Chronobiology of Oestrus ovis (Diptera: Oestridae) in Sardinia, Italy: Guidelines to Chemoprophylaxis

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ABSTRACT Oestrus ovis (Linne 1761) larvae are obligatory parasites of the nasal and sinus cavities of sheep and goats. Infestation is prevalent in hot and dry regions, such as Mediterranean countries. The current work was developed to establish the chronobiology of O. ovis in Sardinia, to determine the most suitable time for chemoprophylaxis. A survey was carried out during 1998, and sheep heads were collected monthly from local flocks. A total of 443 heads was examined, and the prevalence of oestrosis was 73.8%. We collected 2,691 larvae (mean = 6.07 ± 9.52), and the intensity was greatest in November. The humoral immune response against the nasal bot fly was analyzed by means of an indirect-ELISA using second-instar O. ovis excretory and secretory antigens. A seasonal variation in the antibody levels was observed, increasing from April and peaked in June and in September. A significant correlation was observed between first instar intensity and the mean relative humidity (r² = 0.120; P < 0.05), and between second-instar intensity and the mean temperature (r² = 0.241; P < 0.05). Three periods in the chronobiology of O. ovis were defined: diapause (October–February), the active phase of the endogenous cycle (March–September) and the exit phase (May–September). Our results showed that treatment in October–November was suitable, because first instars were in diapause, preventing the development of first into second instars, and second into third instars.

KEY WORDS Oestrus ovis, sheep, prevalence, indirect enzyme-linked immunosorbent assay, Italy

Oestrus ovis (Linne, 1761) is a parasite of sheep and goats, in which the fly larvae are obligatory parasites of nasal and sinus cavities. The female fly deposits larvae in or around the nostrils of the host sheep. The newly deposited first instars enter the nasal cavity and attach to the mucous membranes. Later they migrate to the frontal sinuses before molting into second instars. These larvae move into the frontal or maxillary sinuses where they mature into third instars. These larvae then reenter the nasal cavity before exiting the host to pupate under the soil.

Infestation is associated with chronic rhinitis and sinusitis with a mucopurulent discharge (Goddard et al. 1999). Loss of appetite, emaciation and conjunctivitis occur during development. In addition, to these local effects, the parasites can damage the lungs causing abscesses and interstitial pneumonia (Dorchies et al. 1998). Oestrosis is considered important because its clinical signs may also be similar to those of ovine gid (coenurus), psoroptosis (Bates 1996), listeriosis or scrapie.

Oestrosis is endemic in hot and dry regions, such as Mediterranean countries. It has been reported in Sicily (Caracappa et al. 2000), southern France (Dorchies et al. 2000), in many countries of Africa (Pandey and Ouhelli 1984; Kilani et al. 1986, Horak et al. 2001), in Arabia Saudi (Alahmed 2000), in northern Jordan (Abo-Sehada et al. 2000), and in Egypt (Amin et al. 1997). Oestrosis is also endemic in the warmer southern counties of the United Kingdom (Goddard et al. 1999).

The current study was conducted in an agricultural region of Sardinia, Italy, which is located in a northern Mediterranean climate. The presence of moderate temperature throughout the year produces an environment favorable for infestation of O. ovis. There are ~3.5 million sheep in Sardinia, but the epidemiological features of this parasitosis have not been investigated. In addition, it must be considered that oestrosis is a zoonosis, and farmers can be infested if suitable measures are not employed. There are few data about the zoonotic importance of oestrosis in Italy, although Pampiglione (1958) reported that 58.2% of the conjuntival myasis in Italians was in Sardinia.
The purpose of this work was to establish the prevalence and intensity of *O. ovis* infestations in Sardinian sheep and to determine the chronobiology of the infestations in Sardinia. This information can be used to make recommendations regarding the most suitable time to initiate chemoprophylaxis. In addition, we evaluated the humoral immune response to *O. ovis* in naturally infested sheep, through monthly blood samples obtained from a sheep flock maintained under field conditions.

**Materials and Methods**

**Postmortem Examinations.** The survey was carried out in four slaughterhouses at Sassari (Sardinia, Italy) from January to December 1998. A total of 443 sheep heads was recovered from local animals 6 mo to 10 yr of age. In this region, most flocks are maintained outside and are only brought into paddocks during the night and for milking. Lambing occurs from November to December, and lambs are weaned at ≈1 mo of age.

After slaughter, heads were removed and cut open along their longitudinal axis. The parasites were collected from the nasal cavities, turbinates and sinus following the procedures of Yilma and Dorchies (1991).

**Indirect-ELISA.** Blood samples were obtained monthly from 14 sheep maintained under typical management conditions, and the sera were maintained at −35°C until used.

Enzyme-linked immunosorbent assays (ELISAs) using excretory and secretory products from L2 *O. ovis* were performed on serum samples. The excretory and secretory products of *O. ovis* were obtained by incubating L2 larvae in RPMI culture medium at room temperature for 3 d.

