CLINICOHEMATOLOGICAL STUDIES ON EXPERIMENTAL PNEUMONIA DUE TO **MYCOPLASMA MYCOIDES** SSP **MYCOIDES** (LC) AND **PASTEURELLA HEMOLYTICA** INFECTION IN LAMBS*

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**ABSTRACT**

Experimental pneumonia in lambs could be reproduced by *M. mycoides* ssp *mycoides* (LC) and *P. hemolytica* concurrent infection. High rise in temperature, nasal and ocular discharge, dullness, depression, coughing, sneezing, laboured breathing, arching of back, exhaustion, loss of muscular and eye reflexes, submandibular edema and blood tinged nasal discharge in terminal stages of disease were the manifestation. Hb, PCV and TEC values were in normal range. A significant neutrophilia along with lymphocytopenia upto 10 DPI was also observed.

**INTRODUCTION**

Throughout the world pneumonia still remains an important problem causing huge economic loss to the livestock industry especially sheep. Progress in understanding its pathogenesis has been slow because of its complex etiology and varied epidemiology (Alley, 1991). The major impediment in the development of sheep husbandry is the loss of lamb crop due to pneumonia. Though the pneumonia has a varied etiology, Mycoplasmas causing contagious caprine pleuropneumonia and pneumonia are very common in sheep. Among various Mycoplasmas spp. *M. mycoides* ssp *mycoides* has been implicated as an important organism in causation of pneumonia in sheep (Sreeramulu and Krishnaswamy, 1987; Gupta et al., 1988). The clinicohematological studies in lambs as a result of *M. mycoides* ssp *mycoides* infection have been reported earlier (Batra et al., 2002). Besides *P. hemolytica* being the normal inhabitant of nasopharyngeal region Mycoplasma predisposes lambs and adult sheep to infection by *Pasteurella hemolytica* (Jones, 1983). Since *M. mycoides* ssp *mycoides* (LC) and *P. hemolytica* together have been frequently isolated from pneumonic lungs of sheep and goats in Haryana (Anonymous, 1990), therefore in the present study an attempt has been made to elucidate clinicohematological picture of pneumonia caused by *M. mycoides* ssp *mycoides* (LC) with concurrent infection of *P. hemolytica* and also to compare it with the respiratory infection caused by *P. hemolytica* alone.

**MATERIAL AND METHODS**

*M. mycoides* ssp *mycoides* (LC) and *P. hemolytica* isolated from pneumonic lungs of lambs during a field out break were employed for present studies.

Fifteen lambs of either sex (4-6 months old, 7 male and 8 female) found negative for Mycoplasma by nasal swab isolation as well as serologically using growth inhibition test (Razin and Tully, 1983) procured from Central Sheep Breeding Farm, Hisar were divided randomly into 3 groups comprising 5 lambs each and kept in different enclosures. The lambs were also treated with oxytetracycline (Wockhardt Veterinary Limited) @ 5 mg/kg b. wt. b.i.d. for 3 days prior to experimentation followed by 7 days withdrawal period. The animals maintained in utmost hygienic conditions were provided gram husk, green fodder and clean

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drinking water ad-libitum.

Group I was given 12 ml PPLO broth containing $1.2 \times 10^9$ cfu/ml of 50 hour log phase culture of *M. mycoides ssp mycoides* (LC) by combined respiratory route (8 ml intratracheally and 4 ml intranasally). Two days later they were administered 8 ml nutrient broth containing $5 \times 10^6$ cfu/ml of 24 hour log phase culture of *P. hemolytica* (4 ml intratracheally and 4 ml intranasally). Group II animals were given 8 ml nutrient broth containing $5 \times 10^6$ cfu/ml of 24 hour log phase culture of *P. hemolytica* (4 ml intratracheally and 4 ml intranasally). Group III animals were inoculated sterile broths by combined respiratory route as described earlier.

All animals were examined clinically daily throughout the period of experiment for 28 days. Any untoward clinical signs, general health condition and mortality were duly recorded. Blood samples with EDTA were drawn from Jugular vein of 1-2 lambs at 0, 3, 7, 10, 14, 18, 21 and 28 days post infection (DPI). Fresh blood smears were drawn for differential leukocytic count. Hematological parameters including Hemoglobin (Hb), Packed cell volume (PCV), Total erythrocyte count (TEC), Total leukocytic count (TLC), differential leukocyte count (DLC) were evaluated following standard procedures (Oser, 1965; Schalm et al., 1975).

