Sindh Univ. Res. Jour. (Sci. Ser.) Vol. 48 (3) 671-674 (2016)

SINDH UNIVERSITY RESEARCH JOURNAL (SCIENCE SERIES)

Hemato-biochemical and Pathological Studies on induced *Pastuerella Multocida* Infection in Rabbits

M. F. LEGHARI, Z. A. NIZAMANI, I. H. LEGHARI*T. M. SAMO, A. SETHAR**, F. SAMO

Department of Veterinary Pathology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando Jam.

Received 17th January 2016 and Revised 12rd August 2015

Abstract: The purpose of this work was to investigate hemato-biochemical and pathological changes of Pastuerella Multocida induced infection in rabbits. This study was performed on 12 adult healthy rabbits, assigned into two groups A & B which were challenged with pastuerella multocida intranasal dose 0.5ml/rabbit and 0.5 ml inj (insulin B.D syringes) sub cut doses according to body weights respectively. Results revealed that there was gradual rise in group A body temperatures and rapid rise in group B. The daily feed intake decreased gradually in group A and decreased rapidly in group B from D1 post-inoculation. There were significant decreases in the Hb count and significant increases in WBC, albumin, immature neutrophils, and mature neutrophils and monocytes counts on infected rabbits in comparison with healthy rabbits on D0. In infected rabbits, the concentrations of Globulin were significantly lower and the concentration of P was significantly higher than the healthy goats group. Thus Pasteurella multocida causes disturbances in blood factors and biochemical proteins, and leads to mild to severe injuries of the lung tissue.

Keywords: Pastuerella Multocida, blood, pathology, rabbit, histology

1. INTRODUCTION

Pasteurella multocida is a pathogenic organism and is one of the most common economically significant animal diseases occurring in both developed and developing countries (Kuhnert *et al* 2008). The *P. multocida* can cause disease in a wide range of animal species and is the causative agent of numerous, economically important diseases, that include avian fowl cholera, bovine hemorrhagic septicemia, enzoonotic pneumonia and swine atrophic rhinitis. The species *P. multocida* is subdivided into four subspecies that include the type strain, *multocida,* and three others, *gallicida*, *septica* and *tigris* (Capitini *et al*., 2002). *P. multocida* strains are classified into serogroups (A, B, D, E and F) based on capsule antigens and further classified into 16 serotypes (1–16) based primarily on lipo-polysaccharide antigens using the Heddleston scheme (Carter, 1955; Heddleston *et al.,* 1972). Laboratory rabbits colonized with *P. multocida* often develop clinical disease after being shipped to a research facility and rabbits serve as an excellent laboratory animal model for studying bovine pasteurellosis, as they are natural hosts to *P. multocida* infections, although with their own serotypes. Therefore understanding the pathogenesis of *P. multocida* infection and establishing standardized postmortem gross and histo-pathological findings will prove extremely useful.

The present study was conducted to evaluate the hemato-biochemical changes, along with gross lesions that develop during the course of experimental infection of rabbits with *P. multocida* Robert type-1 B: 2 infections. The work will help in establishing an economical laboratory animal based model for infection with *P. multocida* infection which will facilitate any future studies on many aspects of bovine pasteurellosis in a rabbit model.

2. MATERIALS AND METHODS

Rehydration *P. multocida* vaccine seed and its inoculation in mice

The master seed of *P. Multocida* Robert type-1 Carter type B: 2 (Past. Mult: Robert type Carter type B: 2) was cultured, thawed and rehydrated, and injected I/P with 0.25 ml of seed for experimental infection in mice. Twelve hours after inoculation, as mice died, blood was aseptically collected from the hearts of mice.

Culture on BHI agar plates and BHI Broth

Heart blood from infected mice was inoculated onto BHI Agar by streaking and incubated at 37°C for 24 hours. A colony of *P. multocida* was taken from cultured ager plate with a wire loop and inoculated into a test tube containing broth BHI media mixed with test tube shaker under the bio-safety cabinet.

Plate count or colony count for quantifying number of bacteria

The *P. multocida* in BHI broth cultured was thoroughly mixed with the help of test tube shaker before preparing 10-fold dilutions. 10^{-10} dilution was spread and incubated into different plates and then

++Correspondence Author: Imdad Hussain Leghari, : Email: imdadleghari@hotmail.com

*Department of Poultry Husbandry, Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University Tando Jam.