The antigen was diluted in phosphate buffered saline (PBS, pH 7.4) to a concentration of 3 μg/ml to coat the wells of ELISA plates (Costar, Corning, New York). Serum samples were diluted 1:50 in PBS containing 0.05% Tween and 1% skimmed milk, and Protein G Recombinant-Peroxidase labeled (Sigma-Aldrich, St. Louis, MO) were used at 1:1,500 dilution. Pooled sera from five uninfected and five infected sheep were used as negative and positive controls, respectively. Positive control sera were obtained from sheep that were found to have numerous *O. ovis* larvae when slaughtered. Negative control sera were obtained from five lambs that had been kept housed since birth to avoid *O. ovis* infestation. The cut-off point for a positive result was taken as the mean optical density (OD) of negative sera ± 3 SD The mean OD for the negative sera was 0.1778, with a standard deviation of ± 0.066. Thus, positive values had an OD of 0.3099, or more.

**Statistical Analysis.** Statistical analysis was conducted using analysis of variance (ANOVA), and the Bonferroni multiple comparison test was applied when ANOVA revealed significant differences (*P* < 0.05). Pearson’s correlation test was applied to evaluate the existence of correlation among the different
variables considered. All tests were performed by the statistical package SPSS, version 10.0.6 (SPSS 2000).

Results and Discussion

Prevalence of Oestrosis. The overall prevalence of *O. ovis* was 73.8% and ranged from 62.5 in March to 85.3% in September (Fig. 1). These values were similar to those obtained by Pandey and Ouhelli (1984) in Morocco, and by Kilani et al. (1986) in Tunisia. The prevalence of *O. ovis* was lower in Sicily (Caracappa et al. 2000), South France (Dorchies et al. 2000) and in Aveyron (Bergeaud et al. 1994).

A total of 2,691 (mean = 6.07 ± 9.52) *O. ovis* larvae was recovered from the sheep heads (Table 1). The number of larvae per sheep ranged between 0 and 86.

As represented in Table 1, the percentage of first instars was 67.2, 28.8% of second instars and 4% of third instars with mean larval burdens of 4.08 ± 8.21 for first instars, 1.75 ± 3.29 for second instars and 0.24 ± 0.64 for third instars. These results were also similar to those obtained by Pandey and Ouhelli (1984) in sheep from Morocco. Bergeaud et al. (1994) obtained a mean of 4.9 in Aveyron (France). A higher parasitic burden has been observed in other Mediterranean areas such as South France (Dorchies et al. 2000), Sicily (Caracappa et al. 2000) and Tunisia (Kilani et al. 1986).

Seasonal variations. The maximal and the highest mean larval burden were observed in November (406 and 15.04 ± 18.27, respectively, Table 1), and the lowest in May (84 and 2.47 ± 2.55, respectively). ANOVA showed statistical differences during the year

Table 1. Monthly prevalences and percentages of the different *O. ovis* instars

<table>
<thead>
<tr>
<th>Instar</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Total</th>
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<tr>
<td>No. of examined sheep</td>
<td>32</td>
<td>34</td>
<td>64</td>
<td>46</td>
<td>34</td>
<td>53</td>
<td>48</td>
<td>25</td>
<td>34</td>
<td>27</td>
<td>27</td>
<td>19</td>
<td>443</td>
</tr>
<tr>
<td>No. of infected sheep</td>
<td>26</td>
<td>28</td>
<td>40</td>
<td>38</td>
<td>24</td>
<td>22</td>
<td>19</td>
<td>13</td>
<td>21</td>
<td>15</td>
<td>11</td>
<td>11</td>
<td>327</td>
</tr>
<tr>
<td>L1 larvae</td>
<td>144</td>
<td>136</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
<td>169</td>
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<td>169</td>
</tr>
<tr>
<td>L3 larvae</td>
<td>0</td>
<td>12</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
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<tr>
<td>Total larvae</td>
<td>168</td>
<td>295</td>
<td>237</td>
<td>237</td>
<td>237</td>
<td>237</td>
<td>237</td>
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<td>237</td>
</tr>
<tr>
<td>% L1 larvae</td>
<td>85.7</td>
<td>80.7</td>
<td>57.4</td>
<td>52.7</td>
<td>2.4</td>
<td>36.2</td>
<td>45.4</td>
<td>58</td>
<td>54.7</td>
<td>92.2</td>
<td>90.4</td>
<td>95.2</td>
<td>67.2</td>
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<tr>
<td>% L2 larvae</td>
<td>14.3</td>
<td>15.2</td>
<td>33.8</td>
<td>36.7</td>
<td>71.4</td>
<td>59.6</td>
<td>41</td>
<td>37</td>
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<td>9.4</td>
<td>9.4</td>
<td>9.4</td>
<td>28.8</td>
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<tr>
<td>% L3 larvae</td>
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<td>4.1</td>
<td>8.8</td>
<td>10.6</td>
<td>4.2</td>
<td>2.1</td>
<td>1</td>
<td>1</td>
<td>0.2</td>
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<td>0.2</td>
<td>0.2</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 2. Mean values of the different instars in sheep naturally infested by *O. ovis* (on the left y-axis). On the right y-axis, climatic parameters (mean values of temperature, relative humidity and rainfall) are represented.
(F = 7.485, df = 11, P < 0.05), and a comparison of mean with the Bonferroni test found differences among the means of October and January–June, and among the mean of November and January–September (P < 0.05). Caracappa et al. (2000) found the highest mean number of larvae in July, and Dorchies et al. (2000) in February, whereas the minimal values were in May and June, respectively.

