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Epidemiology of *Oestrus ovis* (Diptera: Oestridae) in sheep in Botucatu, State of São Paulo

Epidemiologia de *Oestrus ovis* (Diptera: Oestridae) em ovinos em Botucatu, São Paulo

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Abstract

The seasonal factors that influence *Oestrus ovis* infestation in sheep were determined in Botucatu, State of São Paulo, Southwestern Brazil, from April 2008 to March 2011. Two tracer lambs were monthly exposed to natural infestation by *O. ovis* larvae for 28 consecutive days, by grazing with a sheep flock. Tracer animals were then euthanized and the larvae of *O. ovis* recovered from nasal and sinus cavities. Of the 72 tracer lambs, 50% were infested with *O. ovis* larvae and the mean intensity of infestation per head infested was 16.8 larvae, with an average of 7.8 L1, 5.3 L2 and 3.7 L3. *O. ovis* larvae were present during the four seasons of the year, but the mean larval burden was significantly higher during the spring and summer months, compared to winter ($P < 0.05$). The highest rate of larvae recovery coincided with mean temperatures between 20 °C and 25 °C and air relative humidity around 70%. Results suggest that evolution and development of *O. ovis* practically occurs throughout the entire year, with larval infestation especially frequent during the spring and summer months.

Keywords: *Oestrus ovis*, epidemiology, sheep.

Resumo

Os fatores sazonais que influenciam a infestação por larvas de *Oestrus ovis*, foram determinados no período de abril de 2008 a março de 2011, em ovinos criados em Botucatu, São Paulo, região Sudeste do Brasil. Mensalmente, dois cordeiros traçadores permaneceram em pastejo com um rebanho ovino durante 28 dias consecutivos, expostos à infestação natural por larvas de *O. ovis*. Em seguida, os cordeiros foram sacrificados, e as larvas de *O. ovis* recuperadas da cavidade nasal e seios frontais. Dos 72 cordeiros traçadores, 50% estavam infestados por larvas de *O. ovis*, com intensidade média de infestação de 16,8 larvas/cabeça infestada, com média de 7,8 L1, 5,3 L2 e 3,7 L3. As larvas de *O. ovis* foram recuperadas durante as quatro estações do ano, mas o número médio de larvas foi significativamente superior durante os meses de primavera e verão comparado aos meses de inverno ($P < 0,05$). A maior taxa de recuperação larval coincidiu com temperaturas médias entre 20 °C e 25 °C e umidade relativa do ar por volta de 70%. Os resultados sugerem que a evolução e desenvolvimento de *O. ovis* ocorre praticamente durante o ano inteiro, mas a infestação é mais frequente nos meses de primavera e verão.

Palavras-chave: *Oestrus ovis*, epidemiologia, ovinos.

Introduction

Oestrosis is a worldwide myiasis caused by larvae of the fly, *Oestrus ovis* (Linné 1761, Diptera: Oestridae), which are obligatory parasites of the nasal and sinus cavities of sheep and goats. The female fly is viviparous and deposits larvae in or around the nostrils of its host. These early first instars attach to the mucous membranes in the nasal cavities, change to second instars and move up to the sinuses, where they complete their development into mature third instars, which are expelled for pupation under

the soil (ZUMPT, 1965). The length of the parasitic life cycle is quite variable: from a few weeks to several months, depending on the season and climatic conditions (HALL; WALL, 1995). The pupation period may vary, on average, from 28 to 43 days and the average life of flies is around eight days (COBBETT; MITCHELL, 1941). Clinical respiratory signs such as seromucous or purulent nasal discharge, frequent sneezing and dyspnea, may severely impair the health of infested animals (DORCHIES et al., 1998). These pathological changes cause considerable economic losses in small ruminant livestock (ALCAIDE et al., 2003), i.e., reduction in live weight gain (HORAK; SNIJDERS, 1974) and decrease in milk production of almost 9% (DORCHIES et al., 2003).

