compared to control. Mean globulin values of control group was 1.87 ± 0.09 g/dl and that of groups II and III were 1.57 ± 0.10 g/dl and 1.28 ± 0.08 g/dl respectively on that day. On 21st day, group III showed significant decrease in the levels (1.35 ± 0.03g/dl)compared to

control (1.68 ± 0.07g/dl). Group II showed significant decrease in the levels (1.47± 0.15g/dl) compared to control (1.86 ± 0.08g/dl) on 28th day .

4.6.4 A: G Ratio

The mean A: G ratio of group I, II and III at weekly intervals are shown in Table 15 and Figure 13. On 7th and 14th days, group III showed significant increase ($p<0.05$) in the levels (3.34± 0.17g/dl and 2.41 ± 0.24 g/dl respectively), compared to control values (2.68 ± 0.48 g/dl and 1.54 ± 0.14 g/dl respectively). Group II showed significant increase in the levels (2.34 ± 0.36 g/dl) compared to control (1.58± 0.10g/dl) on 28th day.

4.7 MORTALITY PATTERN

4.7.1 Group I (control)

No mortality was observed in this group. The birds remained quite active during the whole 30 days study period.

4.7.2 Group II (subcutaneous route)

The onset of mortality was rapid in birds of this group compared to group III. Two birds died within 18 hours after inoculation without showing any symptoms. Three birds were found dead on the second day. On day three, one more death occurred. Remaining six birds survived till the end of experiment.

4.7.3 Group III (intranasal route)

The peracute pattern of mortality seen in group II was not observed in this group. In the first week, there was only one death on the second day of inoculation. Further mortality occurred 22 days after inoculation. On 22nd day, one more death occurred and the remaining birds started to show clinical signs. Six birds were found dead on 23rd day. Remaining four birds were found dead on the very next day.

4.8 CLINICAL SIGNS

4.8.1 Group I (control)

The birds in the control group did not show symptoms of any disease till completion of the study. The feeding habits were quiet normal.

4.8.2 Group II (subcutaneous route)

Two birds died within 18 hours after inoculation without showing any specific symptoms. The birds which died on day two and three showed anorexia, depression, pyrexia and increased thirst. The birds were huddled together and reluctant to move (Fig. 14). They showed increased sensitivity to light and sound. At terminal stages, nervous signs were exhibited. Due to paralysis, they were sitting on hocks and head was drawn in towards the wings (Fig. 15). Prior to death, there was incordination in movements and paralysis of head and neck (Fig. 16). The survived birds showed reduced feed intake for about seven days and then came back to normal. The weight gain by the survived birds was also less compared to the control birds.

4.8.3 Group III (intranasal route)

The bird that died on third day after inoculation showed pyrexia, depression and anorexia. The remaining birds did not show any signs of disease till 22nd day. The feed and water intake were almost normal unlike group II birds. Body weight at the end of first week showed only slight reduction compared to control birds.

On 22nd day, the birds appeared dull and inactive. They showed huddling behavior, increased thirst and refused to take feed. One bird was found dead on that night. On the next day, the signs became prominent. Pyrexia, dullness, depression, inappetence, increased thirst, greenish white diarrhoea, lacrimation, and reluctancy in movements and sitting on hocks (Fig. 15) were the symptoms. One important feature was that the respiratory signs were more severe compared

to the group II birds. The birds expressed dyspnoea, increased respiratory rate, sinusitis and mucous discharge from nostrils and beaks. Paralysis of limbs and neck, incordination and death resulted ultimately. Three birds were found dead in the morning and four deaths occurred in the evening hours on day 23. The remaining three birds were found dead on 24th day morning.

4.9 BLOOD SMEAR EXAMINATION

Heart blood smears and impression smears from spleen and liver of the dead birds on Leishman's staining revealed large number of bipolar stained *P. multocida* organisms (Fig. 17). However the two subcutaneously inoculated birds (group II) which showed peracute death within 18 hours, bipolar organisms were not detected in smears.

4.10 REISOLATION OF *P. multocida*

4.10.1 Cultural Characteristics and Morphology

Pasteurella multocida was reisolated from heart, liver and spleen of the dead birds in Tryptone Soya Agar plates. Colonies suggestive of *P. multocida* were isolated from heart blood, liver and spleen of all the succumbed ducklings on blood agar following incubation at 37°C under five per cent carbon dioxide tension.

The isolate produced typical colonies which were smooth, convex, translucent, and butyraceous and one to three millimetres in diameter, after 18 to 24 h of incubation (Fig. 18). Gram's staining revealed Gram negative coccobacillary organisms arranged singly or in pairs (Fig. 19).

4.10.2 Biochemical Characterization

4.10.2.1 First Stage

The isolate grew aerobically and anaerobically, did not grow on Mac Conkey's agar and was non-haemolytic on blood agar and non motile. It was catalase and oxidase positive and fermentative.

4.10.2.2 Second Stage

The isolate was positive for indole and ornithine decarboxylase, negative for methyl red, Voges-Proskauer and urease reactions, did not produce hydrogen sulphide (H_2S), reduced nitrate and was negative for citrate utilization.

Regarding the fermentation of the sugars, the isolate fermented glucose, galactose, mannose, maltose, trehalose, sorbitol and sucrose but could not utilize dulcitol, lactose, salicin and arabinose.

4.11 PATHOANATOMICAL STUDIES

4.11.1 Gross Lesions

4.11.1.1 Group I (control)

The birds maintained without any treatment were sacrificed on 30th day of experiment. No apparent gross lesions were observed during post mortem.

4.11.1.2 Group II (subcutaneous route)

In this group, major lesions were observed on liver, heart, spleen and intestine. The intensity of lesions in the respiratory system was comparatively less than that of group III birds. Peritonitis was noticed in one bird that died on the third day after inoculation.

4.11.1.2.1 Liver

Hepatomegaly and congestion were noticed in the liver of all dead birds. In birds died on the second and third day, the liver showed multifocal pinpoint

grayish white necrotic foci on surface (Fig. 20). The necrotic foci were less prominent grossly in birds died immediately within 18 hours after inoculation. No apparent changes were observed in the liver of survived birds.

4.11.1.2.2 Spleen

Spleen was enlarged and congested with necrotic foci on the surface in all the dead birds (Fig. 21). Spleen of the survived birds were normal in apperance.

4.11.1.2.3 Brain

Brain lesions were comparatively less severe than that of group III birds. Congestion of meningeal and cerebral vessels was noticed in two birds died on second day and in one bird that died on the third day (Fig. 28).

4.11.1.2.4 Heart

Hemorrhages of varying degrees of severity were seen on the hearts of the dead birds. Of the two birds that died within 18 hours after inoculation, the first one showed absence of any apparent heart lesions and the second one showed only a few minor hemorrhages on the coronary band region. Extensive subepicardial haemorrhages were evident in the heart of all other dead birds (Fig. 22). Haemopericardium was observed in one bird that died on the third day after inoculation (Fig. 23). The fluid inside the pericardial sac was blood tinged. No changes were there in the heart of survived birds.

4.11.1.2.5 Intestine

The two birds which died within 18 hours after inoculation showed haemorrhagic enteritis with blood mixed ingesta (Fig. 24). Same lesion was shown by the bird that died on the third day. Catarrhal enteritis with mucous exudates in the lumen was noticed in three birds died on the second day (Fig. 25). Focal haemorrhages were evident on the mucosa. Intestine of all the survived birds were devoid of any lesions.

4.11.1.2.6 Caecal tonsil

Caecal tonsils were congested. No changes were observed in survived birds.

4.11.1.2.7 Lung

Lungs appeared edematous in all the birds. Two birds which died on the second and third days showed severe congestion and focal haemorrhages in the lungs (Fig. 26). In all other dead birds, moderate congestion was noticed. No lung lesions were there in survived birds.

4.11.1.2. 8 Kidney

Kidneys were enlarged and congested except in survived birds (Fig. 27).

4.11.1.2.9 Bursa

In all the dead birds, bursa appeared enlarged and congested. Survived birds did not show any lesions.

4.11.1.3 Group III (intranasal route)

A more extensive haemorrhagic septicaemic picture was observed in the dead birds on post mortem, compared to group II. There were widespread haemorrhages on all serosal and mucosal surfaces. Petechiae on peritoneum and airsacs were marked. However, individual variation was there in the intensity of expression of lesions.

4.11.1.3.1 Liver

Generally in all the birds the liver was enlarged, congested and friable in consistency. Numerous pinhead sized grayish white necrotic foci were scattered

on the surface (Fig. 20), which was more extensive than that observed in group II birds.

4.11.1.3.2 Spleen

Spleen was enlarged and congested with necrotic foci on the surface (Fig. 21).

4.11.1.3.3 Brain

Extensive submeningeal haemorrhages were noticed in one bird that died on the 24th day after inoculation (Fig. 29 and 30). Moderate meningeal congestion was noticed in three birds died on 23rd day. No lesions were observed grossly in other birds. Compared to group II birds, brain lesions were more evident.

4.11.1.3.4 Heart

Multifocal subepicardial haemorrhages were clearly evident in all the birds (Fig. 22). The intensity varied individually. Some had typical red paint brush appearance. Hydropericardium was another feature observed, especially in the birds which died on 23rd and 24th days after inoculation (Fig. 23). Pericardial sac contained excessive amounts of blood tinged fluid.

4.11.1.3.5 Intestine

Severe haemorrhagic enteritis was noticed in all the birds (Fig. 24). Pinpoint haemorrhages were scattered throughout the length of intestinal mucosa. Intestinal contents were mixed with excess amounts of blood. Serosa appeared highly congested with engorged serosal vessels. Thickening of intestinal wall was also noticed. Severity of all these lesions was more than that of group II birds.

4.11.1.3.6 Caecal tonsil

No changes were observed grossly on caecal tonsils except congestion.

4.11.1.3.7 Lung

In all the dead birds, lung appeared edematous and highly congested. Areas of haemorrhage could also be appreciated grossly (Fig. 26).

4.11.1.3.8 Kidney

Enlargement and congestion were the major changes noticed in kidney (Fig. 27).

4.11.1.3.9 Bursa

Bursa appered enlarged and edematous in all the birds. Subserosal echymotic haemorrhages were evident in three cases (Fig. 31). Mucosal congestion was noticed in six birds.

4.11.2 Histopathology

4.11.2.1 Group I (control)

Brain, heart, liver, spleen, kidney, lung, intestine, caecal tonsil, and bursa on microscopical examination did not reveal any changes.

4.11.2.2 Group II (subcutaneous route)

Histopathological lesions of varying intensity were seen in birds of this group.

4.11.2.2.1 Liver

Congestion of blood vessels and sinusoids, multifocal haemorrhages, degeneration, multifocal coagulative necrosis and focal heterophilic infiltration were evident in the liver (Fig. 32). Lymphocytic infiltration was seen in hepatic portal triads in a few cases. Haemosiderin laden macrophages were scattered throughout in the areas of haemorrhage. Periportal mononuclear hepatitis was also evident. Fatty change, vacuolation and necrosis of hepatocytes, and bile duct

proliferation were seen in the birds which died on the second and third days (Fig. 33). No changes were observed in survived birds.

4.11.2.2.2 Spleen

Multiple randomly distributed foci of coagulation necrosis, lymphoid depletion and plasmacytosis were the main changes in spleen. There was extensive congestion, haemorrhages and necrosis of splenocytes (Fig. 34). Mononuclear cell infiltration was evident in sinusoids. Survived birds didn't reveal any changes.

4.11.2.2.3 Brain

Mild submeningeal congestion was noticed in two birds died on second day and in one bird that died on the third day. Most of the neurons appeared shrunken. Widening of the perineuronal and perivascular space and shrinkage of the neurons were also prominent (Fig. 35). Gliosis, satellitosis and neuronophagia were also observed. No changes were observed in survived birds.

4.11.2.2.4 Heart

Sub epicardial and myocardial haemorrhages, myofibril degeneration, focal myositis, myocytolysis, vacuolation of sarcoplasm, separation and attenuation of fibres were the prominent lesions in the heart (Fig. 36). Survived birds did not reveal any lesions on the tissues.

4.11.2.2.5 Intestine

Haemorrhagic enteritis with increased number of heterophils in lamina propria was evident in birds that died on first and third days after inoculation. There was necrosis and fusion of villi and degeneration and desquamation of villi epithelium (Fig. 37). Necrotic debri was present inside the lumen. The other three birds died on 2^{nd} day showed catarrhal enteritis with focal haemorrhages on mucosa. Necrosis, cystic changes and vacuolation of the submucosal glands were

the other lesions. Goblet cell hyperplasia, congestion of serosa and haemorrhages in sub mucosa were also noticed (Fig. 38). No changes were observed in survived birds.

4.11.2.2.6 Caecal tonsil

Caecal tonsils revealed necrosis and cystic changes in birds which died on the 1st and 2nd days. In one bird that died on the 2nd day after inoculation, necrotic granular substance enclosed within fibrous tissue capsule was seen inside caecal tonsil (Fig. 39). Lymphoid cell atrophy and depletion were evident in most of the birds. The survived birds did not reveal any changes..

4.11.2.2.7 Lung

Congestion, haemorrhages, edema, alveolar cell necrosis, focal heterophilic infiltration and collapse of bronchial mucosa were the major changes (Fig. 40). There were necrosis and desquamation of endothelium of capillaries. Haemorrhages and thrombosis of interlobular and interstitial vessels were observed in lung. No changes were observed in survived birds.

4.11.2.2.8 Kidney

The major changes observed were multifocal haemorrhages in renal parenchyma, widespread coagulation necrosis of tubules and glomeruli, moderate heterophilic infiltration and interstitial haemorrhages (Fig. 41). Congestion, shrinkage, necrosis and hyalinization were prominent in glomeruli. Degeneration, necrosis and desquamation of tubular epithelium were also evident (Fig. 42). Paravenous necrosis of tubules was a prominent feature. Survived birds did not show any lesions.

4.11.2.2.9 Bursa

In all dead birds lymphoid depletion of follicles, haemorrhages, foci of necrosis were evident in bursa (Fig. 43). There was desquamation of the

epithelial lining in some areas. Moderate connective tissue proliferation was noticed in the interstitium in some of the dead birds (Fig. 44) except the two which died within 18 hours after inoculation. No changes were there in survived birds.

4.11.2.3 Group III (intranasal route)

4.11.2.3.1 Liver

Periportal hepatitis with multiple foci of coagulation necrosis, containing heterophil aggregates was the prominent feature in all the dead birds (Fig. 45 and 46). Periductular mononuclear cell infiltration, congestion and obliteration of sinusoids, central venous congestion and Kupffer cell reaction were also seen. Diffuse fatty change was noticed in the birds died on 22nd, 23rd and 24th days. Interlobular connective tissue proliferation and biliary hyperplasia were noticed in birds that died on the 23rd and 24th day.

4.11.2.3.2 Spleen

There was degeneration of periarteriolar reticular cells and multifocal areas of coagulative necrosis in spleen. The other major changes observed were acute splenitis, extensive congestion, haemorrhages, sclerosis of vessels, reticular cell hyperplasia and necrosis in the lymphoid follicles (Fig. 47). Necrosis of splenocytes and mononuclear cell infiltration were seen in the red pulp (Fig. 48).

4.11.2.2.3 Brain

There was localized meningitis with lymphocytic infiltration of pia matter. Heamorrhages in dura and congestion of meningeal and cerebral vessels were also evident. Extensive submeningeal haemorrhages were present in one bird that died on 24th day after inoculation (Fig. 49 and 50). Moderate meningeal congestion was there in three birds died on 23rd day. Congestion, softening, diffuse neuronal degeneration, perineuronal and perivascular edema, gliosis, satellitosis, and neuronophagia, were the other changes observed in brain.

4.11.2.2.4 Heart

Widespread intramuscular and intermuscular haemorrhages was the most prominent feature in all the birds. There was lymphoid cell infiltration in the myocardium. Separation, fragmentation, necrosis and hyalinization of cardiac muscle fibres were also evident (Fig. 36).

4.11.2.2.5 Intestine

Acute haemorrhagic enteritis was the prominent feature. There was necrosis and fusion of villi, degeneration and desquamation of villi epithelium and infiltration of mononuclear cells in lamina propria (Fig. 51). Subserosal congestion and haemorrhages in sub mucosa were also seen. The submucosal glands revealed necrosis, cystic changes and vacuolation. Goblet cell hyperplasia was another lesion observed (Fig. 52).

4.11.2.2.6 Caecal tonsil

Lymphoid cell depletion, necrosis and cystic changes were evident in almost all the birds (Fig. 53).

4.11.2.2.7 Lung

Extensive congestion, marked heterophil infiltration followed by thickening of interlobular septae, pleuritis and vasculitis were the prominent changes in lungs. Peribronchial and bronchiolar haemorrhages, edema of alveoli, collapse of bronchial mucosa and heterophilic infiltration were also noticed (Fig. 54). Severe haemorrhages and thrombosis of interlobular and interstitial vessels were the other important lesions observed.

4.11.2.2.8 Kidney

Diffuse interstitial nephritis was observed with infiltration of intertubular area by mononuclears. Degeneration of tubular epithelium and casts were present in renal tubules. Other major changes observed were paravenous and

intestitial haemorrhages, widespread coagulation necrosis of tubules and glomeruli, and periglomerular inflammatory cell infiltration (Fig. 55). Glomeruli revealed congestion, shrinkage, necrosis and hyalinization. Tubular degeneration, cystic changes, necrosis and desquamation of tubular epithelium were also evident (Fig. 56).

4.11.2.2.9 Bursa

Inter and intrafollicular haemorrhages, edema, loss of lining epithelium, depletion of lymphoid follicles and interstitial connective tissue proliferation were observed in bursa in all dead birds (Fig. 57 and 58).