**Animal Husbandry wing of Livestock & Fisheries Department, Government of Sindh @ Hyderabad

plates were checked for growth of *P. multocida* colonies. Colony counter was used to count the number of colonies in each plate.

Inoculation of *P. multocida* into rabbits

The rabbits were inoculated I/N and S/C with 01 ml of inoculum having 10^7 colony forming units (CFUs)/ ml and with normal saline in control group.

Clinical findings

Daily rectal temperature was taken from animals in all groups. Moreover, daily feed and water intake were also measured. All animals were weighed at the start of experiment and also either at the time of death or termination of experiment.

Blood collection

2 ml blood was collected from each Rabbit form the ear vein and was transferred to vacuum tube (B.D Vacutainer K2 EDTA 3.6mg) and then stored at +4 °C until processed.

Blood analysis

Hematology analysis was performed on Automatic Hematology Analyzer (Mindray BC 2800 Vet) using Kit according to manufacturer instructions. The separated plasma was analyzed for total proteins along with its albumin and globulin concentrations.

Postmortem findings

All rabbits dying at the day 10 were necropsied and lesions were noted from affected organs. Mortality was also recorded for examination of gross pathological lesion and for identification of bacteria postmortem was conducted.

3. RESULTS

Hematology

Our results for RBC count showed significant decrease in the I/N and I/S treated group **(Fig. 2).** Moreover, TLC increased significantly in treated groups in the gradual fashion **(Figure 3).** Contrarily, DLC showed significant gradual drop with the passage of time in Lymphocytic count both in case of I/N and I/Sub route inoculation **(Fig.4).** However, neutrophil count increased in timely fashion after *P. multocida* inoculation in the both treated group **(Fig. 5).** Similarly, Monocytic count increased from the start of the infection but it decreased in both groups at the D-9 gradually in both groups **(Fig. 6).** Besides, both the eosinophil count and basophil count showed slight decrease at the start of the infection.