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Numerous studies regarding the epidemiology of *O. ovis* have been carried out in many countries, but results are likely to be influenced by varying geographical conditions. In southwestern France, for example, infestation by *O. ovis* was present in 65% of the animals with mean intensity of 24.8 larvae/infested head, consisting mainly of first larval instar (YILMA; DORCHIES, 1991). In Sicily, Italy, the prevalence of infestation was 55.8% and all different larval instars were simultaneously recovered in similar proportions (CARACAPPA et al., 2000). In Brazil, there have only been a few studies about this parasite and these are restricted to States in the South of the country, where climatic conditions favorable to *O. ovis* parasitism are observed throughout the year (OLIVEIRA et al., 1999; RIBEIRO et al., 1990; RAMOS et al., 2006), except during periods with temperature of less than 9 °C, when no larvae were recovered from tracer sheep (RAMOS et al., 2006).

Oestrosis is considered a neglected sheep disease in Brazil, despite its increasing occurrence, according to the perception of farmers and veterinary clinicians. Therefore, more research about the epidemiology of the disease is imperative, since such knowledge will allow us to predict environmental factors favoring infestation, as well as enable recommendations regarding the best

strategies for oestrosis prophylaxis. This study was conducted to determine the seasonal variation of *O. ovis* infestation in sheep in Southwestern Brazil over a period of three years.

Materials and Methods

1. Study location

The experiment (approved by the Ethics Committee for Animals of the Institution – 86/07 - CEEA) was carried out in a sheep farm of the “Universidade Estadual Paulista” - UNESP, located at 22° 82' latitude South and 48° 41' longitude West, 613 m a.s.l., in Botucatu, State of São Paulo, Brazil. The climate is Cwa type according to Köppen classification and the data referring to averages of temperatures, relative air humidity and rainfall during the study period were obtained by the Department of Environmental Science, Agronomical Science College, UNESP, located 8 km away from the experimental site (Figure 1a, b).