Table 2: Mean Body Weight of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), g

Groups	BODY WEIGHT (g)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	362.50 [a] ± 10.44	479.17 [a] ± 11.02	597.92 [a] ± 11.29	710.42 [a] ± 9.46	831.25 [a] ± 11.57
II	360.42 [a] ± 11.70	450.00 [a] ± 28.14	554.17 [a] ± 32.54	687.50 [a] ± 21.16	825.00 [a] ± 28.14
III	364.58 [a] ± 10.86	468.18 [a] ± 12.65	584.09 [a] ± 11.81	681.82 [a] ± 10.16	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 3: Mean Packed Cell Volume of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), %

Groups	PACKED CELL VOLUME (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	27.33 [a] ± 0.66	29.67 [a] ± 0.71	30.25 [a] ± 0.80	29.25 [a] ± 0.52	29.83 [a] ± 0.51
II	28.92 [a] ± 0.42	39.00 [b] ± 0.89	31.33 [a] ± 0.71	30.50 [a] ± 0.62	29.83 [a] ± 0.87
III	26.92 [a] ± 0.56	33.27 [c] ± 0.74	35.45 [b] ± 1.32	35.73 [b] ± 1.05	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 4: Mean Haemoglobin Concentration of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), g/dl

Groups	HAEMOGLOBIN CONCENTRATION (g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$11.95^{a} \pm 0.17$	$11.90^{a} \pm 0.12$	$11.71^{a} \pm 0.18$	$11.65^{a} \pm 0.12$	$11.58^{a} \pm 0.15$
II	$11.90^{a} \pm 0.14$	$13.33^{b} \pm 0.18$	$11.65^{a} \pm 0.17$	$11.73^{a} \pm 0.17$	$11.73^{a} \pm 0.13$
III	$11.30^{a} \pm 0.17$	$12.58^{c} \pm 0.13$	$12.23^{b} \pm 0.22$	$12.22^{b} \pm 0.20$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 5: Mean Total Erythrocyte Count of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), $10^6/\mu l$

Groups	TOTAL ERYTHROCYTE COUNT($10^6/\mu l$)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$2.28^{a} \pm 0.14$	$2.10^{a} \pm 0.10$	$1.82^{a} \pm 0.16$	$1.86^{a} \pm 0.12$	$1.82^{a} \pm 0.11$
II	$2.24^{a} \pm 0.11$	$4.02^{b} \pm 0.18$	$3.35^{b} \pm .18$	$2.22^{a} \pm 0.22$	$2.03^{a} \pm 0.13$
III	$2.15^{a} \pm 0.17$	$3.15^{c} \pm 0.16$	$2.98^{b} \pm 0.19$	$3.18^{b} \pm 0.16$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 6: Mean Total Leucocyte Count of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), /mm^3

Groups	TOTAL LEUKOCYTE COUNT(/mm^3)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	22500.00 ^{a} ± 1725.48	21166.67 ^{a} ± 1445.12	19333.33 ^{a} ± 864.57	19500.00 ^{a} ± 1671.96	19000.00 ^{a} ± 1604.92
II	21833.33 ^{a} ± 1313.30	53000.00 ^{b} ± 4024.92	43000.00 ^{b} ± 2236.07	28000.00 ^{b} ± 2529.82	26333.33 ^{b} ± 2703.91
III	22166.67 ^{a} ± 1991.78	35090.91 ^{c} ± 1855.97	33454.55 ^{c} ± 1770.28	34727.27 ^{c} ± 1854.19	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 7: Mean Differential Leucocyte Count – Lymphocytes – of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), %

Groups	DIFFERENTIAL LEUKOCYTE COUNT- LYMPHOCYTES (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	63.25^{a} ± 1.29	64.67 ^{a} ± 1.18	63.67 ^{a} ± 1.01	66.75 ^{a} ± 2.11	64.33 ^{a} ± 1.19
II	62.67 ^{a} ± 1.25	40.17 ^{b} ± 1.35	41.50 ^{b} ± 1.38	42.00 ^{b} ± 0.86	53.67 ^{b} ± 1.99
III	61.83 ^{a} ± 1.34	41.91 ^{b} ± 0.90	39.64 ^{b} ± 0.83	40.55 ^{b} ± 0.99	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 8: Mean Differential Leucocyte Count – Heterophils – of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), %

Groups	DIFFERENTIAL LEUKOCYTE COUNT- HETEROPHILS (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$26.75^{a} \pm 1.24$	$29.58^{a} \pm 2.34$	$28.08^{a} \pm 1.17$	$28.83^{a} \pm 1.24$	$27.75^{a} \pm 1.23$
II	$27.75^{a} \pm 1.17$	$55.33^{b} \pm 1.43$	$52.00^{b} \pm 1.06$	$49.83^{b} \pm 1.14$	$39.83^{b} \pm 1.49$
III	$29.58^{a} \pm 1.32$	$53.18^{b} \pm 0.89$	$51.91^{b} \pm 0.96$	$51.73^{b} \pm 0.81$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 9: Mean Differential Leucocyte Count – Monocytes – of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), %

Groups	DIFFERENTIAL LEUKOCYTE COUNT- MONOCYTES (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$7.75^{a} \pm 0.58$	$6.08^{a} \pm 0.40$	$6.58^{a} \pm 0.51$	$6.50^{a} \pm 0.45$	$6.33^{a} \pm 0.47$
II	$7.58^{a} \pm 0.47$	$3.00^{b} \pm 0.45$	$3.83^{b} \pm 0.31$	$5.83^{a} \pm 0.48$	$4.67^{a} \pm 0.71$
III	$7.92^{a} \pm 0.48$	$3.36^{b} \pm 0.34$	$6.18^{a} \pm 0.84$	$5.45^{a} \pm 0.53$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 10: Mean Differential Leucocyte Count – Eosinophils – of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), %

Groups	DIFFERENTIAL LEUKOCYTE COUNT- EOSINOPHILS (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$1.33^{a} \pm 0.26$	$0.75^{a} \pm 0.18$	$0.92^{a} \pm 0.23$	$0.92^{a} \pm 0.19$	$1.00^{a} \pm 0.25$
II	$1.25^{a} \pm 0.18$	$1.00^{a} \pm 0.26$	$2.33^{b} \pm 0.42$	$2.00^{a} \pm 0.52$	$1.00^{a} \pm 0.26$
III	$0.75^{a} \pm 0.22$	$0.91^{a} \pm 0.21$	$1.64^{b} \pm 0.24$	$1.73^{a} \pm 0.49$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 11: Mean Differential Leucocyte Count – Basophils – of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), %

Groups	DIFFERENTIAL LEUKOCYTE COUNT- BASOPHILS (%)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$0.75^{a} \pm 0.18$	$0.58^{a} \pm 0.15$	$0.67^{a} \pm 0.19$	$0.75^{a} \pm 0.22$	$0.58^{a} \pm 0.19$
II	$0.83^{a} \pm 0.24$	$0.50^{a} \pm 0.22$	$0.33^{a} \pm 0.21$	$0.33^{a} \pm 0.21$	$0.83^{a} \pm 0.54$
III	$0.75^{a} \pm 0.18$	$0.64^{a} \pm 0.15$	$0.64^{a} \pm 0.15$	$0.55^{a} \pm 0.21$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 12: Mean Total Protein of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), g/dl

Groups	TOTAL PROTEIN (g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$4.69^{a} \pm 0.15$	$4.29^{a} \pm 0.22$	$4.62^{a} \pm 0.15$	$4.80^{a} \pm 0.25$	$4.71^{a} \pm 0.13$
II	$4.74^{a} \pm 0.13$	$4.75^{a} \pm 0.30$	$4.26^{b} \pm 0.20$	$4.55^{b} \pm 0.21$	$4.63^{a} \pm 0.11$
III	$4.80^{a} \pm 0.12$	$4.87^{a} \pm 0.11$	$4.18^{b} \pm 0.22$	$4.13^{c} \pm 0.17$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 13: Mean Albumin of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), g/dl

Groups	ALBUMIN (g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$2.98^{a} \pm 0.13$	$2.85^{a} \pm 0.10$	$2.74^{a} \pm 0.17$	$3.12^{a} \pm 0.12$	$2.85^{a} \pm 0.11$
II	$3.11^{a} \pm 0.12$	$3.23^{b} \pm 0.15$	$2.69^{a} \pm 0.14$	$2.88^{b} \pm 0.10$	$3.16^{a} \pm 0.12$
III	$3.11^{a} \pm 0.17$	$3.75^{c} \pm 0.11$	$2.90^{a} \pm 0.12$	$2.78^{b} \pm 0.12$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 14: Mean Globulin of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups), g/dl

Groups	GLOBULIN (g/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$1.71^{a} \pm 0.11$	$1.44^{a} \pm 0.23$	$1.87^{a} \pm 0.09$	$1.68^{a} \pm 0.07$	$1.86^{a} \pm 0.08$
II	$1.63^{a} \pm 0.12$	$1.53^{a} \pm 0.32$	$1.57^{b} \pm 0.10$	$1.67^{a} \pm 0.08$	$1.47^{b} \pm 0.15$
III	$1.68^{a} \pm 0.08$	$1.12^{a} \pm 0.19$	$1.28^{b} \pm 0.08$	$1.35^{b} \pm 0.03$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Table 15: Mean Albumin Globulin Ratio of ducks (control and *Pasteurella multocida* A:1 strain inoculated groups)

Groups	A: G Ratio				
	Day 0	Day 7	Day 14	Day 21	Day 28
I	$1.90^{a} \pm 0.24$	$2.68^{a} \pm 0.48$	$1.54^{a} \pm 0.14$	$1.92^{a} \pm 0.14$	$1.58^{a} \pm 0.10$
II	$2.07^{a} \pm 0.20$	$2.57^{a} \pm 0.44$	$1.75^{a} \pm 0.14$	$1.75^{a} \pm 0.10$	$2.34^{b} \pm 0.36$
III	$1.95^{a} \pm 0.19$	$3.34^{b} \pm 0.17$	$2.41^{b} \pm 0.24$	$2.07^{a} \pm 0.08$	****

(Means bearing same superscript in the same column does not differ significantly)
(a, b, c - Shows significance at five per cent level)

Fig.1: Mean body weight (g)

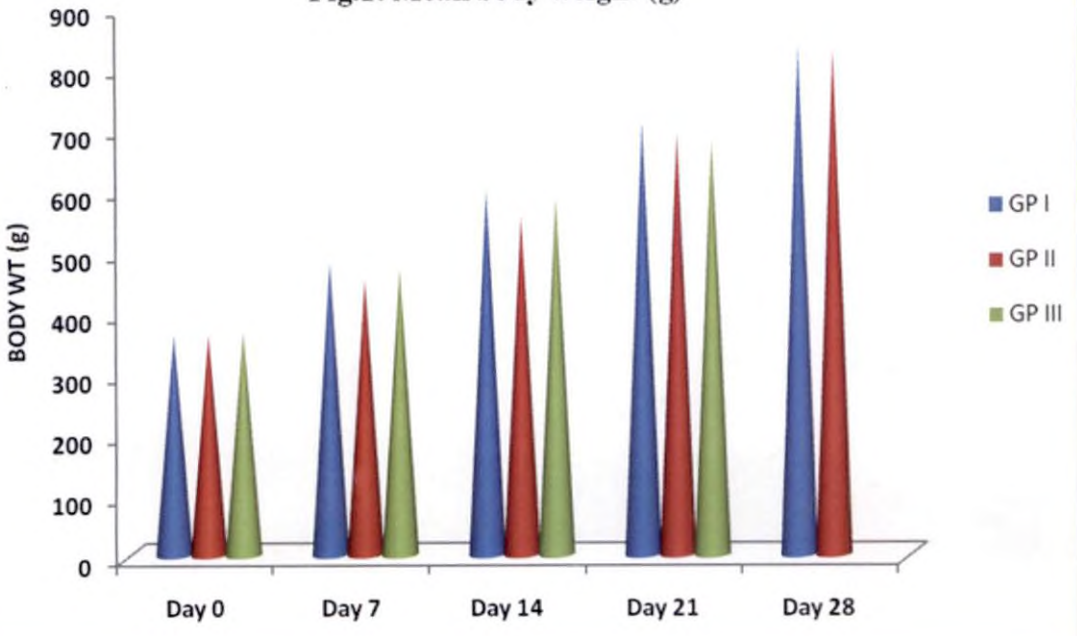

Fig.2 : Mean packed cell volume (%)

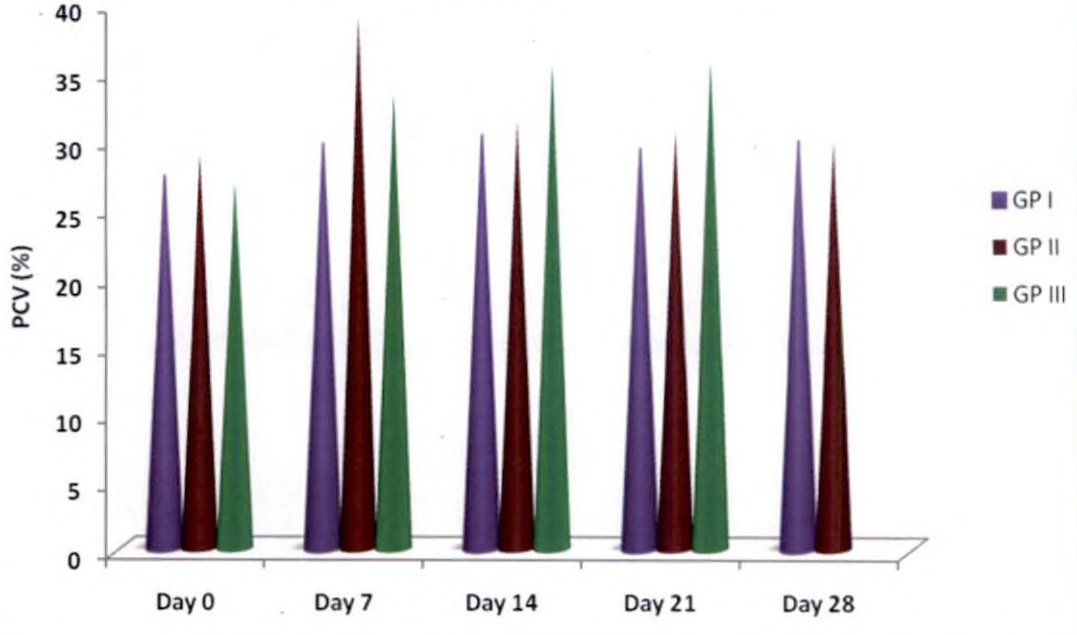

Fig.3 : Mean haemoglobin concentration (g/dl)

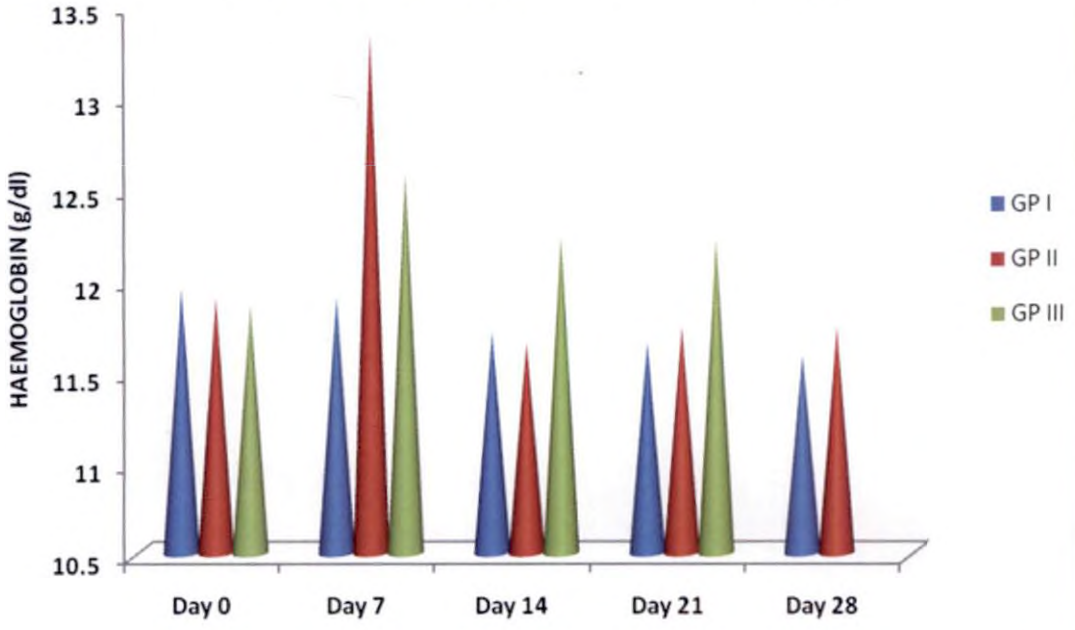

Fig.4 : Mean total erythrocyte count (10^6/µl)

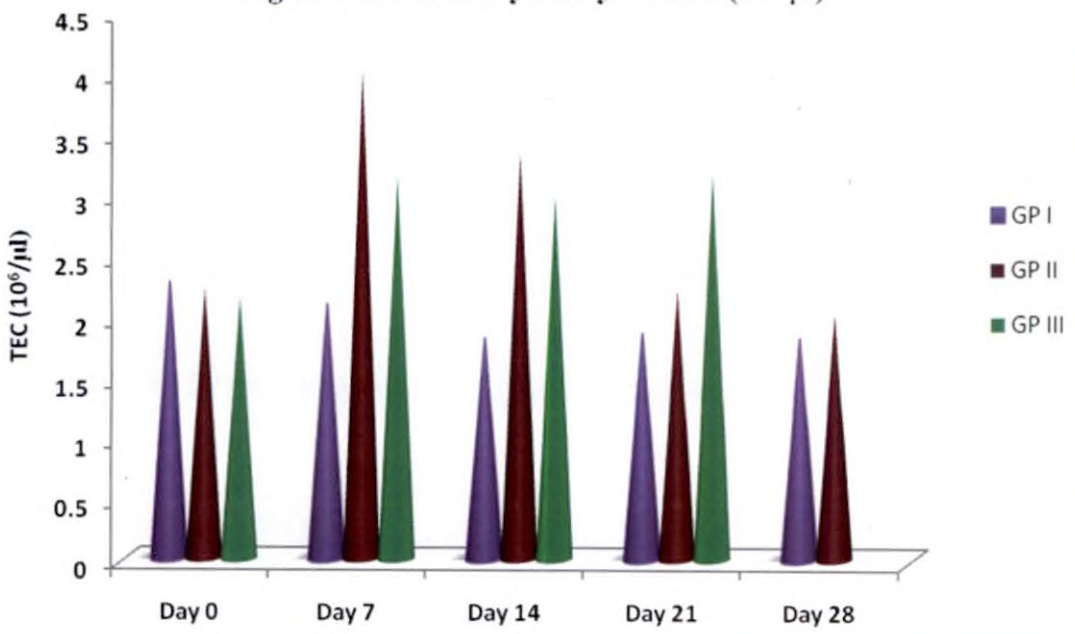

Fig.5: Mean total leucocyte count (1000'S/mm^3)