The highest intensity of first, second and third instars were in November, July, and May, respectively (Table 1, Fig. 2), and the lowest burden were in May (first instars), December (second instars) and October and January (third instars). By means of the ANOVA test, significant differences were proved in first instars between October and March, May, June and August, and between November and January–September, and between February and May (P < 0.05).

Average values of temperature, relative humidity and rainfall are represented in Figs. 1 and 2. By using the Pearson’s correlation test, positive significant correlation was observed between the mean relative humidity and first instars (r² = 0.120; P < 0.05) and between the mean temperature and second instars (r² = 0.241; P < 0.05). Negative statistical correlation was found between the mean temperature and first instars (r² = 0.019; P < 0.05) and between the mean relative humidity and second instars (r² = 0.171; P < 0.05).

Table 1 shows the monthly percentages of each larval stage found. The highest numbers of larvae were observed in December (first instar) and in May (second and third instar), and the lowest for first instars in May, second instars in December and third instars in January and October.

**Indirect-ELISA.** A seasonal variation in mean antibody values was noted (Fig. 3). After slight variations, antibodies began to increase from April and peaked in June and in September (Fig. 3), and then decreased to November–December. ANOVA showed significant differences existed between monthly serological mean values (F = 3.037, df = 11, P < 0.05), and by means of the Bonferroni test, these differences were established between the antibody levels of June and those of November and December (P < 0.05). A significant positive correlation was observed between the IgG values and the average of temperature (r² = 0.169, P < 0.05), whereas the correlation between the IgG response and the average of rainfall was negative (r² = -0.177, P < 0.05). In sheep from Greece, Papadopoulos et al. (2001) noted higher mean antibody levels between March and July than during the wintertime. Serological prevalence in sheep was 100% throughout the year, which agreed with the results of Papadopoulos et al. (2001).

**Chronobiology of O. ovis in Sardinia.** Fig. 4 represents the suspected chronobiology of O. ovis in Sardinia, based on the above data. When climatic conditions were unfavourable (especially low temperatures and high percentages of relative humidity), first instars were in diapause (Dorchies and Alzieu 1997). In Sardinia, first instars were in diapause from October to February, when temperature and relative humidity were least favorable. In the current study, the highest percentages of first instars were obtained during this period (80%). This was coincident with the lowest
percentages of second and third instars which supported the idea that development into second and third instars was not occurring, and first instars were in diapause. Dorchies and Alzieu (1997) reported that diapause occurred when first comprised >80% of the total larval burden.

The IgG response was minimal from October to February, which showed that both the activity and metabolism of *O. ovis* larvae was reduced, and the levels of antigen so. Goddard et al. (1999) reported that in *O. ovis* infestations, during the autumn and winter, first instars deposited in late summer were quiescent in the nasal cavities and therefore presented either no antigen or only low levels of antigen to the immune system.

The active phase of the endogenous cycle took place from March to September. The increment in the mean temperature, and the reduction in the relative humidity and rainfall appeared to stimulate the development of first into second and third instars, which exit the sheep and pupate. This explains the lowest percentage of first instars and the increase in that of second and third instars.

Zumpt (1965) reported that after pupation under the soil, adults emerged at temperature between 20 and 30°C, and the flies grouped and copulated. Then, females deposited larvae in/around the nostrils of sheep. In the current study, eclosion of adult flies occurred primarily from May to September, providing an increased opportunity of flock infestation. Yilma and Dorchies (1991) reported that in the temperate climate of western Europe, adult *O. ovis* were active during the spring, summer and autumn, and that in France, development from first to second instars begins in February. The values of IgG peaked in June and September, reflecting the increase in activity of the nasal bot fly, releasing greater levels of antigen too. Dorchies et al. (1998) reported that a significant increase in antibody titers occurred during the months before the adult flies became active and when second and third instars resume their development.

Our results suggested three periods in the chronobiology of *O. ovis* in Sardinia. Diapause occurred from October to February. The active phase of the endogenous cycle extending from March to September and the exit phase from May to September. Because of the importance of oestrosis in Sardinia, it is necessary to administer a chemical treatment for control. We emphasize that oestrosis is a zoonotic disease, so chemoprophylactic measures should be directed to reduce the infestation of humans as well as sheep. Taking into consideration our proposed chronology, it seems very suitable to administer parasiticides in October–November, when first instars are in diapause. This would prevent the development of first into second, and second into third instars.

### Acknowledgments
The authors thank R. Panadero Fontán (Veterinaria de Lugo, USC) for critically reviewing the manuscript. This work was supported, in part, by a research grant from the Xunta de Galicia (XUGA, Spain), R. Sánchez-Andrade Fernández, Ph.D. dissertation and by the Research Project PGIDT00PX126102PR (XUGA, Spain).

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Received for publication 10 December 2001; accepted 26 March 2002.