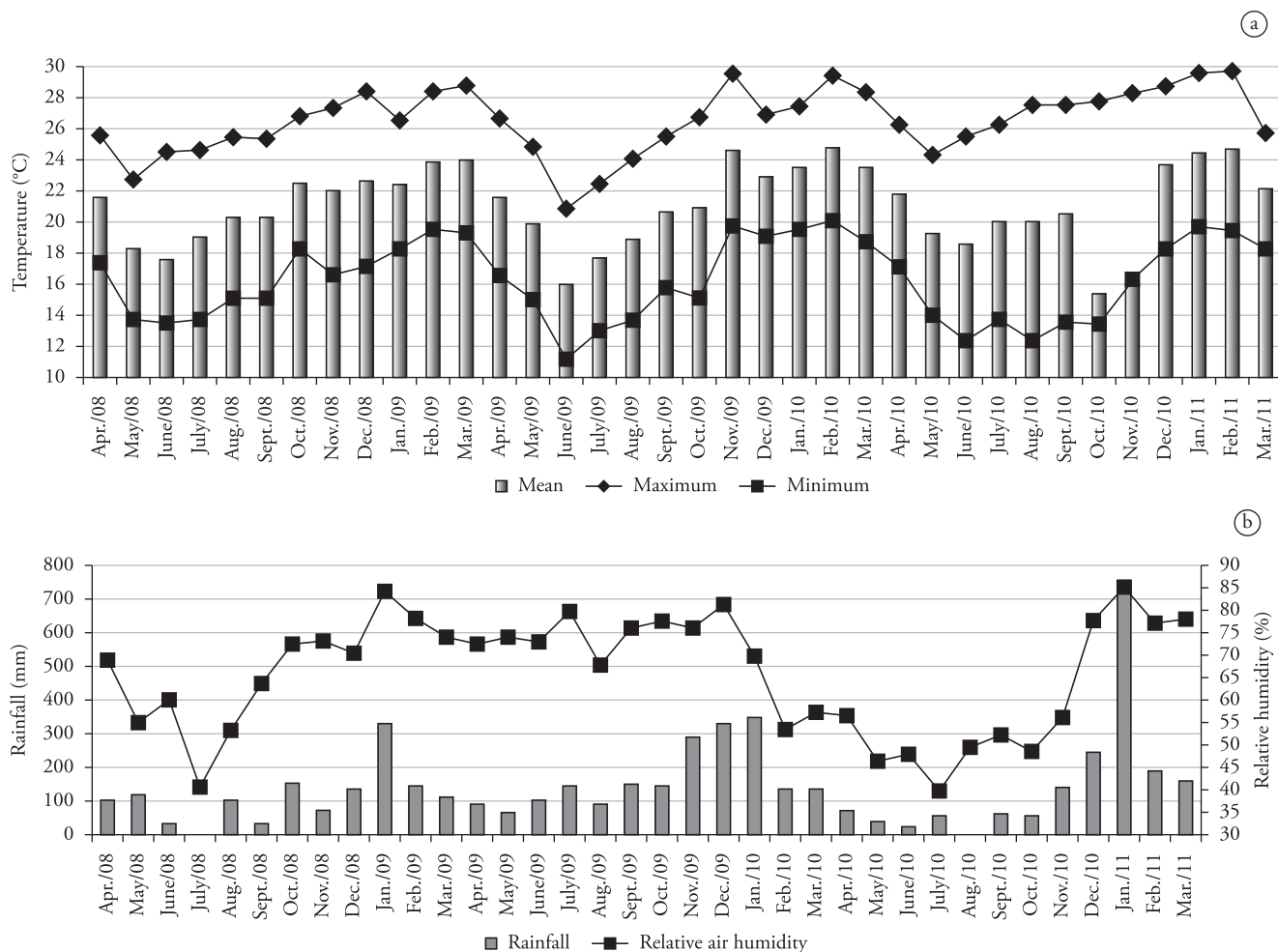


Figure 1. Average maximum, minimum and mean monthly temperatures (a) monthly rainfall and relative air humidity from April 2008 until March 2011 (b) Source: Department of Environmental Sciences, Agronomical Science College, São Paulo State University – UNESP, Botucatu, São Paulo State.

2. Management of tracer lambs

In total, during the three years of observations, 72 Ile de France weathered male lambs were purchased from a farm located in the State of São Paulo. Twelve lambs were purchased every six months, with initial ages between two and five months.

Immediately after arrival at the University facilities, animals were housed, vaccinated against clostridial infections (Sintoxan Polivalente®, Merial, Brazil) and orally drenched, once daily, for three consecutive days with levamisole phosphate (10 mg/kg, Ripercol® L 150 F, Fort Dodge) and albendazole (10 mg/kg, Valbazen® 10 Cobalto, Pfizer). One week later, the same protocol was carried out with triclofon (100 mg/kg, Neguvon®, Bayer S.A.) to remove any existing infestation with *O. ovis* and infection with gastrointestinal nematodes. Tracer lambs that had remained housed for more than one month received an additional treatment with triclofon (100 mg/kg; Neguvon® - Bayer S.A.) one week before being taken to the farm, to remove any infestation with *O. ovis*.

While indoors, the animals were fed on concentrate (Tech Ovin Unique, Socil®, with 18% of crude protein) with an amount corresponding to 1% of their mean live weight and had free access to Tifton hay and tap water. Decoquinate (Deccox®, Alpharma) was added to the commercial feed to prevent coccidiosis.

Every month, from April 2008 until March 2011, two tracer lambs were exposed to natural infestation with *O. ovis* larvae for 28 consecutive days, while grazing together with the same sheep flock. Immediately thereafter, the tracer lambs were euthanized. Heads were removed and cut open along their longitudinal and sagittal axis. Nasal cavity (nasal passage, septum, middle meatus and conchae) and paranasal sinuses (frontal and maxillary) were carefully examined and all larvae present were collected and counted. Larvae were preserved in 70% alcohol and identified according to their stage of development based on the descriptions by Zumpt (1965) and Capelle (1966).

3. Sheep flock management

The sheep farm where the tracer lambs were placed held 156 Bergamacia sheep at the beginning of the study and 202 animals

at the end of the study. The sheep were kept permanently in rotational grazing on *Panicum maximum* cv Tanzania grass. However, due to the low amount of forage during winter and early spring (from June to October), the animals received additional corn silage feed daily.

4. Statistical analyses

Analysis of variance was performed in order to compare the degree of infestation throughout the year. Data were transformed using $\log_{10}(x + 1)$ prior to analysis and grouped according to the season of the year, autumn (March-April-May), winter (June-July-August), spring (September-October-November) and summer (December-January-February). Significant differences between season means were determined by the Student's t-test at 5%. Spearman's rank correlation coefficients were estimated between larval burden and climatic conditions (temperature, rainfall and air relative humidity). All analyses were performed using SAS (release 9.2).

