

# Type I Hypersensitivity in Lambs With Coughing Syndrome

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### Summary

**Lambs from two flocks with a chronic respiratory disease characterized by paroxysmal cough and rectal prolapses were tested for their skin reactivity to *Mycoplasma ovipneumoniae* (MO) and *M. arginini* (MA) antigens (Ags). There was a marked, immediate skin reaction to intradermal injection of MO Ag in many of the tested lambs. Some of these positive lambs also reacted to MA Ag. Phosphate buffered saline (PBS) used as a negative control gave no skin reaction in any of the tested animals. In addition, simultaneous serological tests revealed low antibody levels against MO and MA. However, both agents could be routinely isolated from nasal swabs of the affected lambs. There is reason to suspect that an immediate type hypersensitivity to MO Ag and perhaps to other allergens that develops in association with the mycoplasmal infection contributes to this coughing syndrome.**

### Introduction

*M. ovipneumoniae* has been routinely recovered from the respiratory tract of sheep with respiratory disease worldwide. Recently, in our laboratory, Khan (1993) also consistently isolated both MO and MA from lambs with a coughing syndrome. This respiratory disease is a major problem in Iowa and neighboring states due to widespread occurrence in young lambs predisposing to rectal prolapses and secondary bacterial pneumonia, as well as reducing the rate of gain.

The mechanisms of pathogenesis of MO infection in sheep still remain unclear. However, we believe that an immediate type hypersensitivity to the organism contributes to the clinical syndrome. This was based on reports of temporary abatement of clinical signs in lambs treated with antihistamines (Kaerberle, personal communication) and also demonstration of respiratory allergy in association with mycoplasmal infection in people (Yano et al., 1994). Production of IgE antibodies specific for the allergen during the infection is responsible for the allergic reactions involving mast cells or basophils. Diagnosis of an allergy is usually based upon detection of specific IgE antibodies or immediate skin reactions following intradermal injection of the allergen.

Since an antibody specific for sheep IgE is not available the only suitable means of allergy diagnosis in sheep is skin testing. This is a classical test that has been widely used for indirect demonstration of IgE antibodies specific for allergens. Our objective was to investigate the immediate skin reactivity of lambs with coughing syndrome to MO and MA Ags as a demonstration of allergic respiratory disease in these animals.

### Materials and Methods

Coughing lambs in the Iowa State University McNay sheep research farm or purchased from the same flock (Table 1) and housed in our facilities were utilized for this experimentation. These lambs were approximately 10 to 12 weeks of age and of mixed breeding. As Ags., cultures of MO and MA organisms were washed, sonicated and diluted to 5 ug protein/ml. Lambs were injected intradermally on the inside of the thigh with 0.1 ml. of each Ag. preparation and PBS as a control preparation. To improve the observation of the skin reaction, some animals were injected intravenously with 5 ml. of a suspension of 0.5% Evans blue just before testing. The sites were observed for at least 30 minutes for development of blue stained wheals indicative of positive skin reactions. In lambs which were not injected with Evans blue, development of a wheal and flare response (swelling, redness) was considered as a positive skin reaction. Reactions were graded on the basis of size as a moderate

reaction of 5-10 mm. diameter (1+) and a strong reaction of 10-25 mm. diameter (2+).

Also, sera collected from the lambs were tested with an ELISA using sodium dodecyl sulfate (SDS)-treated MO and MA organisms and capsular material of MO as Ags.

### Results

The results of the skin tests on animals originating at McNay farm are shown in Table 1. There was an immediate skin reaction in about 56% (19/34) of the lambs injected with MO Ag, whereas only 29% (10/34) reacted to MA Ag. All the lambs that were positive to MA Ag. reacted also to MO Ag. This response usually began within 5 minutes after injection and reached its maximum within 30 minutes. Although some of the wheals lasted for more than 6 hours, no induration at the site of injection was observed at 48 hours post-injection indicating absence of delayed hypersensitivity.

ELISA tests conducted on serum of animals in group B demonstrated low circulating antibody levels in many of the tested animals. However, this lack of response was more evident against MO capsular Ag. (average mean OD values = 0.385, range 0.152 to 0.785) and MO SDS treated Ag (average mean OD values = 0.385, range 0.135 to 0.863) than MA Ag. (average mean OD values = 0.456, range 0.054 to 1.026).

Sheep from a second flock of purebred Suffolks were also skin-tested. Strong reactions to MO AG were observed in some coughing lambs of approximately 10 to 12 weeks of age and also in some yearling ewes that had apparently recovered from the clinical disease.

### Discussion

Results of this study indicate that an immediate type of hypersensitivity specific for MO can develop during infection of lambs with this microorganism. The reaction is apparently specific, since not all the lambs injected showed positive reaction, the

resultant wheals in various lambs were not always equal in size and PBS injection induced no reaction. Whether or not the responses to MA are specific is unclear since lambs responding to Ag. from this microorganism also responded to MO Ag. Therefore, the possibility of cross reactivity exists.

Skin testing has been routinely used to demonstrate allergic responses of humans and animals to allergens from microorganisms (Yano, et al., 1994; Chen et al., 1990; Gershwin and Craig, 1984). The characteristics of the response observed in this study are quite similar to those previously described in sheep hypersensitivity induced by *Ascaris suum* extract (Chen et al., 1990) and in the human allergic reaction to *M. pneumoniae* (Yano et al., 1994), including formation of raised blebs immediately after injection with no late reaction.

The relatively low levels of IgG specific antibodies to both MO and MA during the early stages of the clinical disease, as seen here, is likely an indication of a predominant IgE response. In such a situation, this IgE may play a role in the development of allergic airway reactions in these lambs involving release of histamine and serotonin mainly from mast cells and basophils as has been suggested in asthmatic people infected with *M. pneumoniae* (Yano et al., 1994). Unfortunately however, due to lack of anti-sheep IgE we cannot at the moment measure the level of IgE antibodies in these sheep.

### Selected References

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**Table 1. Response of lambs to intradermal administration of MO and MA antigens.**

Group of Lambs	# Tested	# Reacting to MO		# Reacting to MA		# Reacting to PBS
		2+	1+	2+	1+	
A	19	5	7	1	5	0
B	15	2	5	1	3	0