

Immune Response in Lambs Naturally Infected With *Mycoplasma* Species

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Summary

During the past several years, an ovine coughing syndrome characterized by paroxysmal cough leading to rectal prolapses has been observed in Iowa and neighboring states. Preliminary studies conducted by Kaerberle and Eness (1) several years ago indicated the presence of relatively high levels of *M. ovipneumoniae* (MO) antibody in lambs from affected flocks. In the present study, serum samples obtained from six flocks around the state of Iowa, at various stages of the clinical disease, were compared by ELISA for antibody to MO and *M. arginini* (MA). Results indicated low antibody levels to MO in flocks sampled at the early stages of infection whereas increased levels of antibody were evident in lambs from flocks that had apparently recovered from the disease. On the other hand, antibody levels to MA were more likely to increase earlier in the disease process. Our results suggest that the chronic nature of this disease may result from the failure of the immune system to produce antibodies that are protective against MO infection. At such a time that appreciable levels of specific antibodies appear in the serum (several weeks following infection) lambs seem to recover from the clinical disease. In addition, this lack of circulating antibody levels against MO would not be inconsistent with a predominant IgE response during early stages of the clinical disease as we have suggested in another entry in this issue of Sheep Research Reports.

Introduction

M. ovipneumoniae is one of the most commonly isolated microorganisms from sheep with respiratory disease worldwide. Recently in our laboratory, this microorganism as well as MA have been routinely recovered from young lambs with a respiratory disease that we have termed the "coughing syndrome" since a severe paroxysmal cough leading to rectal prolapses is the primary clinical response. This syndrome is widespread in Iowa and neighboring

states with morbidity and severity variable among flocks. The disease is chronic and persists for several weeks in most affected lambs.

The extended persistence of the MO organism in the respiratory tract of lambs and the chronic nature of the disease may be due to failure of the immune system to develop protective immunity. Immunosuppressive effects for several mycoplasma species associated with long term colonization of the host respiratory tract have been reported (2). Reasons for that are not known but probably strain variation, expression of a polysaccharide capsule, mycoplasma-host cell interaction, or immune deviation may be involved. The objective of this experiment was to measure the levels of Ab to MO and MA antigens in the serum of naturally-infected lambs to better understand the nature of the immune response against these organisms.

Materials and Methods

Lambs in six flocks around the State of Iowa, in which outbreaks of clinical respiratory disease occurred, were bled between 1990 and 1995. These lambs were of mixed breeding. In some flocks, lambs were bled during the early phase of the disease process, when they were about 10 to 12 weeks of age, while in others this was done in lambs that either were in late phase of the disease process or had apparently recovered from the clinical disease (approximately 20 weeks of age) (see Tables 1 and 2). Sera were harvested within 24 hours of bleeding and stored at -20°C until tested.

Serum was diluted at 1:800 and tested for presence of antibodies to MO and MA by ELISA using crude capsular material of MO and SDS-treated MA organisms as antigens. The ELISA was performed using standard methods and optical density (OD) values were measured with an automated microplate reader at 405 nm. Negative and positive control sera were included in each plate. The mean OD values for each test serum (S) was divided by the mean OD values of three positive control sera (P), and results were multiplied by 100 and referred to as S/P ratios. Values in excess of three standard deviations from the mean S/P ratios of 20 sera from lambs free of mycoplasmas at the time of bleeding and with no history of clinical respiratory disease were considered as positive.

Results and Discussion

The presence of both MO and MA organisms in these flocks was indicated by the presence of serum antibodies and isolation of the agents from nasal swabs. The results of serological testing of lambs

from the six flocks are provided in Tables 1 and 2. Clinical disease was not evident in two flocks (HD and MY) at the time of bleeding while flocks KP and SN were at mid-stage of the disease and only a small percentage of lambs were still coughing in flocks HR and MR. While there was extensive variability in titers among lambs in each flock, interesting trends were noted. Appreciable levels of antibodies to MA were present in lambs without clinical disease. High levels of antibody to MA were present in lambs at the mid-stage of the disease process. Low levels of antibody to MO were present in lambs in the unaffected flocks and there was only a moderate rise during the first several weeks of clinical disease. However, lambs had developed high levels of

antibody to MO late in the disease process when most lambs had apparently recovered.

Results of this experimentation indicate that lambs readily produce antibodies in response to MA but the response to MO is delayed. This slow development of circulating antibodies to MO has recently been confirmed by sequential bleeding of lambs during the course of the disease. There is an apparent failure to generate an immune response to MO that will eliminate the organisms from the respiratory tract until late in the clinical disease. However, non-protective immune responses do develop (immediate hypersensitivity, ciliary autoantibodies) indicating that infection with the microorganism has manipulative effects upon the respiratory immune system.

Table 1. Antibody levels to MO in lambs from flocks sampled at different disease stages.

Flock	# Tested	Mean Titer(\pm SD)	Range of Titer	Disease Stage
HD	12	11.573 \pm 4.715	5.508-20.469	No Disease
MY	12	18.296 \pm 10.181	4.946-38.910	No Disease
KP	20	25.510 \pm 13.752	6.453-57.342	Middle
SN	8	26.777 \pm 12.456	13.433-51.102	Middle
HR	15	56.686 \pm 16.139	29.562-90.069	Late
MR	10	50.874 \pm 21.556	33.696-107.325	Late

Table 2. Antibody levels to MA in lambs from flocks sampled at different disease stages.

Flock	# Tested	Mean Titer(\pm SD)	Range of Titer	Disease Stage
HD	12	26.100 \pm 3.462	22.050-32.264	No Disease
MY	12	32.299 \pm 12.521	11.153-50.379	No Disease
KP	20	53.529 \pm 27.910	14.731-113.342	Middle
SN	8	40.813 \pm 13.470	18.204-62.611	Middle
HR	15	44.952 \pm 24.468	20.109-96.468	Late
MR	10	35.786 \pm 17.518	12.720-64.292	Late

Selected References

1. Kaeberle, M.L. and Eness, P. 1990. Beef-Sheep Research Report. Iowa State University, Ames, IA. pp. 44-45.
2. Howard, C.J. et al., 1987. Veterinary Immunology and Immunopathology, 17:401-412.