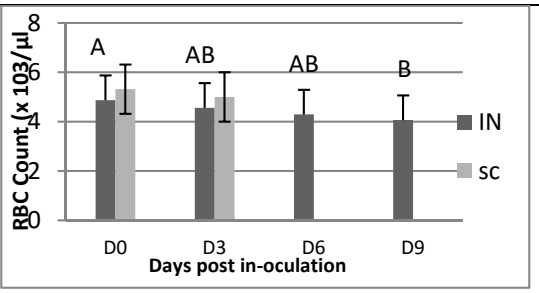


Figure 1

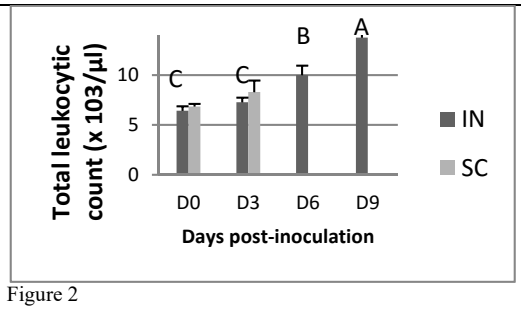


Figure 2

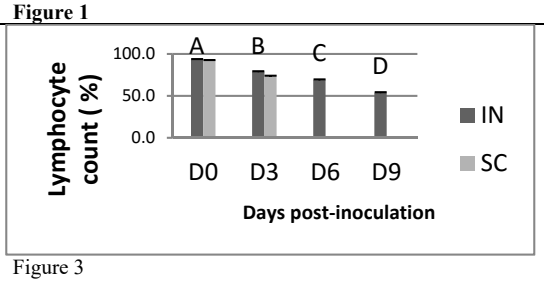


Figure 3

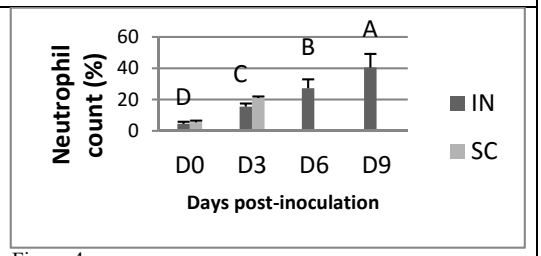


Figure 4

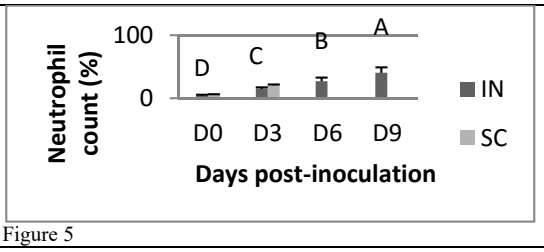


Figure 5

Fig. 1: RBC count in rabbits intra-nasally and sub-cutaneously infected with *P. multocida.*
Fig. 2: Total Leukocyte count in rabbits intra-nasally and sub-cutaneously infected with *P. multocida.*
Fig. 3: The lymphocyte count in rabbit intra-nasally and sub-cutaneously infected with *P. multocida*
Fig.4: The neutrophil count in rabbits intra-nasally and sub-cutaneously infected with *P. multocida.*
Fig. 5: The hemoglobin concentration in rabbits intra-nasally and sub-cutaneously infected with *P. multocida.*

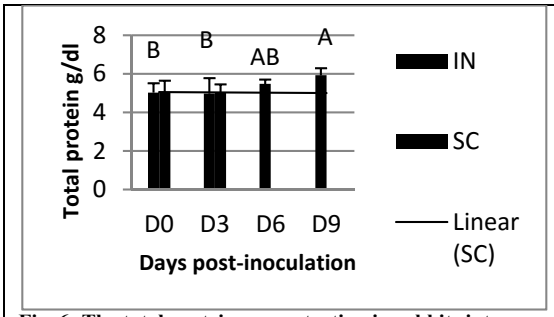


Fig. 6: The total protein concentration in rabbits intra-nasally and sub-cutaneously infected with *P. multocida*.

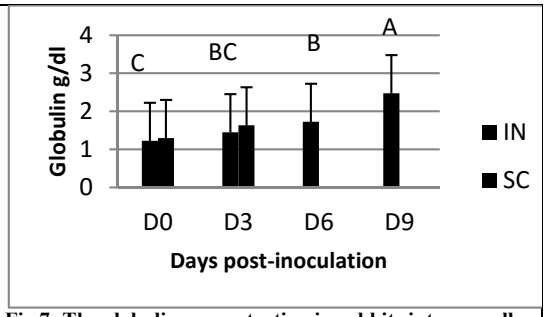


Fig.7: The globulin concentration in rabbits intra-nasally and sub-cutaneously infected with *P. multocida.*

Blood chemistry

The result showed there was timely decline in the Hb conc. in all the groups post-inoculation, which shows decrease in the Hb conc. However, there was no any significant difference in the average total protein conc. among the groups and in albumin conc.. However, there was gradual rise in the globulin concentration (g/dl) in rabbits of group A and B.

Clinical findings

The clinical findings in rabbits inoculated with *P. multocida* by different routes showed marked difference .Daily rectal temperature recorded in group A and B showed gradual rise in the body temperature taken on daily basis .However, their was no significant difference between two groups.

Necropsy findings

Lungs were the most severely and commonly affected organs. The prominent lesion observed in intranasal infected rabbits after lifting the rib cage was accumulation of fibrin .The pathological scoring of affected organs showed congestion, consolidation with presence of fibrino purulent exudates on lung exterior as well as in the thoracic cavity. Mean pleurisy scores recorded in rabbits in group A were 2.75 and 1 in group B ,while their average lung consolidation was 68.75 % in group A and 50% in group , respectively. Hearts of both groups A and B were found congested. While hearts in the intranasally inoculated rabbits was frequently found to have fibro purulent exudates probably originating from inflamed left lung concomitant pleurisy. Livers in both groups were inflamed, hardened, congested and having milliary multifocal petechial hemorrhages

4. DISCUSSION

Rabbits are excellent laboratory animal models for bovine pasteurellosis as rabbits have their own strain of *P. multocida* (Somya *et al*., 2011). In the present study, pasturella multocida strain commonly found in cattle's and buffaloes when inoculated in rabbits showed high rise in temperature but no mortality in first three days which is in congruity with Kluger and Vaughn, (1978). Our study also showed drop in the RBC after bacterial inoculation which could be partly due to toxins released by *P. multocida* and partly due to reduced feed intake by the infected animals. In the present study, post infection T. L. C. increase was observed and the similar findings have also been reported by the Richardson *et al* (1997) in mice. In addition to this, Neutrophilia was seen in rabbits infected with *P. multocida* which is congruity with Ashraf, *et al* (2014). Moreover, our study also showed Monocytosis throughout the course of infection in both cases, which is contrarily in mice Faez *et al*., 2013. Similarly our findings were not also in congruity with Sirous *et al.* 2011 and Katoch et al. 2015 in goats who reported decrease in monocyte count.

