

The pathology of *Oestrus ovis* and an investigation on the use of a skin hypersensitivity test for the diagnosis of sheep oestrosis

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Abstract. Studies on *Oestrus ovis* infection in a sheep flock in Romania showed that 50% of the sheep exhibited upper respiratory symptoms during winter and early spring. The second larval stage was the most prevalent on post-mortem examination. Experimental intra-dermal inoculation of *O. ovis* larval extracts induced an edematous skin reaction in sheep, suggesting the possibility of using skin hypersensitivity tests for the early diagnosis of prepatent infection.

Keywords: *Oestrus ovis*; hypersensitivity test; diagnosis.

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Introduction

Myiasis due to *Oestrus ovis* is a major economic disease of sheep in Romania (Cozma et al., 1999; Cosoroaba, 2000; Șuteu and Cozma, 2007) and in other European countries with temperate climate (Jacquiet and Dorchies, 2002) but also in the Mediterranean countries (Papadopoulos et al., 2010). The diagnosis of sheep oestrosis is difficult during the prepatent stages of the life cycle of the parasite. There has been one report on the use of serological and skin hypersensitivity assays for the early diagnosis of this disease (Dorchies and Alzieu, 1997). This paper presents the studies on the development of a single intra-dermal hypersensitivity assay for the early diagnosis of *O. ovis* infection.

Materials and methods

Experimental animals

A total of 280 Transylvanian Merino sheep (1-5 years old) were used in this study. The sheep were subjected to a clinical examination periodically, during the period December-May to determine the presence or absence of respiratory symptoms.

Preparation of antigen

A total of nine sheep from the above mentioned population were euthanized in December, March and April and their nasal cavities and sinuses examined. *O. ovis* larvae were collected and identified according to their development

stage. Whole stage II larvae were homogenized in physiological saline and the total protein was measured using the biuret method.

Skin hypersensitivity tests

In April, 20 sheep were divided into four groups, each of five sheep. Sheep in three of the groups received intra-dermal inoculations of homogenized *O. ovis* larvae (100 µl/inoculum), with various protein content (table 1). The fourth group was injected with physiological saline.

Table 1. Protein concentration of *Oestrus ovis* extract injected into sheep

Group	Number of animals	Total protein in inoculum (µg/ml)
1	5	1.58
2	5	3.15
3	5	6.32
4	5	0

The skin thickness at each inoculation site was measured at 30 and 60 minutes and at 24 hours post-inoculation (p.i.). Immediately after this experiment all 20 sheep were treated with an ivermectin (0.2 mg/kg, s.c.) in single dose. Three weeks after treatment the sheep were

subjected to a second skin hypersensitivity test.

Results

Clinical symptoms of mild upper respiratory tract infection were observed in 50% of the 280 sheep examined between December and May. The sheep had a mucopurulent nasal discharge with repeated sneezing. Otherwise, the general health status of the sheep appeared unaffected as they maintained normal appetite and behavior. *Oestrus ovis* larvae were found in 66.6% of the sheep subjected to a post-mortem examination. A total of 67 larvae were collected and identified from the 9 sheep examined by necropsy (table 2). The majority of the larvae (86.6%) were in stage II. The larvae induced a moderate to severe catarrhal sinusitis and rhinitis, which in some animals became hemorrhagic and purulent.

Intra-dermal inoculation of *O. ovis* extract produced an edematous reaction in the skin. The size of the reaction increased as the protein concentration of the inoculum increased (table 3). The oedematous reaction was more intense 60 minutes p.i. The skin reaction however, was significantly reduced 24 hours p.i. Ivermectin treatment resulted in a lower skin reaction after inoculation with *O. ovis* extract.

Table 2. Number of *Oestrus ovis* larvae found and their stage of development in sheep

Month	Number of sheep examined	Number of positive sheep	Number of identified larvae			
			Total	L ₁	L ₂	L ₃
December	5	2	8	2	6	-
March	2	2	47	0	45	2
April	2	2	12	0	7	5
Total	9	6	67	2	58	7

Table 3. Skin reaction thickness (mm) in sheep injected with *Oestrus ovis* extract

Group	Date	Time post inoculation			
		0	30'	60'	24 h
Group 1	Pre ivermectin treatment	2.24 ± 0.35	4.06 ± 0.70	5.10 ± 0.36	3.38 ± 0.94
	Post ivermectin treatment	2.26 ± 0.37	3.82 ± 0.78	4.58 ± 0.76	3.02 ± 0.21
Group 2	Pre ivermectin treatment	2.30 ± 0.22	4.50 ± 0.47	5.66 ± 0.43	3.28 ± 0.52
	Post ivermectin treatment	2.08 ± 0.46	3.86 ± 0.43	5.62 ± 0.58	3.00 ± 0.74
Group 3	Pre ivermectin treatment	2.62 ± 0.31	5.64 ± 0.75	7.40 ± 0.99	5.10 ± 0.97
	Post ivermectin treatment	2.36 ± 0.08	5.16 ± 0.77	6.28 ± 0.90	4.02 ± 1.23
Group 4	Pre ivermectin treatment	1.78 ± 0.58	2.08 ± 0.58	2.22 ± 0.45	1.82 ± 0.56
	Post ivermectin treatment	1.82 ± 0.56	2.10 ± 0.46	2.22 ± 0.45	1.82 ± 0.74

Discussions

Since *O. ovis* larvae were found in all sheep subjected to post-mortem examination, it is reasonable to assume that the sheep used for the skin hypersensitivity experiment had also been infested as they came from the same population. Thus, the results reported here suggest that skin hypersensitivity tests may be useful in the early diagnosis of sheep oestrosis. However, to date there is no standardized procedure for interpreting skin reactions (Şuteu, 1998), and further work is required before this method is acceptable as a diagnostic technique. Nevertheless, these studies demonstrated that low-grade upper respiratory symptoms are common in sheep infected with *O. ovis* in Romania. and they suggest that skin hypersensitivity may be useful in the early diagnosis of *O. ovis*.

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