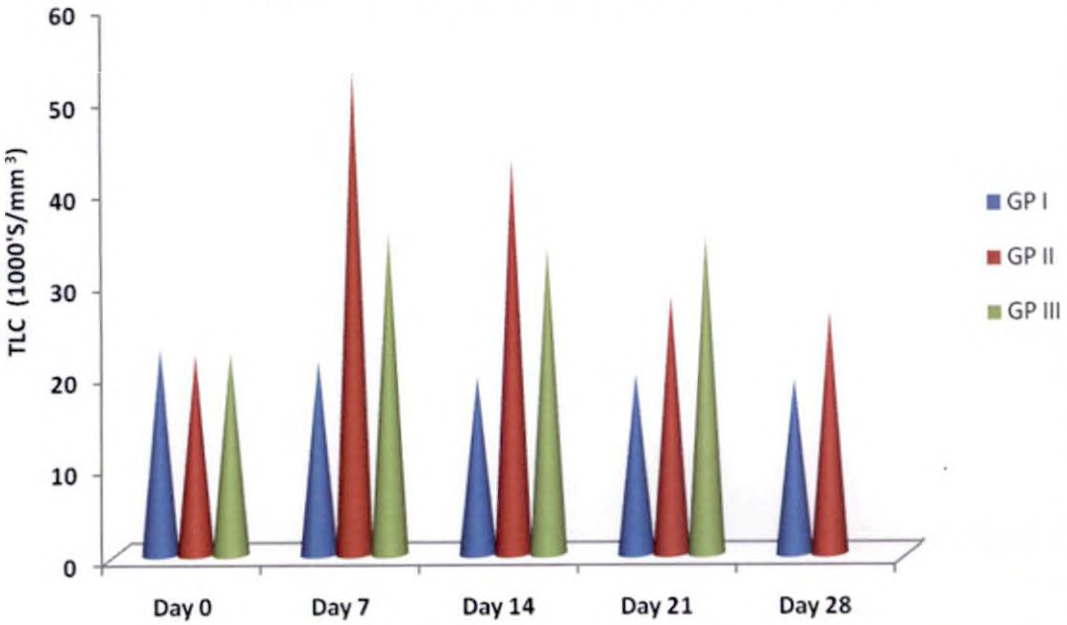

Fig.6 : Mean differential leucocyte count -Lymphocytes (%)

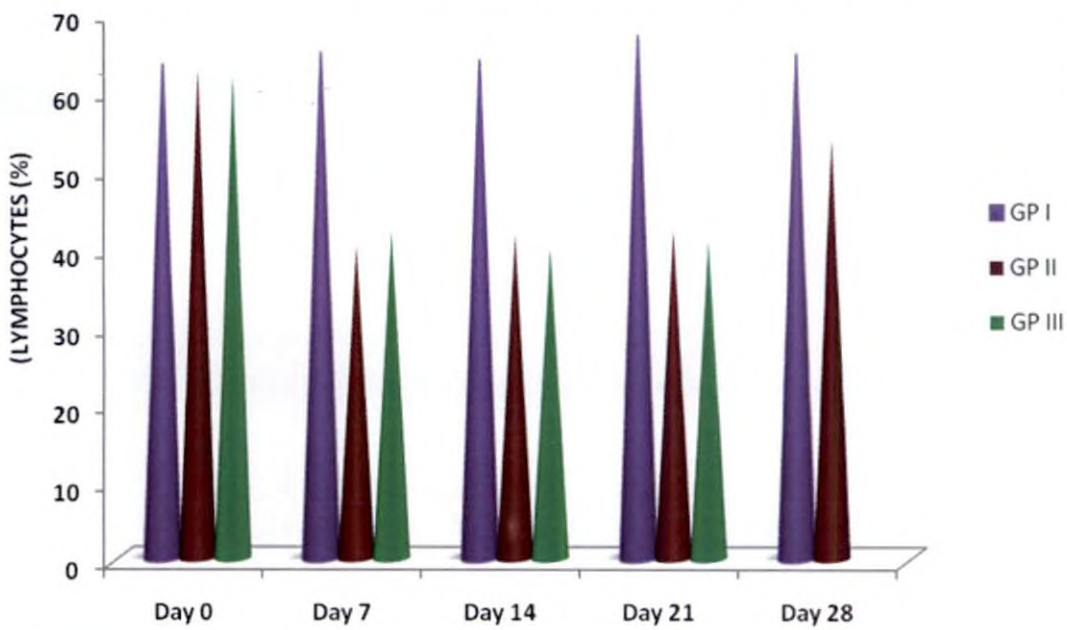

Fig.7 : Mean differential leucocyte count -Heterophils (%)

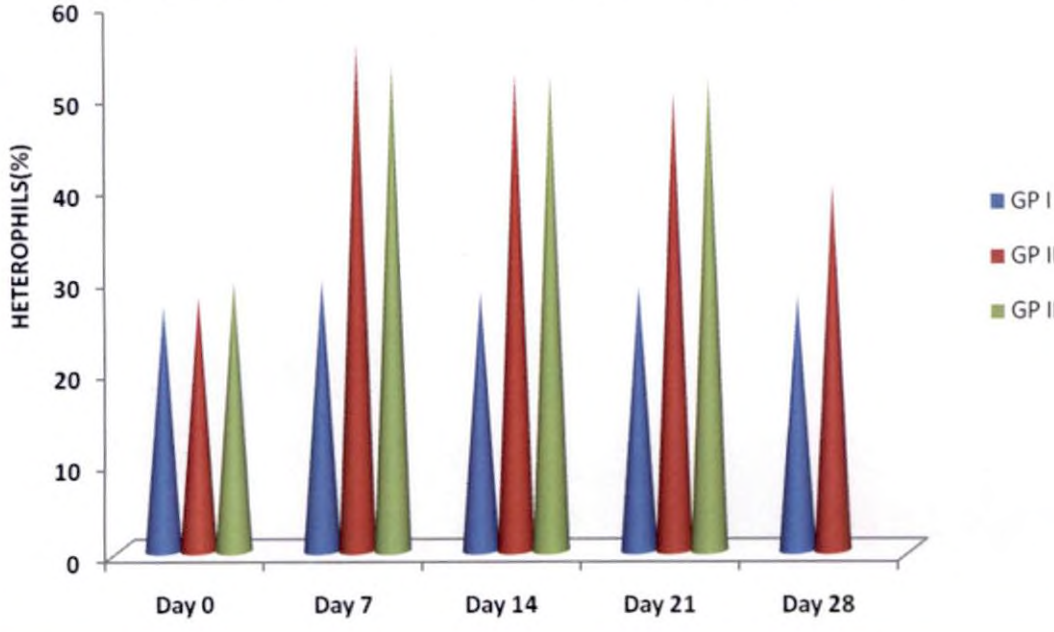

Fig. 8 : Mean differential leucocyte count -Monocytes (%)

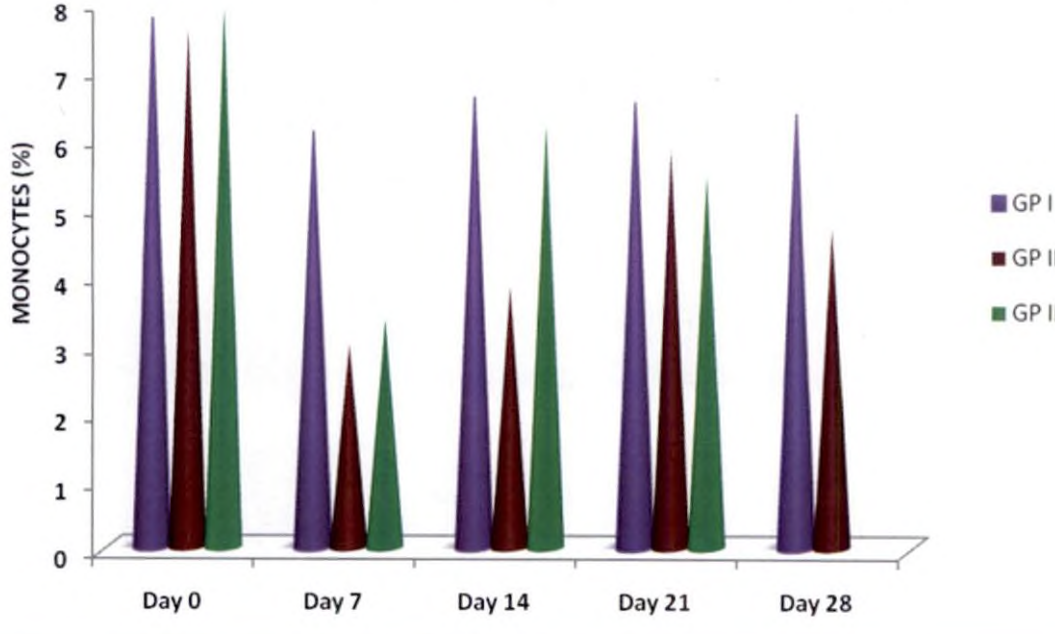

Fig.9 : Mean differential leucocyte count -Eosinophils (%)

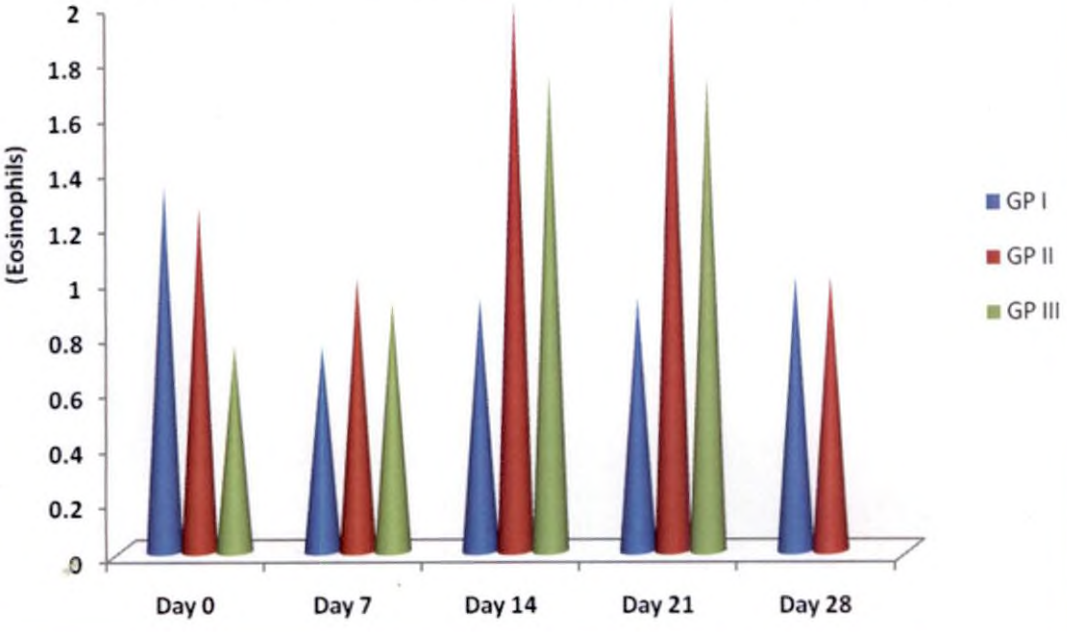

Fig.10 : Mean total protein (g/dl)

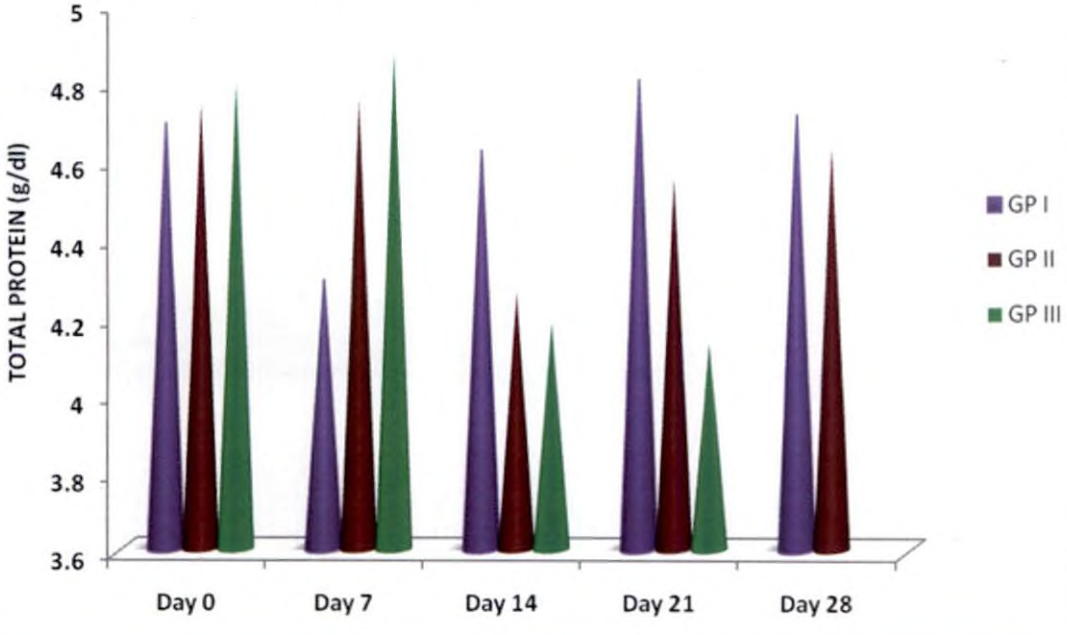

Fig.11 : Mean Albumin (g/dl)

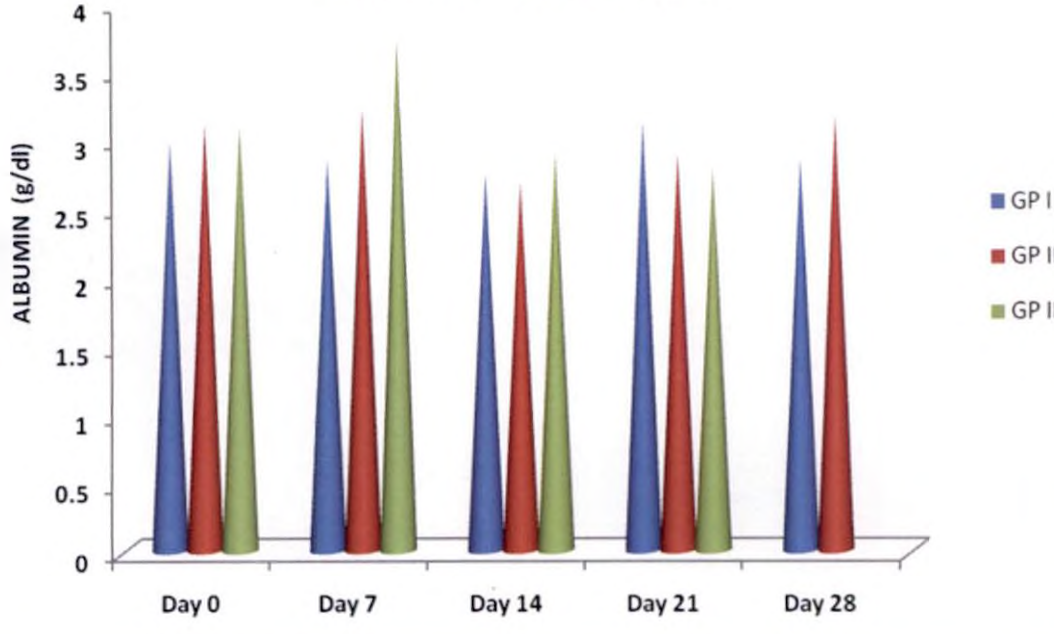

Fig.12 : Mean Globulin (g/dl)