RESULTS AND DISCUSSION

Group I

Clinical signs appeared 1 DPI onwards and a high rise in temperature (102.36°F to 107°F) resulted. Animals exhibited dullness, depression, anorexia and serous to mucus nasal and ocular discharges. Lambs also manifested laboured breathing, sneezing and arching of back. Animals were reluctant to move. Later animals were completely exhausted, lying down with poor eye and muscular reflexes few hours prior to death. Submandibular edema, blood tinged nasal discharge in terminal stages of disease were observed. Similar signs have been noticed by Gilmour *et al.* (1982) and Jones *et al.* (1982) in sheep in pneumonia due to *M. ovipneumoniae* and *P. hemolytica* and also by Gharagozlou *et al.* (1992) in pneumonia in sheep due to *M. arginini* and *P. multocida* infection. The severity of signs noticed presently as compared to *M. mycoides ssp mycoides* (LC) alone (Batra *et al.*, 2002) may be ascribed to stress caused by dual infection. Edematous submandibular swelling might be the result of increased capillary permeability associated with toxic effect of *P. hemolytica* toxins and blood tinged nasal discharge could be the ultimate effect of \( \beta \) hemolysin (Smith and Phillips, 1990). Sudden mortality in short span i.e. 3 and 4 DPI might be due to stress of combined infections.

There was no significant difference in Hb, PCV, TEC values at different DPIs and they were within normal range (Fig. 1, 2 and 3). Batra *et al.* (2002) reported significant decline in these values in *M. mycoides ssp mycoides* (LC) alone infection in lambs. It seems that little or no change in Hb, PCV and TEC values in the present study might be the balancing effect of endotoxins of *M. mycoides ssp mycoides* (LC) as well as enhanced febrile reaction induced by *P. hemolytica*. TLC values showed significant increase from 3 DPI onwards (Fig. 4) and the values were maximum at 10 DPI. Afterwards the values showed a declining trend and reverted back to normal range.

DLC revealed that there was also significant increase in relative percentage of neutrophils with consequent decrease in number of lymphocytes starting from 3 DPI upto 10 DPI (Fig. 5 and 6). Eosinophilic, basophilic and monocytic counts were in normal range. It is well known that neutrophilia results due to systemic response to injury. A significantly higher neutrophilic count as compared to *M. mycoides ssp mycoides* (LC)
Fig. 1. Mean hemoglobin content of different groups of lambs

Fig. 2. Mean packed cell volume of different groups of lambs
Fig. 3. Mean total erythrocyte count of different groups of lambs

Fig. 4. Mean total leukocyte count of different groups of lambs
Fig. 5. Mean neutrophil count of different groups of lambs

Fig. 6. Mean lymphocyte count of different groups of lambs
infection alone might be due to stress of dual infections.

Group II

No mortality was observed and slight febrile reaction (102.24°F to 104°F), scanty serous nasal discharge were the only clinical manifestations. Generally pasteurellosis results due to stress or by other concurrent infections (Jubb et al., 1993; Radostits et al., 1994). Further, immune status of experimental lambs employed in production of pasteurellosis in present studies cannot be overlooked as serum antibody titre at 0 DPI against *P. hemolytica* was 1:640.

Hemogram (Hb, PCV and TCV) showed an significantly increasing trend from 3 DPI to 7 DPI. Rise in Hb, PCV and TEC might be correlated with dehydration and hemoconcentration characteristic of pasteurellosis as suggested by Kamil et al. (1994).

In this group TLC values revealed a significant increasing trend from 3 DPI onwards upto 7 DPI and then reverted towards normal range. DLC depicted a significant increase in neutrophils associated with decline in lymphocytes upto 7 DPI in group II alone might indicate an early recovery phase in pasteurellosis. A similar trend of neutrophilia associated with lymphocytopenia (upto 8 DPI) was observed by Kamil et al. (1994) in *P. hemolytica* infection. Eosinophils, Basophils and monocytes maintained their normal range values throughout the period of experimentation.

Group III

Neither the clinical signs nor the mortality was observed in this group. Blood values remained within normal range throughout the period of experimentation.

The disease resulted in severe manifestation in *M. mycoides* ssp *mycoides* (LC) and *P. hemolytica* dual infection group. It can be concluded from the present studies that *M. mycoides* ssp. *mycoides* (LC) predisposes the animals to pasteurellosis.

REFERENCES