Moreover, over results showed statistically significant difference in the total proteins and albumin to globulin ratio in the serum after infection in all the groups. A gradual decrease in total proteins (%) was recorded in rabbits of both groups .The reduction in total proteins was due to a reduction in albumin component to a major part and a slighter increase in globulins. The significant rise the globulins after infection in rabbit may be due to the stimulation of the immune system against infection. In addition to this our study revealed more severe lesions of the heart accompanied with fibrino purulent exudates by intranasal route than sucut route where signs were almost negligible. The similar observations were also reported by Debey *et al.,* (1992) in rabbits. This Inflammation and presence of fibrino-purulent exudates on pericardium may be due to the extension of *P. multocida* infection from infected lung as lungs are the prominent site of bacterial multiplication. Moreover, both the intranasal and sub cut routes showed acute pasteurellosis by severe congestion in the lungs and mucopurulent exudates from the nostrils respectively in each case.

Conclusively it was observed that an induced Pastuerella Multocida infection via intranasal and subcut route brings disturbances in blood factors and biochemical proteins and leading to the severe injuries of the lung tissues.

REFERENCE:

Ashraf, A., S Mahboob, K. Al-Ghanim, and T. H. M. Shah, (2014). Immunogenic Activity of Lip polysaccharides From Pasteurella Multocida In Rabbits. J Anim. Plant Sci. 24(6), 1780-1785.

Capitini C. M, I. A Herrero, R Patel, M. B Ishitani and T. G. Boyce (2002). Wound infection with Neisseria weaveri and a novel subspecies of *P. multocida* in a child who sustained a tiger bite. Clin. Infect. Dis. 34: e74–e76.

Carter G. R (1955) Studies on *Pasteurella multocida*. A haemaggtination test for the identification of serological types. Am. J. Vet. Res. 16: 481–484.

DeBey, B. M., R. C. Cutlip, K. A Brogden, and G. H. Frank (1992) A comparison of the intratracheal, intravenous and intratonsillar routes of inoculation of goats with *Pasteurella haemolytica*. Vet. Res. Comm. 16 (4), 247-251.

Faez Firdaus, J. A., A. Lawan, Y. O. Abdinasir and Z. Zunita, (2013). Biochemical and Hematological Alterations in mice inoculated with outer membrane protein, Lipopolysacchrides and whole cells of *P. multocida* TYPE B: 2. Amer. J. An. Vet. Sc. 8 (3): 152-158.

Heddleston, K. L, J. E. Gallagher and P. A. Rebers. (1972) Fowl cholera: gel diffusion precipitin test for serotyping *P. multocida* from avian species. Avian. Dis. 16: 925–936

Kluger, M. J., and L. K. Vaughn.,(1978). Fever and Survival in Rabbits infected With *P. multocida.* J. Pay. Bio. 282: 243-251.

Katoch, S., L. Verma, M., Sharma, R. K Asrani, S. Kumar, R., Chahota, and S. Verma, (2015). Experimental Study of the Pathogenicity of Pasteurella multocida Capsular Type B in Rabbits. J. of compa. Path.,153(2), 160-166.

Kuhnert P; and H Christensen (editors) (2008). Pasteurellaceae: Biology, Genomics and Molecular Aspects. *Caister Academic* Press. ISBN 978-1-904455-34-9

Richardson, M., Fletch, A. Delaney, K.. DeReske, M. Wilcox, L. H. K. Rathbone, R. Sirous, R. M.. Mokhber, and A., Gholam, (2011). *P. multocida* pneumonic infection in goat: Hematological, biochemical, clinical and pathological studies. J. Smal. Rum. Res. 100: 189-194.

Somya A., H. Nassar, A. Mohamed, S. Soad M. Nasr and K. M. Mahran. (2011). Immunostimulant Effect of Egyptian Propolis in Rabbits. Sci. Word. J. 9: 1100-901516.