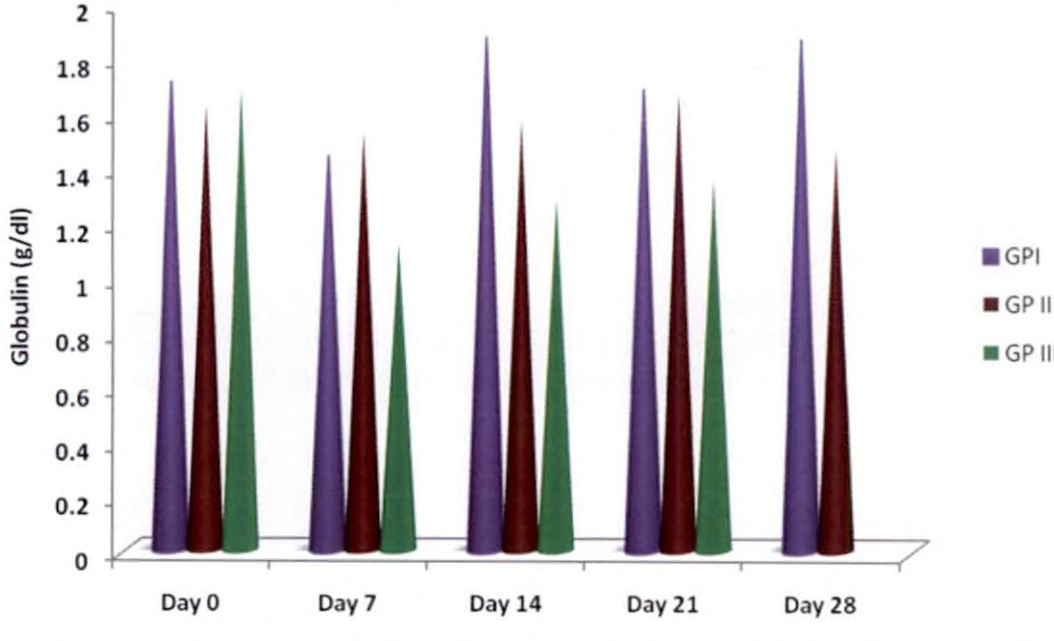

Fig.13 : Mean Albumin Globulin Ratio

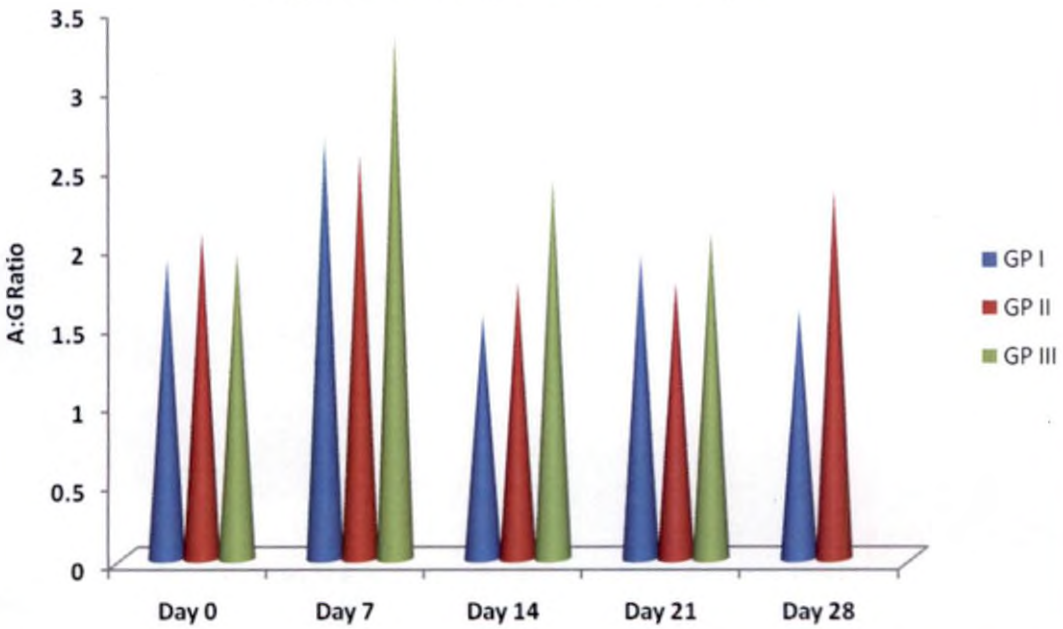

Fig. 14: Huddling behaviour

Fig. 15: Sitting on hocks

Fig. 16: Dullness, depression and paralysis

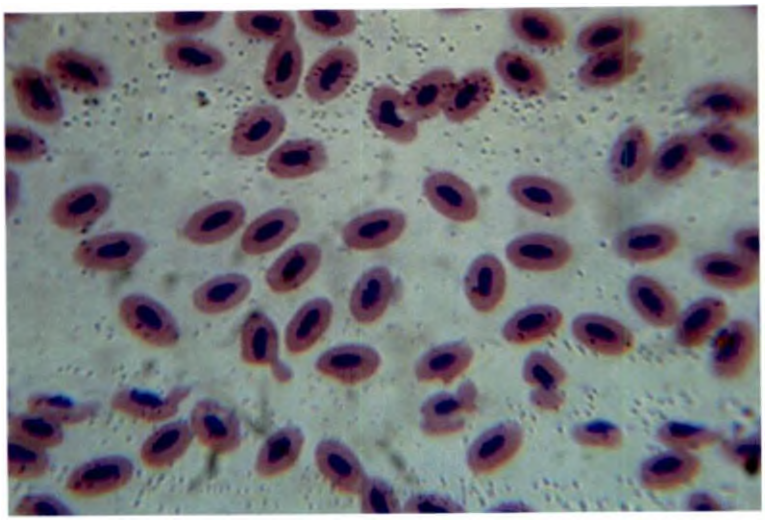

Fig.17: *Pasteurella multocida* in blood smear

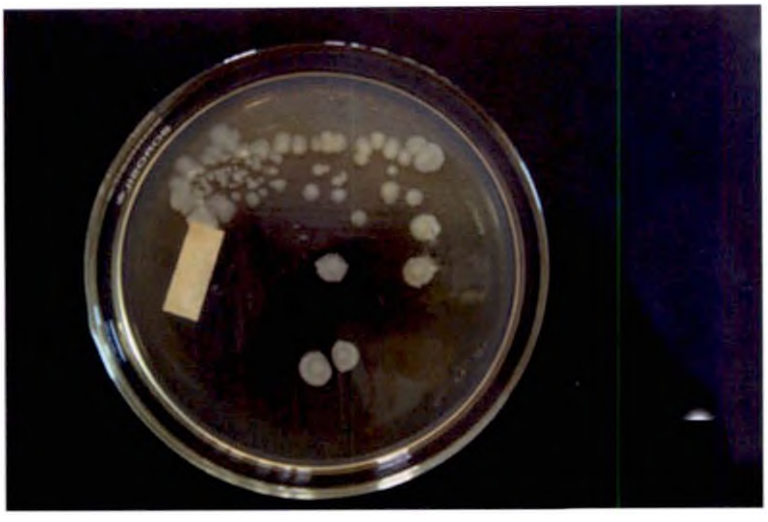

Fig. 18: Reisolation - Colonies of *Pasteurella multocida* on TSA agar

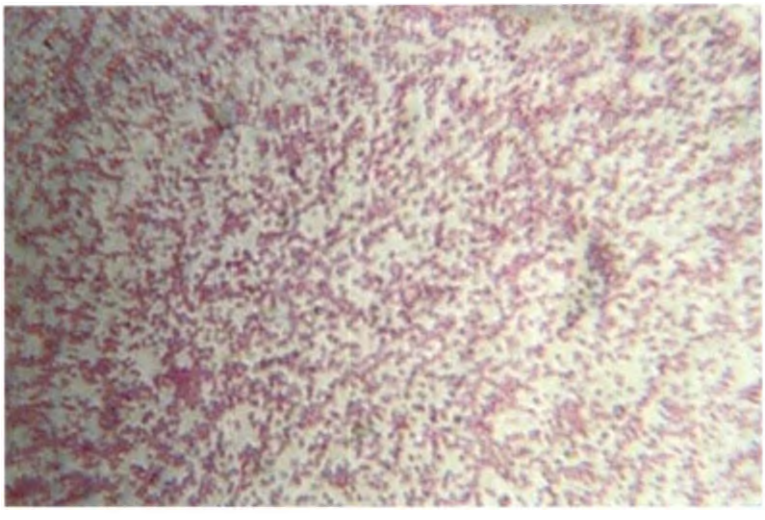

Fig. 19: Gram negative *Pasteurella multocida* organisms in culture

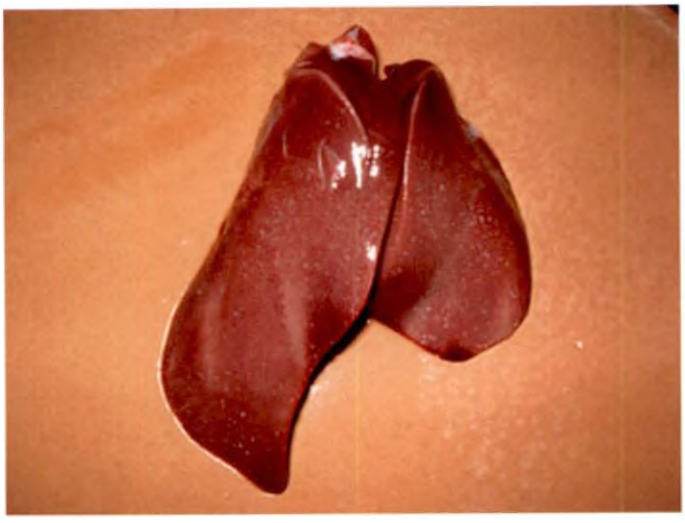

Fig. 20 : Liver - Hepatomegaly with congestion and multifocal necrosis

Fig. 21: Spleen - Congestion and enlargment with necrotic foci on surface.

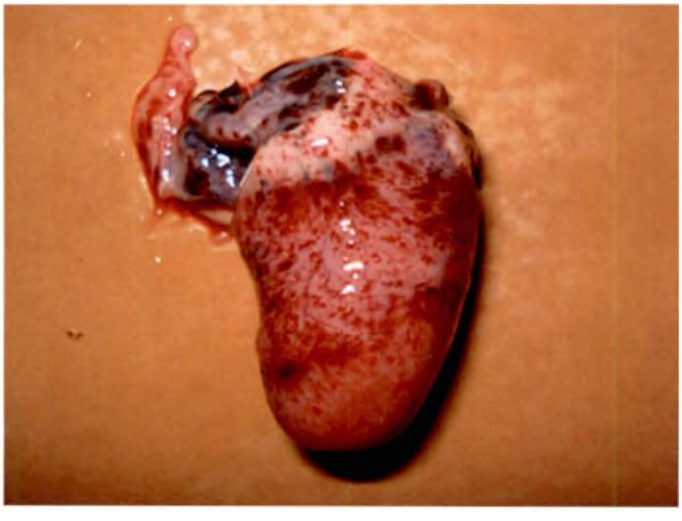

Fig. 22: Heart - widespread subepicardial haemorrhages

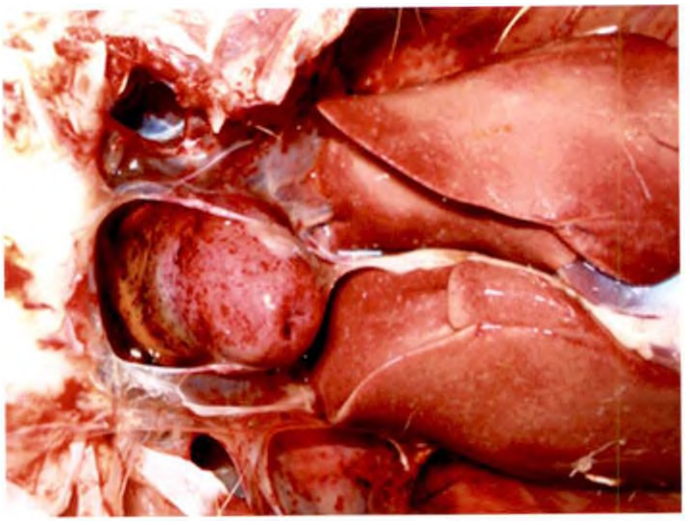

Fig. 23: Haemopericardium

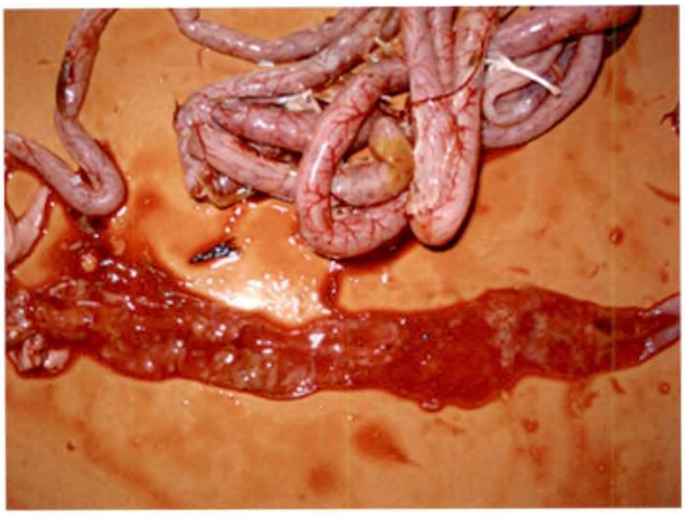

Fig. 24: Intestine - Haemorrhagic enteritis

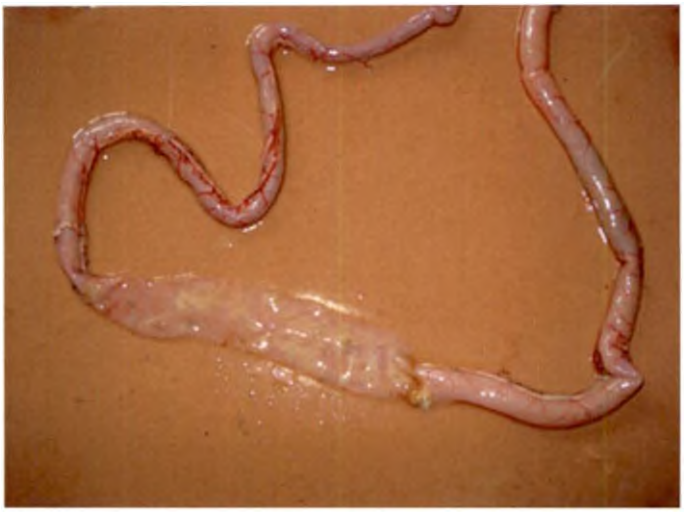

Fig. 25: Intestine - Catarrhal enteritis

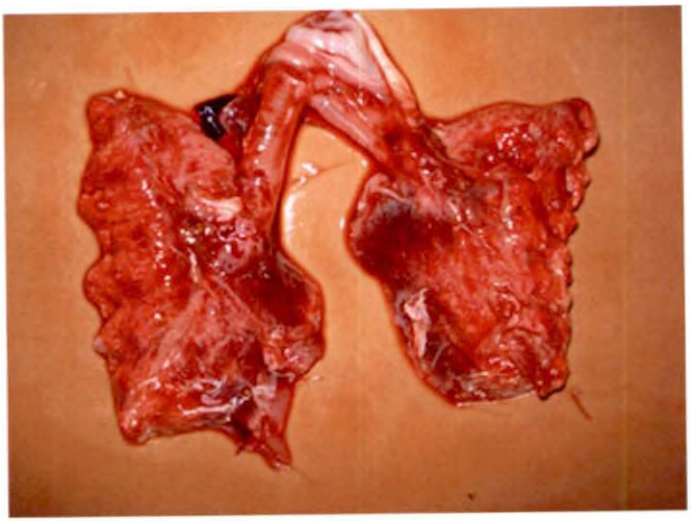

Fig. 26: Lungs - Congestion and haemorrhages

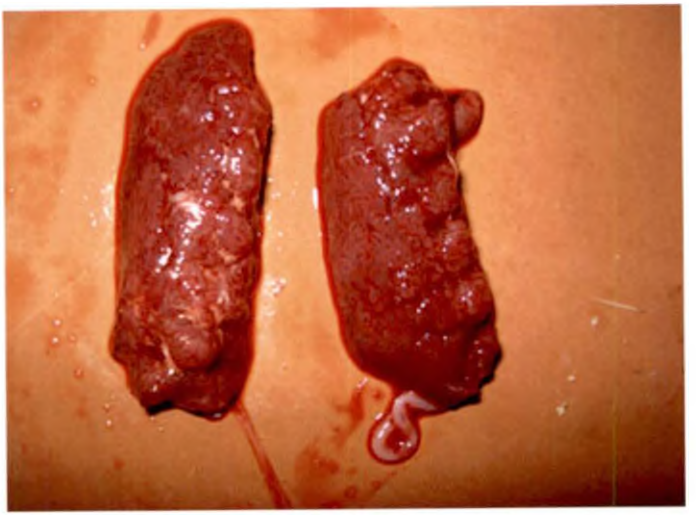

Fig. 27: Kidney - congestion

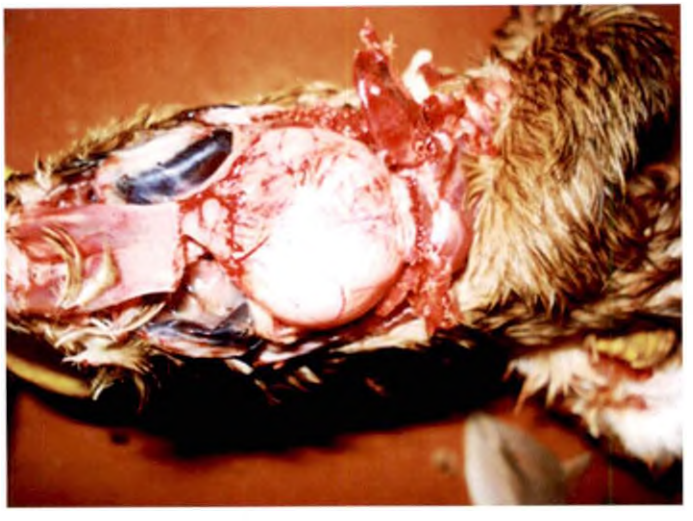

Fig. 28: Brain - Congestion of meningeal vessels

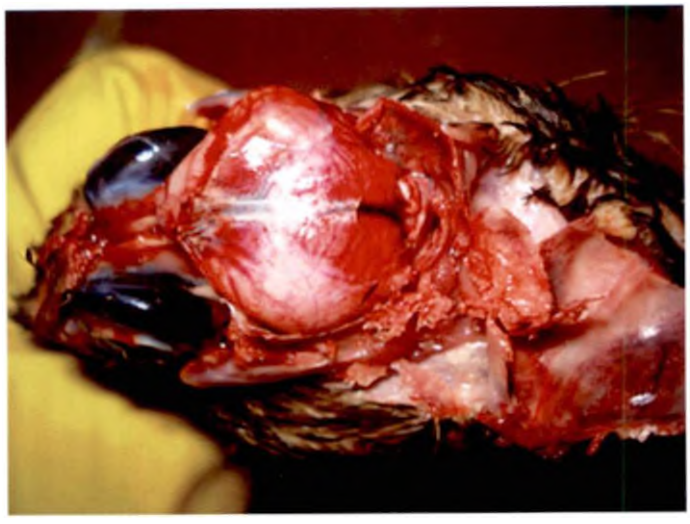

Fig. 29: Brain - extensive submeningeal haemorrhages

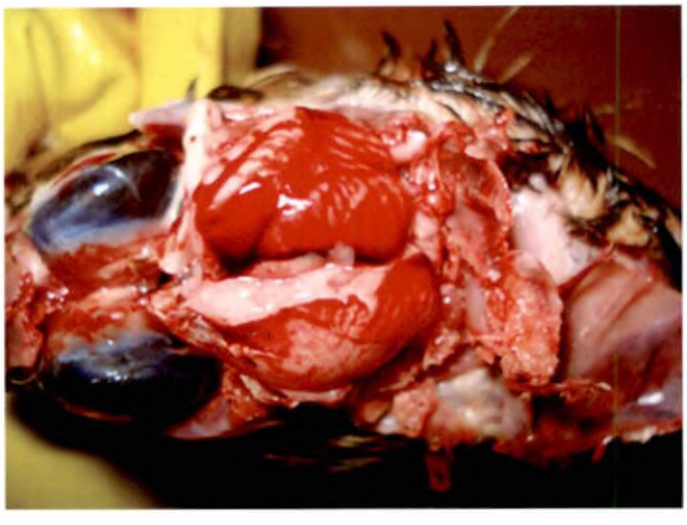

Fig. 30: Brain - Haemorrhages

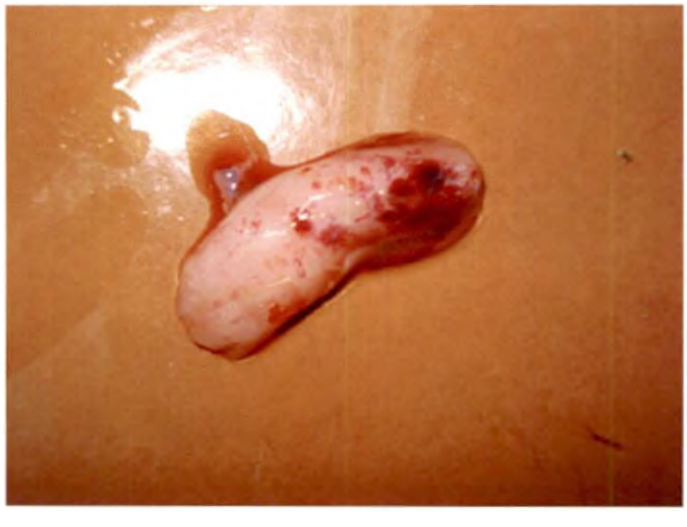

Fig. 31: Bursa - haemorrhage on surface

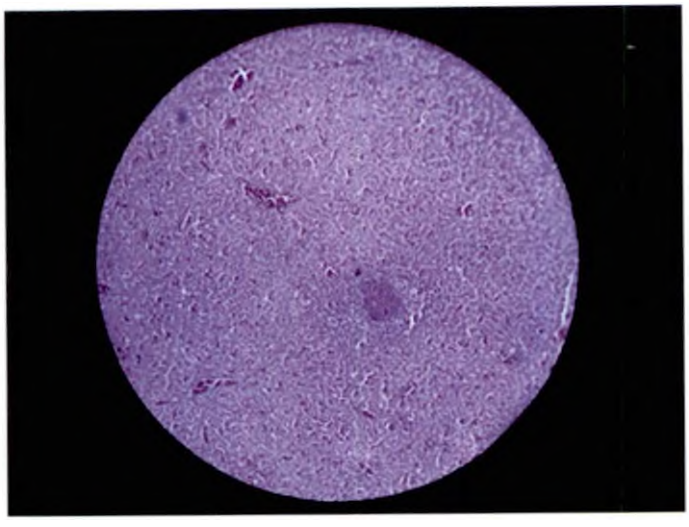

Fig. 32: Liver - Congestion and necrotic foci (H&E x 100)

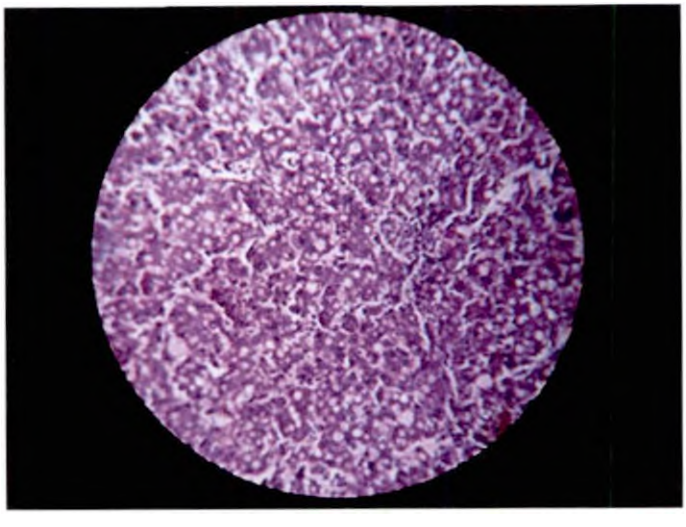

Fig. 33: Liver - Fatty change, inflammatory cell infiltration in sinusoids (H&E x 400)

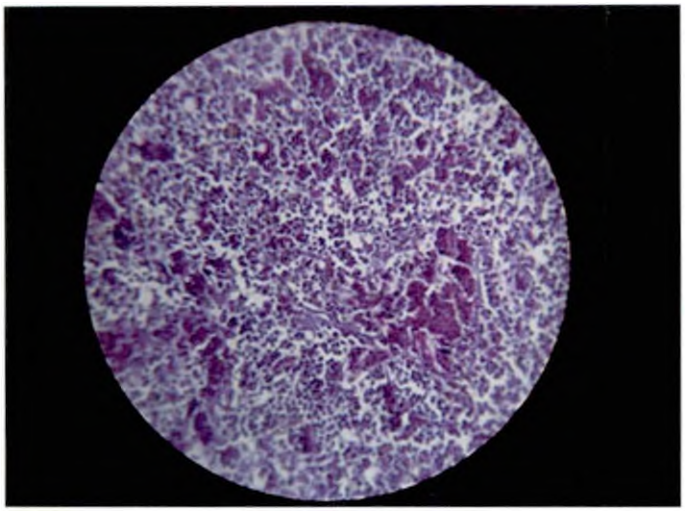

Fig. 34: Spleen - Lymphoid depletion and haemorrhages (H&E x 400)

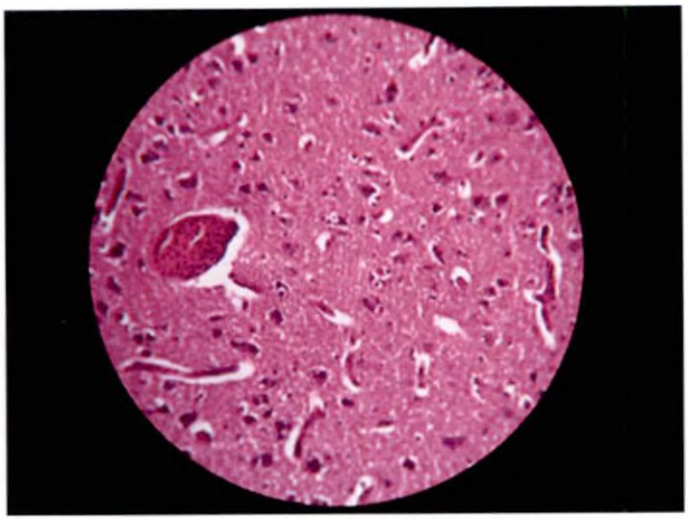

Fig. 35: Brain - Congestion, perineuronal and perivascular vacuolation (H&E x 400)

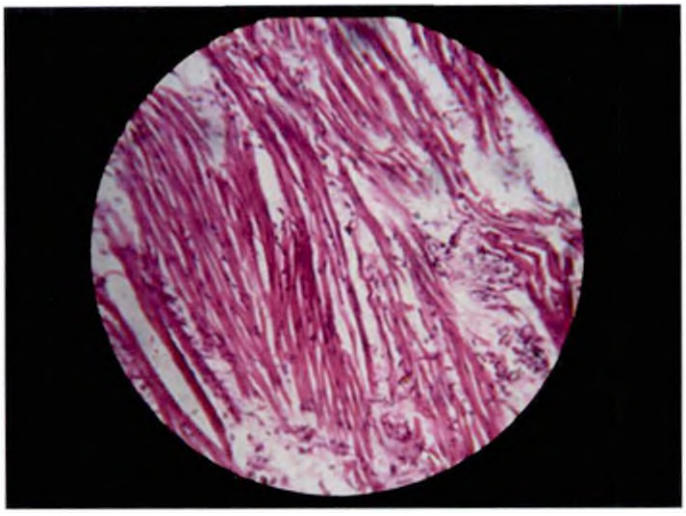

Fig. 36: Heart - Intermuscular haemorrhages, separation and fragmentation of muscle fibres (H&E x 400)

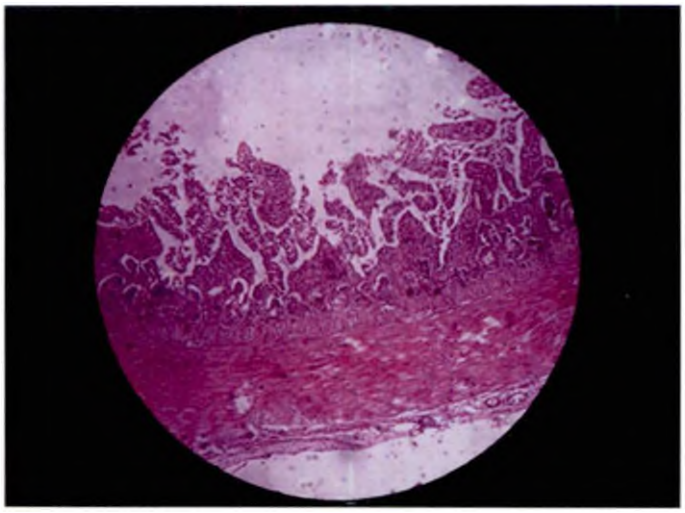

Fig. 37: Intestine - Necrosis and fusion of villi (H&E x 100)

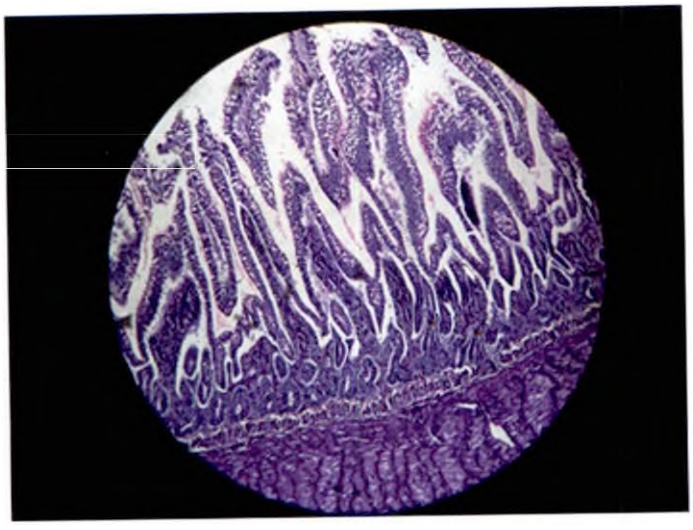

Fig. 38: Intestine - Goblet cell hyperplasia (H&E x 100)

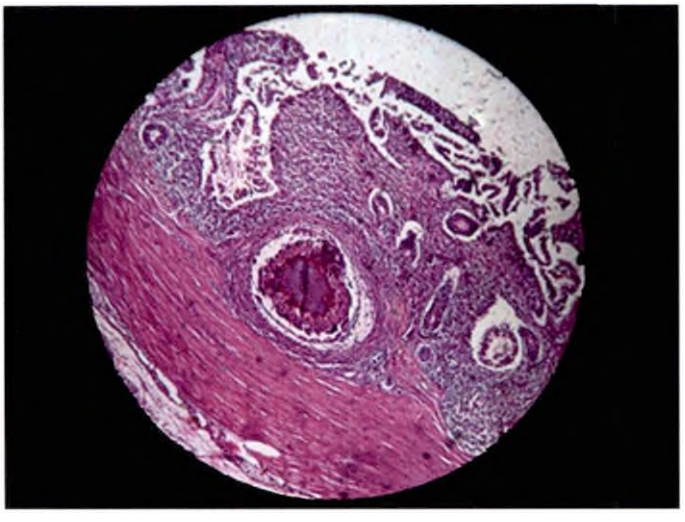

Fig. 39: Caecal tonsil - Necrotic granular substance enclosed with in fibrous tissue capsule (H&E x 100)

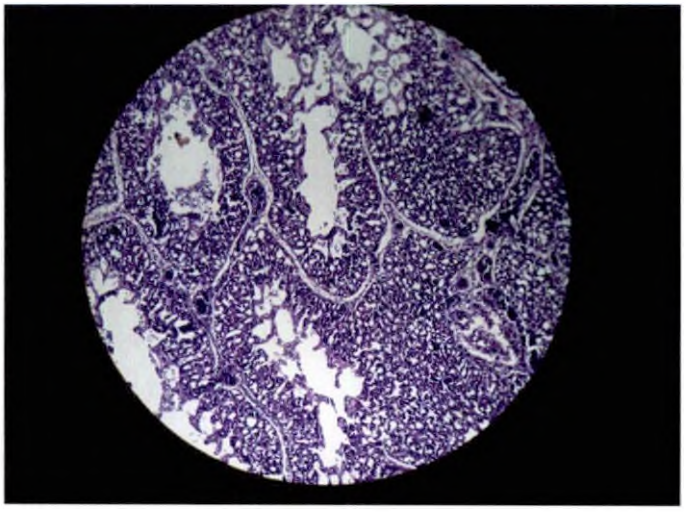

Fig. 40: Lung - Congestion and haemorrhages (H&E x 100)

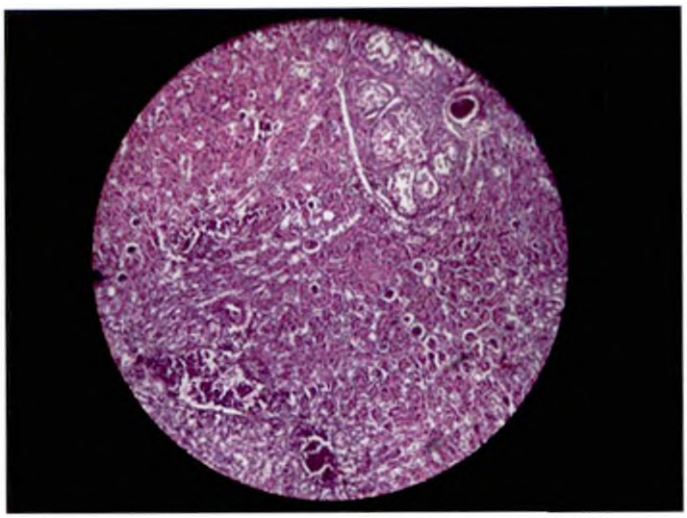

Fig. 41: Kidney - Paravenous necrosis of tubules and glomeruli, interstitial haemorrhages (H&E x 100)

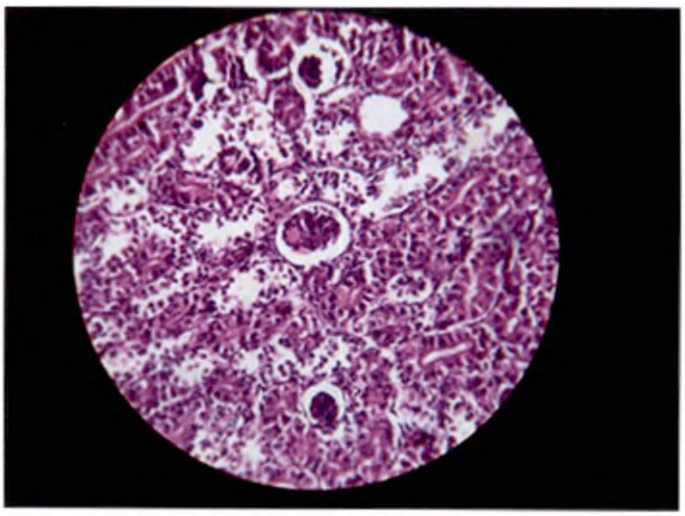

Fig. 42: Kidney - Necrosis of tubules, desquamation of tubular epithelium , degeneration and hyalinisation of glomeruli (H&E x 400)

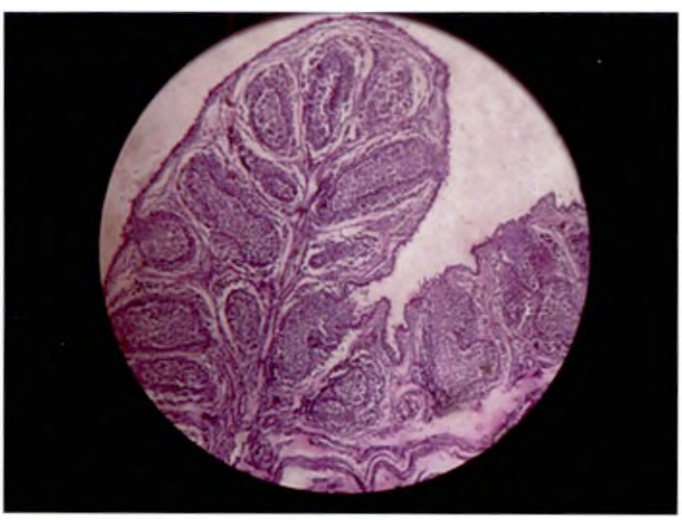

Fig. 43: Bursa - Lymphoid depletion and connective tissue proliferation (H&E x 100)

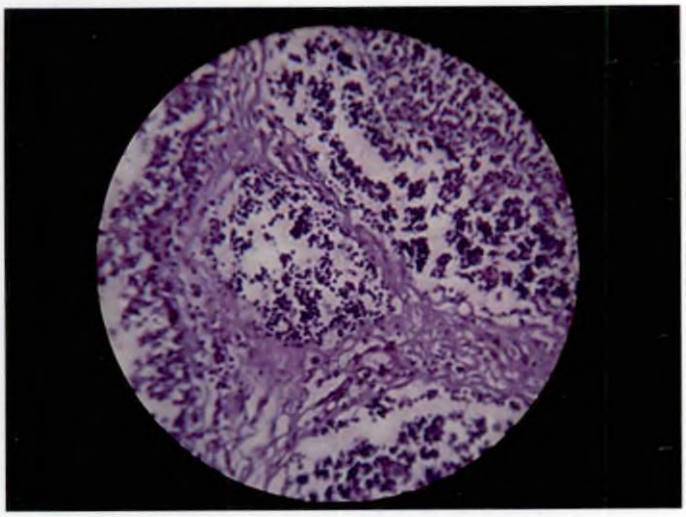

Fig. 44: Bursa - Lymphoid depletion in bursal follicles (H&E x 400)

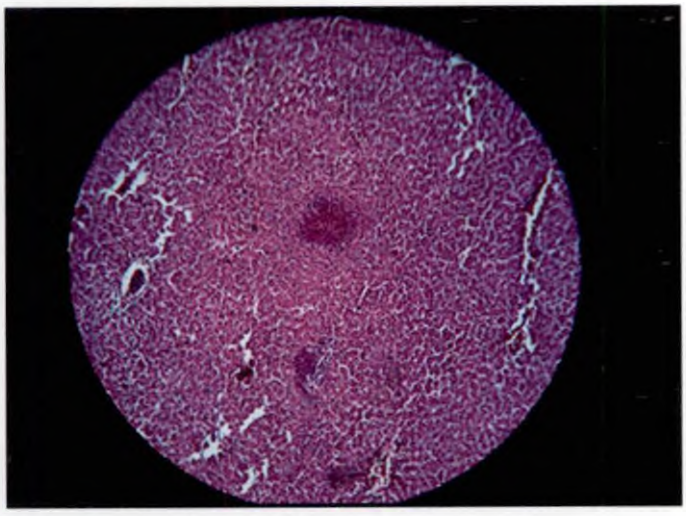

Fig. 45: Liver - Congesion, necrotic foci and infiltration of inflammatory cells (H&E x 100)

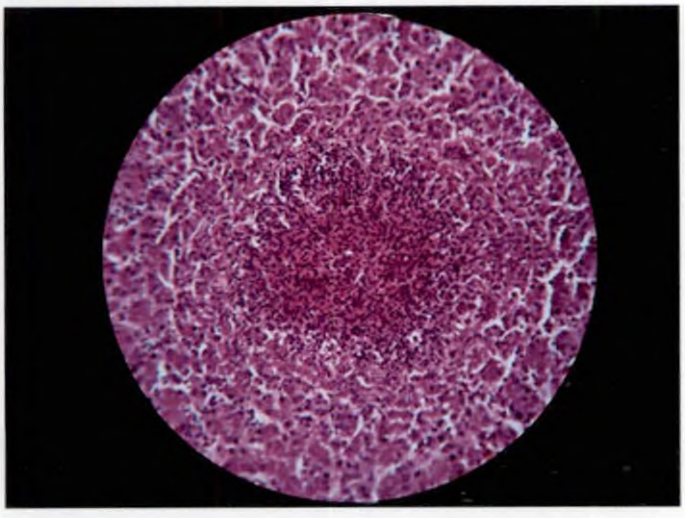

Fig. 46: Liver - Focal coagulation necrosis (H&E x 400)

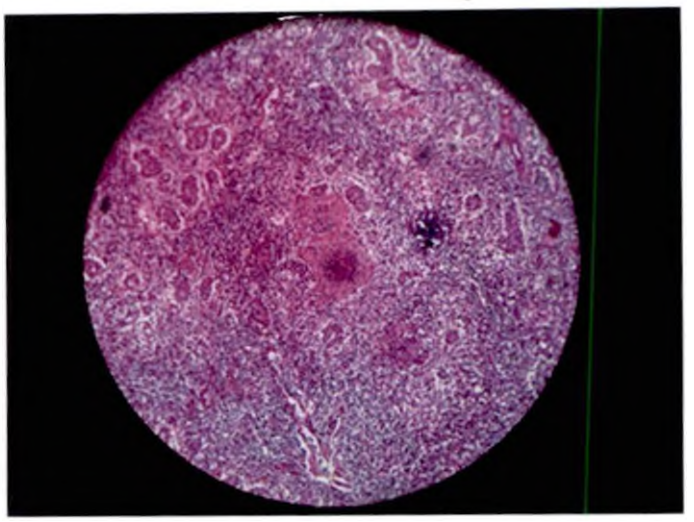

Fig. 47: Spleen - Congestion, focal coagulation necrosis (H&E x 100)

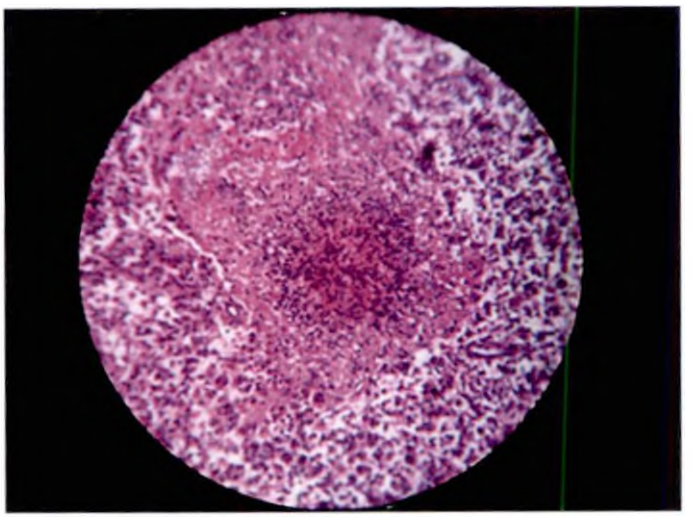

Fig. 48: Spleen- focal coagulation necrosis (H&E x 400)

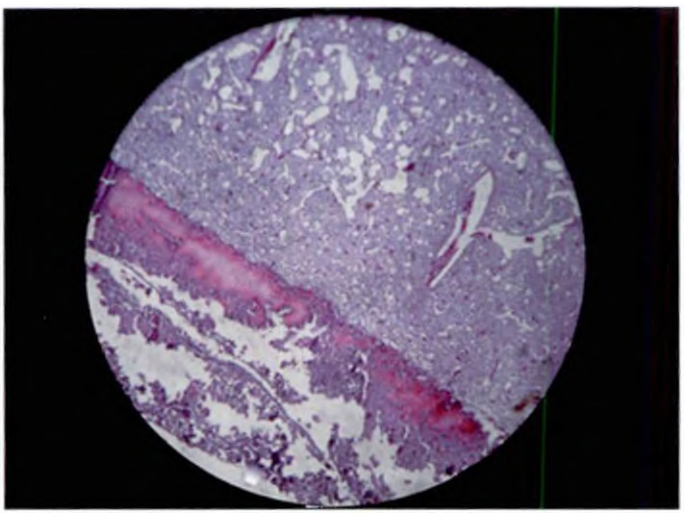

Fig. 49: Brain - Extensive sub-meningeal haemorrhages, perineuronal and peri-vascular vacuolation (H&E x100)

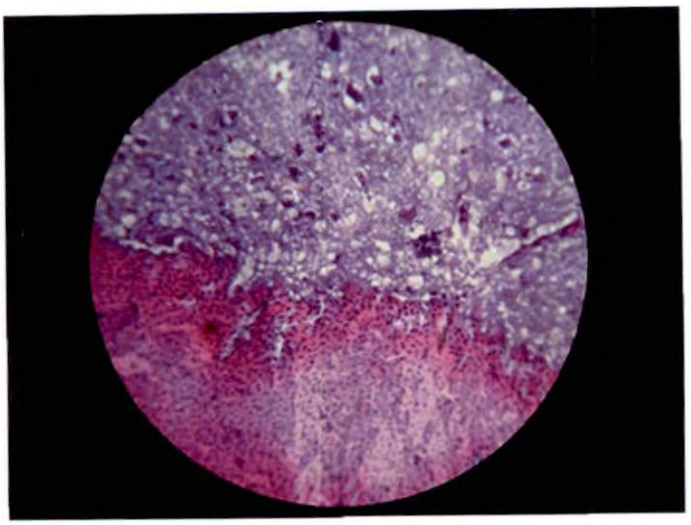

Fig. 50: Brain - Haemorrhage
(H&E x 400)

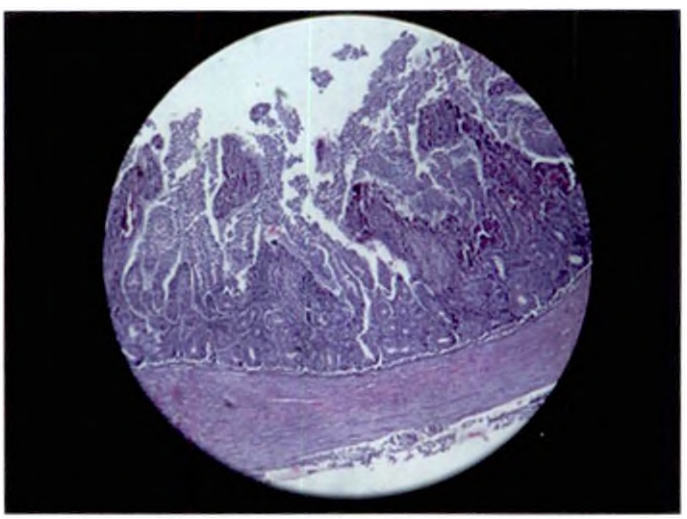

Fig. 51: Intestine - Haemorrhages, necrosis and fusion of villi, desquamation of villous epithelium and mononuclear cell infiltration (H&E x 100)

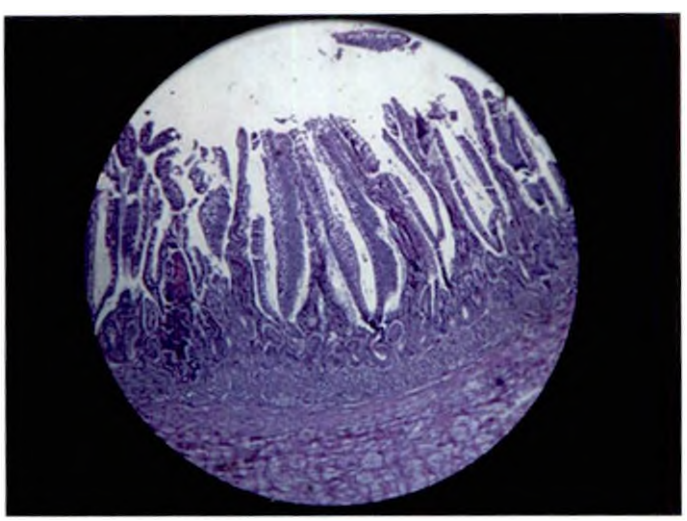

Fig. 52: Intestine -Glandular necrosis, goblet cell hyperplasia
(H&E x 100)

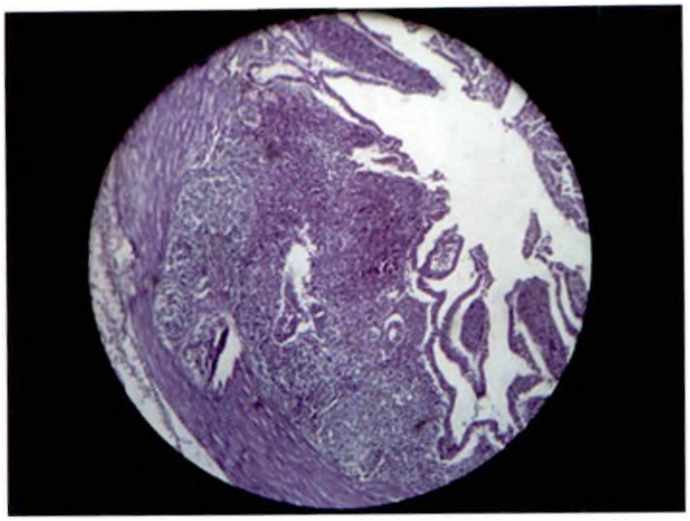

Fig. 53: Caecal tonsil - Lymphoid depletion, necrosis (H&E x 100)

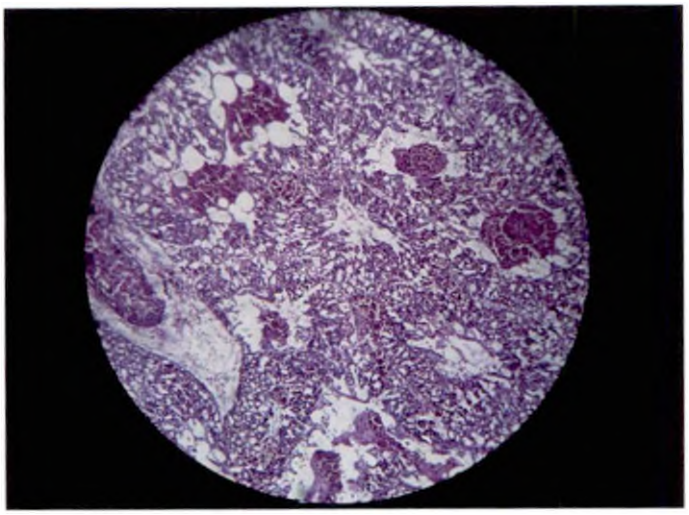

Fig. 54: Lung - widespread congestion and haemorrhages (H&E x 100)

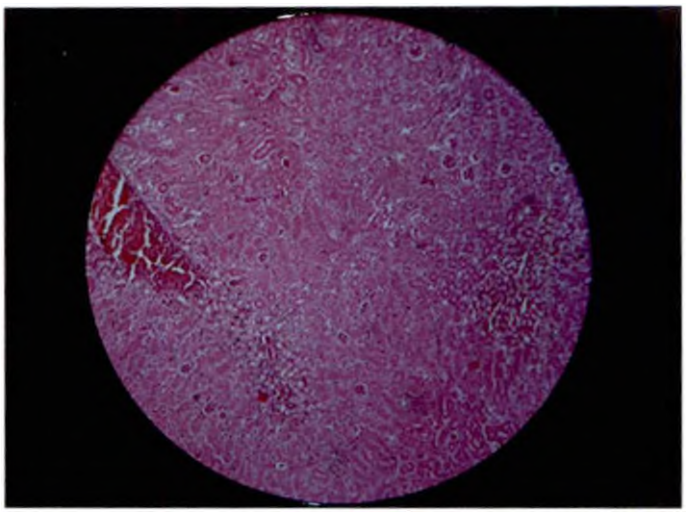

Fig. 55: Kidney - Congestion and paravenous necrosis of tubules and glomeruli (H&E x 100)

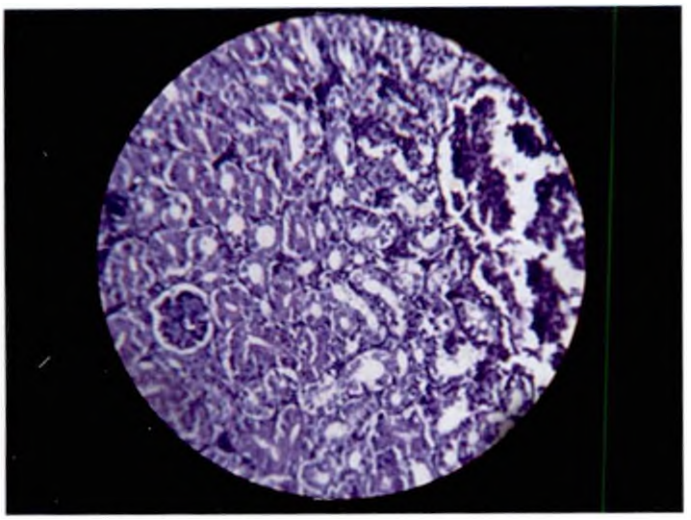

Fig. 56: Kidney - Interstitial haemorrhages, tubular necrosis (H&E x 400)

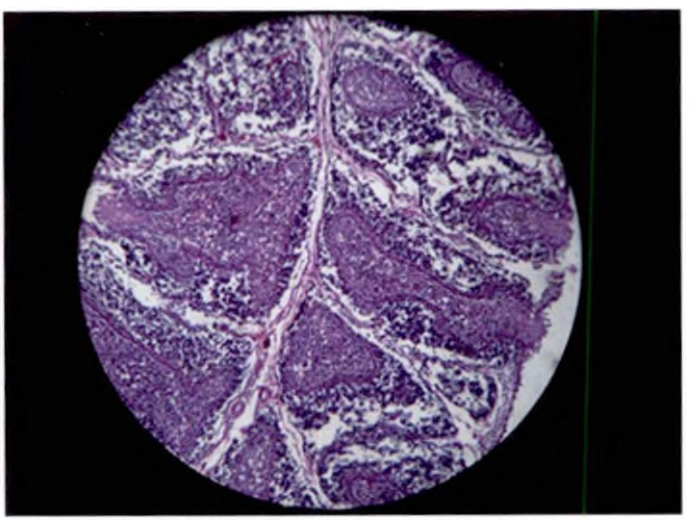

Fig. 57: Bursa - Lymphoid depletion and haemorrhage (H&E x 100)

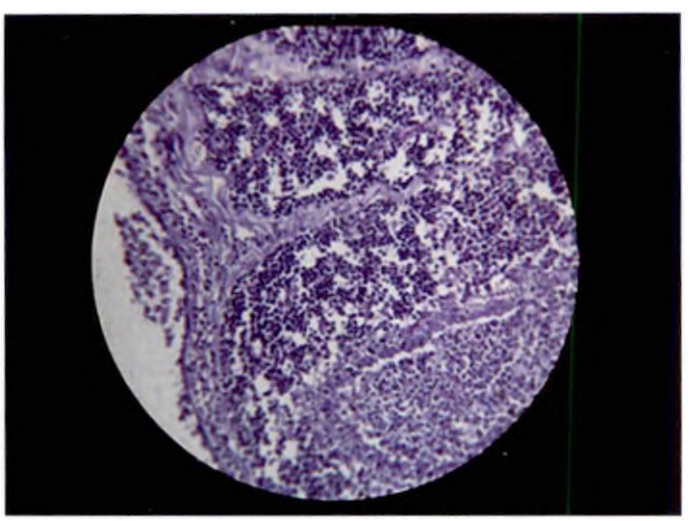

Fig. 58: Bursa - Lymphoid depletion (H&E x 400)

Discussion

5. DISCUSSION

Fowl cholera is a septicaemic disease of poultry caused by avian strains of the bacteria, *P. multocida.* This infectious disease is a serious threat to both domestic and wild birds. Among the birds, ducks are highly susceptible to pasteurellosis and it is a potential threat causing high mortality and morbidity. Incidence of this disease has been reported from Kuttanad region of Kerala and also from University duck farms. It is observed that most of the outbreaks occurred consequent to immunosuppression caused by ducks feeding on crustaceans in the fields, which have accumulated residues of pesticides. Hedge *et al.* (1996) reported an outbreak of duck pasteurellosis due to prior exposure of the birds to aflatoxins with resultant immunosuppression.

The exact pathological features of this disease have not been delineated properly. Perusal of literature also reveals only a few reports on the pathological aspects of pasteurellosis in ducks. Therefore the present experimental study was carried out using the local strain isolated from ducks of Kuttanad area, with the objective of evaluating the pathological changes associated with the infection which would help in formulating proper control measures.

5.1 Routes of inoculation of *P. multocida*

Intranasal and subcutaneous routes of exposure were tried for establishing the infection. The route and dose for inoculation were selected so as to simulate the natural exposure. The intranasal route of inoculation was selected because the usual portal of entry of *P. multocida* in cases of systemic fowl cholera outbreaks is through mucous membranes of the pharynx and upper air passages. Another common route of entry is via cutaneous wounds. So subcutaneous route of inoculation was also selected. These two routes of exposure were tried to delineate the differences in the onset of disease and manifestations. Rhoades and Rimler, (1990) studied the routes of entry of *P. multocida* in poultry and observed that the common routes were through the mucous membranes of the

pharynx or upper air passages, the membranes of the eye or through cuts and abrasions in the skin.

5.2 Dose of inoculum.

Lethal Dose fifty (LD_{50}) dilution of *P. multocida* was the dose of inoculum used to establish the infection through both the routes. As the organism was highly virulent, large doses would have resulted in rapid mortality. Therefore this dose was selected to study the progressive pattern of development of symptoms and lesions. The LD_{50} was determined by the method described by Reed and Muench (1938). For a practical and reliable measurement of pathogenicity, use of LD_{50} for experimental studies was supported by Cruickshank *et al.* (1975).

5.3 Mortality pattern

The acute onset of the disease with rapid mortality was more evident in birds which were subcutaneously inoculated with *P. multocida.* Two birds died within 18 hours after inoculation, followed by four deaths within three days. In the intranasally inoculated group, first death occurred only on the 2^{nd} day of inoculation. After that, no deaths were there in that group in the first week. From these observations, it can be inferred that the time taken for death depended on the route of inoculation. The parentral (subcutaneous) inoculation resulted in direct entry of the organism into the system, rapid multiplication and death. The virulence of the organism may also be dependent on the route of inoculation. Non parentral route of administration (intranasal) took more time for establishment of infection and death. The native defences of the respiratory tract might have resisted the progression of the disease up to a certain extent. The enhanced heterophil counts and the activity of which in the lungs on histopathological examination indicated improved resistance. Pillai *et al.* (1993), Pehlivanoglu *et al.* (1999) and Wilkie *et al.* (2000) found from their experimental trials that the parentral (intravenous, intramuscular and subcutaneous) routes of inoculation of

P. multocida caused more deaths in ducks while the non parentral (occular, nasal and oral) routes caused comparatively lower mortality, when challenged with *P. multocida* isolates by different inoculation routes.

5.4 Symptoms

The birds which died within 18 hours after subcutaneous inoculation did not show any symptoms. This might be due to the rapid multiplication and massive endotoxin release by the organism, resulting in a peracute form of infection. A reduction in the natural immunity might also have favoured rapid multiplication. The histopathological findings of lymphoid depletion and necrotic changes in the spleen, caecal tonsils and bursa confirmed this. Ramasastry and Ramarao, (1989), Hegde *et al.* (1996) and Rao (1990) observed sudden death without any signs in per acute forms of fowl cholera.

After the first two peracute deaths, symptoms such as pyrexia, depression, inappetence, increased thirst, mucous discharge from beaks, diarrhoea, paralysis of limbs and neck and incoordination were expressed by the subcutaneously inoculated birds before death. Similar observations were made by Fisher *et al.* (1998) and Sarkozy *et al.* (2002) in experimental fowl cholera infection. The gradual endotoxaemia and subsequent damage to the vital organs, which developed after inoculation might have been responsible for the expression of symptoms. All the birds which died showed vascular and necrotic lesions in organs including brain. Hunter and Wobeser (1979) and Collins (1977) reported toxaemia as a cause of vital organ damage and death. Further, sufficient time gap was there before death for the development of typical symptoms of toxaemia. The action of toxin was so extensive that it affected the thermoregulatory centre which resulted in pyrexia.

Besides the above symptoms, the intranasally inoculated birds showed severe sinusitis. This could be attributed to the local irritation of the nasal mucosa by the organisms and subsequent development of inflammation. Songserm *et al.*

(2003) also observed sinusitis in the ducks intranasally inoculated with the nasal discharge of fowl cholera infected birds.

5.5 PHYSIOLOGICAL PARAMETERS

5.5.1 Body weight

A statistically significant alteration in body weight was not observed in the treatment groups when compared to the control group. However, the birds in the treatment groups didn't gain weight as much as the control group as there was in appetence and reduction in feed intake caused by the infection. The affected birds showed a statistically significant reduction in total protein which also contributed to the loss of gain in weight. Proteins are the major components which contribute to the body muscle mass. The body weight never reached up to that of control birds at any point of time. Similar observation of reduced weight gain by *P. multocida* inoculated birds was made by Shilpa *et al.* (2005) and Shivachandra *et al.* (2005) in their experimental studies.

5.6 HAEMATOLOGICAL PARAMETERS

The leucocytosis observed after inoculation of *P. multocida* at all time intervals was the result of the heterophilia. The heterophilic leucocytosis might be viewed as the primary response to an acute bacterial infection. Corresponding to heterophilia, reduction in the monocyte and lymphocyte counts were observed which may be attributed to the rapid activation and influx of heterophils by the body defense system. Rose *et al.* (1988) observed similar variation in DLC when *Pasteurella multocida* was inoculated intravenously into turkeys. Towards the second week after inoculation in both the treatment groups, an increased eosinophil count was observed. Chansoria *et al.* (1993) stated that unlike mammalian inflammation, eosinophils have a major role in later stages of avian inflammation. With the help of strong enzymes present within the granules these cells actively take part in phagocytosis along with heterophils. This findings correlates with the observations of Latimer *et al.* (1988) and Shilpa *et al.* (2005).

They observed pathological leucocytosis in infections, localized and generalized tissue necrosis and acute inflammatory diseases including fowl cholera.

Mean Hb and PCV values of the ducklings of both treatment groups markedly increased on day 7 and on day 14 and reached normal level on day 28. During these periods, all the birds remained without manifesting any symptoms except reduced feed and water intake. Therefore the increasing trend of these values can be attributed to the resultant dehydration and haemoconcentration. The level reaching normal on the 28th day indicated improvement of the defense system subsequent to the development of antibodies resulting in clearance of infection.. These observations were in agreement with that of Latimer *et al.* (1988) and Shilpa *et al.* (2005). Contrary to this, Rameshkumar *et al.* (2006) reported a reduction in Hb, PCV and TEC values in rabbits infected with pasteurellosis which was attributed to anaemia in the infected animals.

5.7 SEROLOGICAL ALTERATIONS

On day seven, the mean serum total protein values of the treatment groups were found slightly increased although not statistically significant. Meanwhile on day 14 and 21, significant decrease in the level was observed. This may be attributed to the hepatic necrosis caused by the prolonged endotoxaemia. The albumin values of both treatment groups were found increased on seventh day post infection whereas the globulin values remained reduced on days 14 and 21 which could only be a relative change subsequent to a reduced blood volume. Similar changes in albumin, and total protein values were reported by Mbuthia *et al.* (2008) and Latif and Dessouky (2006) in experimental avian cholera infection.

5.8 PATHOANATOMICAL STUDIES

5.8.1 Gross lesions

The two birds in the subcutaneously inoculated group which died within 18 hours didn't reveal any lesions except a few petechiae on the heart in one bird.

This may be due to the rapid multiplication and massive endotoxin release by the organism, resulting in peracute death, leaving no time for the establishment of infection and development of visible gross lesions. Ramasastry and Ramarao (1989) also observed no grossly visible changes in any of the organs in per acute fowl cholera deaths.

The major lesions observed in birds which died from second day after inoculation were haemorrhages on epicardium, hydropericardium with blood tinged fluid in pericardial sac, scattered pin point necrosis of liver, haemorrhagic enteritis, congestion and necrosis of spleen, congestion of kidney, caecal tonsils and bursa, petechiae on airsacs and serosal surfaces and congestion and haemorrhages in the lungs. The gross lesions observed in this study were in accordance with those reported by others (Christensen & Bisgaard, 1997; Glisson *et al.*, 2003). The predominant vascular lesions could be attributed to the endotheliotropism of the bacteria and their multiplication within the vascular endothelial cells. Further, the endotoxins produced also contributed to the damage. Mir *et al.* (2001) and Collins (1977) highlighted the role of an endotoxin in the pathogenesis of pasteurellosis. More pronounced lesions were observed in the birds intra nasally inoculated with *P. multocida,* which died on 23rd, 24th and 25th day post- inoculation. The heart of the dead birds showed typical red paint brush appearance. The multifocal necrotic foci on liver were numerous and more prominent. This may be attributed to the longer survival time of those birds and persistence of infection. Unlike peracute deaths, enough time was there for the development of appreciable pathological lesions. Based on the results obtained in an experimental study of intravenous *P. multocida* inoculation in ducks, Mbuthia *et al.* (2008) opined that the longer the survival time after infection, the more abundant and drastic might be the lesions.

5.8.2 Histopathology

The histopathological lesions observed in the various organs in both the treatment groups were comparable, but varied in intensity within and between the

groups. Liver, kidney, lungs, intestine, heart, brain, spleen, caecal tonsils and bursa were the organs examined and showed lesions typical of an acute bacterial septicaemia. Vascular lesions like congestion and haemorrhage, necrosis and inflammatory lesions with heterophilic infiltration were predominant in all the organs. The brain lesions such as meningeal congestion, submeningeal haemorrhage and associated neuronal changes were prominent in the birds which were inoculated through intranasal route, besides the marked lung lesions. The lung lesions varied from congestion, haemorrhage and oedema to inflammatory cell infiltration. None of the organs and the infiltrating heterophils revealed the presence of *Pasteurella* organisms on histopathological examination.

The various histopathological lesions observed were in accordance with the observations of various research workers. (Collins, 1977; Hunter and Wobeser, 1980; Prantner *et al.*, 1990; Christensen and Bisgaard, 1997; Bojesen, 2004; Rameshkumar *et al.*, 2006 and Mbuthia *et al.*, 2008). The lymphoid organs such as spleen, caecal tonsils and bursa in all the infected cases revealed lymphoid depletion, necrosis, congestion and haemorrhage which clearly indicated the immunosuppression suffered by the dead birds due to infection. Prantner *et al.* (1990) and Fisher *et al.* (1998) also observed lymphoid depletion and foci of necrosis in spleen, bursa and thymus on experimental inoculation of *P. multocida* in broilers and turkeys. Mir *et al.* (2003) observed immunosuppression in rabbits infected with *Pasteurella multocida*.

All the gross and histopathological lesions observed might have been produced by the *Pasteurella* endotoxins and septicaemia. Bojesen (2004) observed that the heterophils recruited during infection mediated tissue damage.

Six birds in the subcutaneously inoculated group survived the infection, though showed reduced feed intake and reduction in body weight for two weeks. The non development of clinical illness can be attributed to the better clearance of the organism from the system as evidenced by the failure of reisolation of organism from the organs. None of the immune organs like the spleen, caecal

tonsils and bursa showed any pathological changes in the survived birds whereas those succumbed to the disease had severe lesions in these organs. This indicated that the recovered birds were immunologically well protected and heterophil activation might have been high. Hunter and Wobeser (1980) indicated that heterophil activation played a dual role for the outcome of *Pasteurella multocida* infection in birds where it initially seemed to promote invasion and systemic spread, but subsequently helped to limit the infection by bacterial clearance.

5.9 REISOLATION OF *P. multocida*

Organisms could be reisolated from all the infected birds except the six birds which survived after subcutaneous inoculation. Bacterial concentrations were found to be high in the spleen on reisolation. This indicated the sequestration of *P. multocida* in the spleen and subsequent multiplication. The spleen from infected birds showed extensive necrotic and inflammatory changes on histopathological examination.

From this experimental study it was noticed that the route of inoculation has considerable significance in the development of the disease and the different routes resulted in different patterns of progression of the disease. More acute pattern of infection was observed when *P.multocida* was inoculated subcutaneously. The course of disease took more time by the non parentral intranasal route. However intranasally inoculated birds displayed classical symptoms and lesions of fowl cholera. This might be due to persistence of infection and resultant endotoxaemia for comparatively longer period than the subcutaneously inoculated birds.. The disease was found to be immunosuppressive as there was depletion, necrosis and cystic changes in the bursa, caecal tonsils and spleen. These accounts for the increased morbidity due to pasteurellosis, as these immunosuppressed birds become highly susceptible to other infections.

Summary

6. SUMMARY

The experiment was designed to evaluate the pathological features of pasteurellosis in ducks using locally prevalent strain of *P .multocida* and to compare the route of administration and production of disease.

Thirty six one month old ducks were maintained under identical feeding and managemental conditions in the laboratory and were randomly divided into three groups of twelve each and designated as group I to group III. The Group I birds were maintained as healthy control. *Pasteurella multocida* LD_{50} dilution was injected subcutaneously in the Group II birds at the dorsal neck region @ 0.1 ml/bird. In Group III, *P. multocida* LD_{50} dilution was administered by intra-nasal inoculation @ 0.1 ml/bird.

The parameters studied included body weight changes at weekly intervals, mortality pattern, gross pathological changes, histopathological examination of heart, liver, lung, kidney, brain, intestine, spleen, caecal tonsils and bursa. Haematological and biochemical parameters such as total erythrocyte count, total leucocyte count, haemoglobin concentration, differential leucocyte count, total protein, albumin and globulin values were estimated at weekly intervals. Attempts were made to reisolate *P. multocida* from heart, liver and spleen of the dead birds in Tryptone Soya Agar plates.

A statistically significant alteration in body weight was not observed in the treatment groups when compared to the control group. However, the birds in treatment groups didn't gain weight as much as the control group, although it was not statistically significant. Mean Hb, TEC and PCV values of the ducklings of both treatment groups were markedly increased on day 7 and on day 14 after inoculation and reached normal level by day 28. The mean heterophil count of both treatment groups showed a significant increase in the levels on all periods while the mean lymphocyte count showed a corresponding decrease. Monocytopaenia and eosinophilia were also noticed. On days 14 and 21, the

infected birds showed reduced total protein values when compared to the control. The albumin values of both treatment groups were found significantly increased on seventh day post infection. Significantly reduced globulin values were noticed at days 14 and 21, especially in the intranasally inoculated birds.

The acute onset of the disease with sudden mortality in the first a few days was more evident in the group of birds which were subcutaneously inoculated with *P. multocida.* Two birds died within 18 hours after inoculation, followed by four deaths within three days. Remaining six birds survived till the end of experiment. The peracute pattern of mortality seen in this group was not observed in intranasally inoculated group. In the first week, there was only one death on the 2nd day of inoculation. Further mortality occurred 22 days after inoculation. On the 22rd day, one more death occurred in that group and the remaining birds started to show clinical signs. Six birds were found dead on the 23th day and the remaining four birds on the very next day.

The birds which died within 18 hours after subcutaneous inoculation didn't show any symptoms. After the first two peracute deaths, symptoms such as pyrexia, dullness, depression, inappetence, increased thirst, mucous discharge from beaks, diarrhoea, lacrimation, reluctancy to move, paralysis of limbs and neck and incoordination were expressed by the birds before death. Intranasally inoculated group showed severe sinusitis besides the above symptoms.

On postmortem, the two birds in the subcutaneously inoculated group which died within 18 hours didn't reveal any lesions except a few petechiae on the heart in one bird. The major lesions observed in birds those died from second day after inoculation were haemorrhages on epicardium, hydropericardium with blood tinged fluid in pericardial sac, scattered pin point necrotic foci in the liver, haemorrhagic enteritis, necrosis of spleen, congestion of kidney, caecal tonsils and bursa, petechiae on airsacs and serosal surfaces and congestion and haemorrhages in lung. These lesions were more pronounced in the birds inoculated intranasally with *P. multocida,* which died on 23rd, 24th and 25th day

post- inoculation. The heart of the dead birds showed typical red paint brush appearance. The multifocal necrotic foci in the liver were numerous and more prominent. Severe lung lesions which varied from congestion, edema and extensive haemorrhages were observed. Brain lesions like meningeal congestion and submeningeal haemorrhages were more prominent in this group.

Histologically, there was severe pulmonary necrosis in intranasally inoculated birds. Coagulative necrosis in liver and spleen, epicardial haemorrhages, haemorrhagic enteritis along with destruction of villus epithelium and necrosis of submucosal glands, extensive paravenous necrosis of tubules of the kidney, lymphoid depletion in caecal tonsils and bursa were evident in all the dead birds, although intensity varied individually.

Reisolation of *P. multocida* was carried out from the liver, heart and spleen of all the dead birds except control and survived birds.

The result of this study proved that the route of inoculation has considerable significance in the development of the fowl cholera. Immediate mortality was noticed when *P. multocida* was inoculated subcutaneously. However, the severity of clinical signs and lesions were more in intranasally inoculated birds. The damage of lymphoid tissues like spleen, caecal tonsils and bursa revealed on histopathology shows that *P. multocida* infection caused immunosuppression. Hence the affected birds became more prone to other infections, leading to heavy economic losses.

References

REFERENCES

Anon. 1996. *XV Quinquennial livestock census – 1996.* Directorate of Animal Husbandry, Government of Kerala. Tiruvananthapuram. p. 56

Bancroft, J. D. and Cook, H. C. 1984. Manual of Histological Techniques and their Diagnostic Applications. *Second edition. Churchill Livingstone, Edinburgh. p. 457*

Barrow, C. I. and Feltham, R. K. A. 1993. *Cowan and Steel's manual for identification of medical bacteria.* Third Edn. Cambridge University Press. p. 331

Bhadury, S. K. 1948. Fowl cholera- some interesting features in a sub acute outbreak. *Indian Vet. J.* 25: 278-280

Bhattacharya, A. 2005. Isolation, characterization and antibiotic sensitivity of *Pasteurella multocida* from incidences of duck cholera in khaki Campbell and vigova super-M ducks in Tripura. *Indian Vet. J.* 82: 203-205

Bhaumik, A. and Dutta, S. 1995. Studies on bacteriological parameters of *Pasteurella multocida* isolated from Khaki Campbell ducklings in Tripura. *Indian Vet. J.* 72: 1092-1093

Bojesen, M. A., Petersen, K. D., Nielsen, O. L., Christensen, J. P. and Bisgaard, M. 2004. *Pasteurella multocida* Infection in Heterophil-Depleted Chickens. *Avian Dis.* 48: 463-470

Chakraborthy, T., Nayak, N. C., Guha, C. and Bhaumik, M.K. 1989. Some unusual records on natural infection with *Pasteurella multocida* in breeder fowls. *Indian Vet. J.* 66: 270-271

Chandran, N. D. J., Prabakar, T. G., Albert, A., David, B. P. and Venkatesan, R. A. 1995. Pasteurellosis in Japanese quails. *Indian Vet. J.* 72: 876-877

Chansoria, M. Awadhiya, R. P., Vegad, J. L. and Katiyar, A. K. 1993. Studies on the cellular response in avian inflammation using a simple subcutaneous pouch model. *Avian Pathol.* 22: 593-598

Char, N. L., Rao, M. R. K., Sarma, D. R. L. and Khan, D. I. 1987. Report on mortality in ducklings due to fowl cholera. *Poult. Adv.* 20: 45-47

Christensen, J. P. and Bisgaard, M. 1997. Avian pasteurellosis: taxonomy of the organisms involved and aspects of pathogenesis. *Avian Pathol.* 26: 461-483.

Christensen, J. P., Dietz , H. H. and Bisgaard M. 1998. Phenotypic and genotypic characters of isolates of *Pasteurella multocida* obtained from backyard poultry and from two outbreaks of avian cholera in avifauna in Denmark. *Avian Pathol:* 27: 373-381.

Collins, F. M. 1977. Mechanisms of acquired resistance to *Pasteurella multocida* infections: A review. *Cornell Veterinarian.* 67:103–138.

Cruickshank, R., Duguid, J. P., Marmion, B. P. and Swain, R. H. A. 1975. *Medical Microbiology*, 12th edn. Churchill Livingstone, Edinburg. p.1068

Curtis, P. E. and Ollerhead, G. E. 1978. *Pasteurella multocida* infection of broiler chickens. *Vet. Rec.* 103: 312-313

Das, V., Biswas, G., Bhattacharya, H. M. and Mukherjee, M. 1991. Prevalence of duck cholera in West Bengal. *Indian J. Poult. Sci.* 26: p.60

Devi, V. R., Rao, T. S., Aruna, P. and Sujani, G. 2000. Incidence of duck cholera in Krishna district, Andhra Pradesh – a field report. *Indian Vet. J.* 79: 535-536

Dougherty, E. 1953. Disease problems confronting the duck industry. *Proc. Annu. Meet. Am. Vet. Med. Assoc.* pp 359-365

Doumas, B., Watson, W. A. and Blaggo, H. G. 1971. Photometric determination of serum albumin concentration. *Clin. Chem.* 31: 87-96

Dwivedi, P. N. and Sodhi, S. S. 1989. Morphological, cultural and biochemical characterization of *Pasteurella multocida* isolated from poultry. *Poult. Adv.* 22: 69-71

Eigaard, N. M., Permin, A., Christensen, J. P., Bojesen, A. M. and Bisgaard, M. 2006. Clonal stability of *Pasteurella multocida* in free-range layers affected by fowl cholera. *Avian Dis.* 35: 162-171

Feldman, F. B., Zinkal, G.J. and Jain, C.N. 2000. *Schalm's Veterinary Haematology.* 5th Edn. Lippincott Williams and Wilkins, USA. 1344p

Fisher, M. E., Trampel, D. W., Griffith, R. W. 1998. Postmortem detection of acute septicaemia in broilers. *Avian Dis.* 42: 452—461

Fujihara, Y., Onai, M., Koizumi, S., Satosh, N. and Sawada, T. 1986. An outbreak of fowl cholera in wild ducks in Japan. *Jpn. J. Vet. Sci.* 48: 35-43

Glass, S. E. and Panigrahy, B. 1990. Dermal necrosis caused by *Pasteurella multocida* infection in turkeys. *Avian Dis.* 34: 491—494

Glisson, J. R. 1994. Fowl cholera in commercial poultry. *Poult. Adv.* 27: 53-58

Goto, Y., Nakura, R., Nasu, T., Sawada, T. and Shinjo, T. 2001. Isolation of *Pasteurella multocida* during an outbreak of infectious septicaemia in Japanese quail (Coturnix coturnix japonica). *J. Vet. Med. Sci.* 63: 1055-1056

Gustafson, C. R., Cooper, G. L., Charlton, B. R. and Bickford, A. A. 1998. *Pasteurella multocida* infection involving cranial air spaces in white leghorn chicken. *Avian Dis.* 42: 413-417

Heddleston, K. L. 1976. Physiologic characteristics of 2,268 cultures of *Pasteurella multocida*. *Am. J. Vet. Res.* 37: 745-747

Hegde,V., Gowda, R. N. S. and Vijayasarathy, S. K., 1996. Pathology of pasteurellosis in ducks. *Indian J. Vet. Pathol.* 20: 130-132

Henry, R. J., Sobel, C. and Berkman, S. 1957. Photometric determination of total protein in plasma. *Annl. Chem.* 45: 1491-1499

Hunter, B. and Wobeser, G. 1980. Pathology of experimental avian cholera in mallard ducks. *Avian Dis.* 24: 403-414

Jayakumar, P. S. 1998. Comparative efficacy of different vaccines against pasteurellosis in ducks. M.V.Sc thesis submitted to Kerala Agricultural University, Thrissur. p.126

Jeffrey, J. S., Shivaprasad, H.L., Duran, L., Cardona, C. J. and Charlton, B. R. 1993. Facial cellulitis associated with fowl cholera in commercial turkeys. *Avian Dis.* 37: 1121-1129

Joshy, V.B., Baxi, K. K., Dwivedi, P. N. and Sambyal, D. S. 1987. A case of bumble foot in a chicken due to *Pasteurella multocida*. *Poult. Adv.* 20: 51-52

Karim, R. 1987. An outbreak of clinical pasteurellosis in ducks and its control. *Indian Vet. J.* 64: p.722

Kumar, A. 1990. Fowl cholera in poultry and its control. *Poult. Adv.* 23: 49-53

Kwon, Y. K. and Kang, M. I. 2003. Outbreak of fowl cholera in Baikal teals in Korea. *Avian Dis.* 47: 1491-1495

Latif, M. M. A. and Dessouky, S. A. 2006. Studies on some bacterial causes and blood serum biochemical changes of respiratory infections in lambs. *Ass. Vet. Med. J.* 56: 104-106

Latimer, S, K., Tang, K., Goodwin, M. A., Steffens, W. L. and Brown, J. 1988. Leucocyte changes associated with acute inflammation in chicken. *Avian Dis.* 32: 760-772

Lowes, N. 1990. Fowl cholera in overwintering mallard ducks. *Can. Vet. J.* 31: p.846

Mariana, S. and Hirst, R. 2000. The immunogenicity of *Pasteurella multocida* isolated from poultry in Indonesia. *Vet. Microbiol.* 72: 27-36.

Mbuthia, P.G., Njagi, L.W., Nyaga, P.N., Bebora, L.C., Minga, U., Kamundia, J. and Olsen, J.E. 2008. *Pasteurella multocida* in scavenging family chickens and ducks: carrier status, age susceptibility and transmission between species. *Avian Pathol.* 37: 51-57

Miguel, B. E., Wang, W.R., Maslin, R.W., Keirs, K. and Glisson, J. R. 1998. Subacute to chronic fowl cholera in a flock of Pharaoh breeder quail. *Avian Dis.* 42: 204-208.

Mir, M. S., Dwivedi, P. and Charan, K. 2001. *Pasteurella multocida*- 12:A induced clinic-pathological changes in rabbits. *Indian J. Vet. Pathol.* 25: 24-27

Morishita, T. Y., Lowenstine, J. L., Hirsh, D. C. and Brooks, D.L. 1996. *Pasteurella multocida* in psittacines: prevalence, pathology, and characterization of isolates. *Avian Dis.* 40: 900-907

Morishita, T. Y., Lowenstine, J. L., Hirsh, D. C. and Brooks, D.L. 1997. Lesions associated with *Pasteurella multocida* infection in raptors. *Avian Dis.* 41: 203-213

Muhairwa, A. P., Christensen, J. P. and Bisgaard M. 2000.Investigations on the carrier rate of *Pasteurella multocida* in healthy commercial poultry flocks and flocks affected by fowl cholera. *Avian Pathol.* 29: 133–142

Mulbagal, A. N., Kulkarni, W. B. and Paranjape, V. L. 1972. Some observations on pasteurellosis in ducks. *Indian Vet. J.* 49:544-546

Murugkar, H. V. and Ghosh, S. S. 1995. *Pasteurella multocida* serotype A: 1 in ducks in Tripura. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 16: 109-111

Mutters, R.S., Ihm, P., Pohl, S., Frederiksen, W. and Mannheim, W. 1985. Reclassification of the genus *Pasteurella trevisan 1887* on the basis of deoxyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis, Pasteurella canis, Pasteurella stomatis, Pasteurella anatis and Pasteurella langaa. Int. J. System. Bacteriol. 35: 309-322*

Nakamine, M., Ohshiro, M., Ameku, Y., Oshiro, K., Keruma, T., Sawada, T. and Ezaki, T. 1992. The first outbreak of fowl cholera in muscory ducks (Ca*rina moschata*) in Japan. *J. Vet. Med. Sci.* 54: 1225-1227

Natt, M. P. and Herrick, C. A. 1952. A. new blood diluent for counting the erythrocytes and leucocytes of the chicken. *Poult. Sci.* 31: 731-735

Panda, S. N., Misra, B., Das, B.C. and Nayak, B. C. 1981. An outbreak of avian pasteurellosis in Orissa. *Indian Vet. J.* 58: 418-420

Pehlivanoglu, F., Morishita, T. Y., Aye, P. P., Porter, R. E., Angrick, E. J., Harr, B. S. and Nersessian, B. 1999. The effect of route of inoculation on the virulence of raptorial *Pasteurella multocida* isolates in pekin ducks (*Anas platyrhynchos*). *Avian Dis.* 43: 116-121

Petersen, K.D., Christensen, J.P., Permin, A. and Bisgaard, M. (2001). Avirulence of *P. multocida* subsp. *multocida* isolated from outbreaks of fowl cholera in wild birds for domestic poultry and game birds. *Avian Pathol.* 30: 27-31

Pillai, R. M., James P. C., Punnoose, K. T., Sulochana, S., Jayaprakasan, V. and Nair, G. K. 1993. Outbreak of pasteurellosis among duck population in Kerala. *J. Vet. Anim. Sci.* 75: 34-39

Prantner, M. M., Harmon, B. G., Glisson, J. R. and Mahaffey, E. A. 1990. The pathogenesis of *Pasteurella multocida* serotype A:3,4 infection in turkeys: A comparisos of two vaccine strains and a field isolate. *Avian Dis.* 34: 260-266

Prantner, M. M., Harmon, B. G., Glisson, J. R. and Mahaffey, E. A. 1990. Septicaemia in vaccinated and non vaccinated turkeys inoculated with *Pasteurella multocida* serotype A:3,4. *Vet. Pathol.* 27: 254-260

Prasad, V., Madhubala, K., Sharief, S. K. A., Murty, K. K., Murty, P. R. and Rao, T. V. J. 1997. Studies on avian pasteurellosis in TANUKU, Andhra Pradesh. *Indian Vet. J.* 74: 635-637

Praveena, P. E., Singh, N., Sivakumar, P. and Kumar, A. A. 2007. Studies on haemato-biochemical changes in *Pasteurella multocida* serotype A: 1 infection in mice. *Indian J. Vet. Pathol.* 31: 122-125.

Rajagopal, R. 2007. Production and evaluation of vaccines employing *Pasteurella multocida* A: 1 grown under different growth conditions. M.V.Sc thesis submitted to Kerala Agricultural University, Thrissur. p.147

Rajiny, R., Seshagiri Rao, A., Dhanalakshmi, K. and Sarma, B. J. R. 1995. Studies on avian pasteurellosis in Andhra Pradesh. *Indian Vet. J.* 72: 115-118

Ramanath, K. R. and Gopal, T. 1993. A note on the isolation and characterization of *Pasteurella multocida* from ducks. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* 14: 32-33

Ramasastry, P. and Ramarao, M. 1989. Acute pasteurellosis in ducks- report of an outbreak and its control. *Poult. Adv.* 22: 43-44

Rameshkumar, P., Ramkumar., Kumar, A. A., Ravindran, R. and Paliwal, O. P. 2006. Studies on experimental pasteurellosis in rabbits inoculated with *Pasteurella multocida* serotype A: 3. *Indian J. Vet. Pathol.* 30: 32-35

Rao, V. S. R. 1990. Diseases of the chicken. *Poult. Adv.* 23: 55-57

Reddy, M. R. 1996. Fowl cholera: A threat to poultry industry. *Poult. Adv.* 29: 21-24

Reed, J. L., Muench, H. 1938. A simple method of estimating fifty per cent end points. *Am. J. Hyg.* 27: 493-497

Rhoades, K.R. and Rimler, R.B. 1990. Virulence and toxigenicity of capsular serotype D *Pasteurella multocida* strains isolated from avian hosts. *Avian Dis.*34: 384-388

Rhoades, K.R., Rimler, R.B. and Bagley, R. A. 1992. Fowl cholera epornitic: antigenic characterization and virulence of selected *Pasteurella multocida* isolates. *Avian Dis.*36: 84-87

Rose, D. M., Giri, S. N., Zinkl, J. G., Snipes, K. P., Davidson, J. N., McCapes, R. H. and Conzelman, G. M. 1988. Changes in circulating prostaglandins, cloacal temperature and leucocyte counts in turkeys inoculated with *P. multocida*. *Avian Dis.* 32: 204-208

Roy, P., Ramaswamy, V. and Maity, B. 1993. An outbreak of pasteurellosis in ducks. *J. Vet. Anim.* Sci. 24: 76-77

Roy, P., Edwin, P. G., Sakthivelan, S. M., Purushothaman, V. 2004. An outbreak of pasteurellosis among Japanese quails. *Indian J. Anim. Sci.* 74: 728-729

Roy, S. S. and Misra, S. K. 1989. Common duck diseases and their control. *Poult. Adv.* 22: 33-39

Sahoo, P. K., Reddy, M. R. and Chawak, M. M. 1994. Characteristic lesions of common diseases of poultry. *Poult. Adv.* 27: 21-30.

Sambyal, D. S., Soni, G. L., Sodhi, S. S. and Baxi, K.K.,1988. Characterization of *Pasteurella multocida* (serotype 1) from an outbreak of fowl cholera in ducks. *Indian J. Anim. Sci.* 58: 1059-1060

Samuel, M. D., Shadduck, D. J., Goldberg, D. R., Wilson, M. A., Joly, D. O. and Lehr, M. A. 2003. Characterization of *Pasteurella multocida* isolates from wetland ecosystems during 1996 to 1999. *J. Wildlife Dis.* 39: 798-807

Sarkozy, G., Semjen, G., Lakzay, P. and Horvath, E. 2002. Treatment of Experimentally Induced *Pasteurella multocida* Infections in Broilers and Turkeys: Comparative Studies of Different Oral Treatment Regimens *J. Vet. Med. B.* 49: 130–134.

Sheehan, D.C. and Hrapchak, B.B.1980. *Theory and Practice of Histotechnology.* Second edition. Mosby Company Ltd, London, p.481

Shilpa., Verma, P.C. and Minakshi. 2005. Different vaccines against local fowl cholera isolate. *Indian J. Anim. Sci.* 75: 199-202

Shilpa., Verma, P.C., Minakshi and Kamil, S. A. 2005. Clinico-haematological studies on *Pasteurella multocida* infection in layers. *Indian J. Anim. Sci.* 75: 422-424

Shivachandra, S. B., Kumar, A. A., Singh, S. D., Shivakumar, B. M. and Prasanna, K. 2005. Pathogenicity of *Pasteurella multocida* A: 1 in experimentally infected chickens and chicken embryo. *Indian J. Anim. Sci.* 75: 411-414

Shivachandra, S. B., Kumar, A. A., Joseph, S., Saxena, M. K. and Srivastava, S. K. 2006. Biochemical characterization of avian strains of *Pasteurella multocida* in India. *Indian J. Anim. Sci.* 76: 429-432

Snedecor, G.W. and Cochran, W.G. 1994. *Statistical methods*. 8th Edn. The Iowa State University Press, Ames, Iowa, USA. 564p

Songserm, T., Viriyarampa, A. S., Sae-Heng, N., Chamsingh, W., Bootdee, O. and Pathanasophon, P. 2003. *Pasteurella multocida*- associated sinusitis in Khaki Campbell ducks. *Avian Dis.* 47: 649-655

Srilatha, C., Ramadevi, V., Sujatha, K. and Nisar Ahamed, M. 2003. An outbreak of fowl cholera in Japaneese quails. *Indian Vet. J.* 80: 6-7

Takahashi, S., Sato, H., Yamada, T., Takenouchi, T., Sawada,T., Nakano, K. and Saito, H. (1996). Outbreaks of fowl cholera in muscovy ducks (*Cairina moschata*) on a farm in Aomori prefecture. *J. Vet. Med. Sci.* 58: 269-272

Thrall, M.A., Baker, D.C., Campbell, T.W., De Nicola, D., Fettman, M.J., Lassen, E.D., Rebar,A. and Weiser, G. 2004. *Veterinary Haematology and Clinical Chemistry*. Lippincott Williams and Wilkins, U.S.A. 3p

Toth, T. E., Pyle, R. H., Caceci, T., Siegel, P. B. and Ochs, D. 1988. Cellular defense of the avian respiratory system: influx and nonopsonic phagocytosis by respiratory phagocytes activated *by Pasteurella multocida. Infect. Immun.* 56: 1171-1179

Verma, P. C. and Mohanty, P. K., 1983. Infectious disease affecting ducks and their control. *Poult. Adv.* 16: 41-47

Wilkie, I. W., Grimes, S. E., O'Boyle, D. and Frost, A. J. 2000. The virulence and protective efficacy for chickens of *Pasteurella multocida* administered by different routes. *Vet. Microbiol.* 72: 57-68

Windingstad, R. M., Kerr, S. M., Duncan, R. M. and Brand, J. C. 1988. Characterization of avian cholera epizootic in wild birds in western Nebraska. *Avian Dis.* 32: 124-131

Wobeser, G. and Leighton, T. 1988. Avian cholera epizootic in wild ducks. *Can. Vet. J.* 29: 1015

PATHOLOGY OF EXPERIMENTAL PASTEURELLOSIS IN DUCKS

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

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2009

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ABSTRACT

The present study was undertaken to evaluate the pathological aspects of pasteurellosis in ducks following experimental inoculation by different routes – subcutaneous and intranasal. The haematobiochemical changes associated with the infection were also studied.

Body weight changes at weekly intervals, mortality pattern, haematological and biochemical parameters such as total erythrocyte count, total leucocyte count, haemoglobin concentration, differential leucocyte count, total protein, albumin and globulin values estimated at weekly intervals were the parameters used, to study the changes caused by the infection. Gross pathological changes and histopathological changes of heart, liver, lung, kidney, brain, intestine, spleen, caecal tonsils and bursa were also studied.

Statistically significant alteration was not observed in body weight. Mean Hb, TEC, PCV, albumin, heterophil and eosinophil percentage values were markedly increased after infection. Correspondingly, the lymphocyte and monocyte counts and the total protein values showed a significant decrease.

Acute onset of the disease with sudden mortality was noticed in subcutaneously inoculated birds. Peracute death without any symptoms and lesions was shown by two birds in this group. The peracute pattern of mortality seen in this group was not observed in intranasally inoculated group. But the severity of clinical signs and lesions were more in intranasally inoculated birds. After the first two peracute deaths, symptoms of acute toxaemia such as pyrexia, dullness, depression, inappetence, reluctancy to move, paralysis of limbs and neck and incordination were expressed by the birds before death.

The birds died from second day after inoculation onwards revealed an acute haemorrhagic septicaemic picture on all visceral organs. Haemorrhages on epicardium and scattered pin point necrotic foci in the liver, the prominent lesions

of fowl cholera, were clearly evident. These lesions were more pronounced in the birds inoculated intranasally with *P. multocida,* which died on 23rd, 24th and 25th day post- inoculation. The lung and brain lesions were more severe in these birds.

Reisolation of *P. multocida* was carried out from the liver, heart and spleen of all the dead birds except control and survived birds.

The present study proved that the route of entry of organism played a significant role in the progression of infection, onset of mortality, number of deaths and symptoms and lesions in fowl cholera outbreaks. The immune suppression due to lymphoid organ damage in fowl cholera make the birds more susceptible to other infections.