Descriptive statistical analyses were used to summarize the data in agreement with Bush et al. (1997), using the following terms:

Prevalence: the number of hosts infested with *O. ovis* larvae divided by the number of hosts examined;

Intensity of infestation: the number of *O. ovis* larvae in a single infested host;

Mean intensity of infestation: the total number of *O. ovis* larvae found divided by the number of hosts infected with that parasite.

Results

The prevalence of *O. ovis* infestation was 50% for the tracer lambs, i.e., half of the 72 animals displayed larvae during the three years of the experiment. *O. ovis* larvae were present during the four seasons of the year with the highest prevalence (61.1%) in summer and autumn (Figure 2). The highest rate of larvae recovery coincided with mean temperatures between 20 °C and 25 °C and air relative humidity around 70% (Figure 1a, b). Nevertheless, no significant correlations were observed between monthly larval

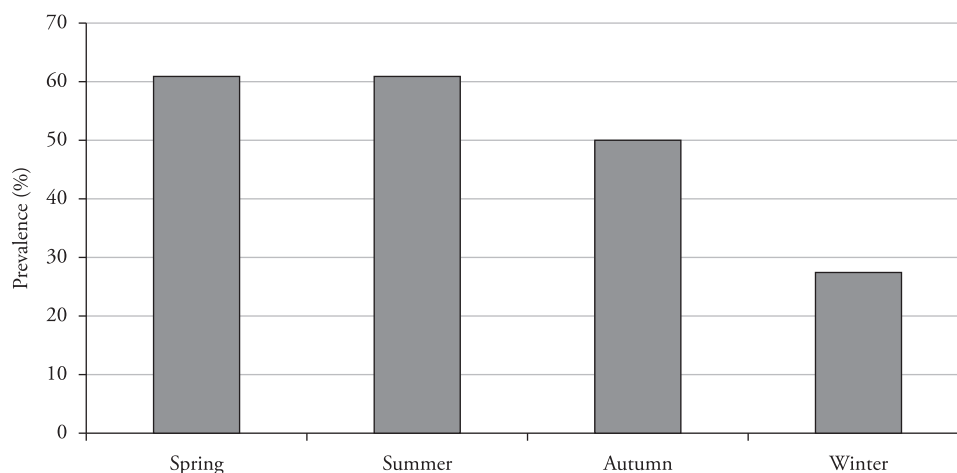


Figure 2. Seasonal prevalence of *Oestrus ovis* in tracer sheep from April 2008 until March 2011, Botucatu, São Paulo State.

numbers and the climatic variables, i.e., temperature, rainfall or air relative humidity ($P > 0.05$).

The mean intensity of infestation was 16.8 larvae per infested head with an average of 7.8 first larval instar (L1), 5.3 second larval instar (L2) and 3.7 third larval instar (L3) (Figure 3). The highest number of larvae recovered from a single sheep was 66, in September 2008: 64 L1 and two L2. The highest mean intensity of infestation was found at the beginning of spring in September and October 2008 (40 and 41 larvae/infested head, respectively). The mean larval burden was significantly higher in spring and summer compared to winter ($P < 0.05$).

From the total 606 larvae, 46.4% was L1, while L2 and L3 represented 31.3% and 22.3% of the total larval burden, respectively. The majority of L1 were located in the nasal cavity (99.6%). In contrast, L3 were found especially in the frontal sinus (97.8%). L2 were recovered from both sites: 40% from the nasal cavity and 60% from the frontal sinus. No larvae were recovered from maxillary sinus.

Most of the tracer lambs parasitized with *O. ovis* (30 of 36) did not show any clinical signs of oestrosis. Only six animals presented signs, e.g., dyspnea and seromucous discharge; their larval burden was variable, ranging from 12 to 66 larvae, constituted especially of L1 and L2.

Discussion

The prevalence of *O. ovis* in tracer lambs was 50%, which is less than that observed in Bagé, State of Rio Grande do Sul, Brazil, where 85.4% of sheep were infested (RIBEIRO et al., 1990). The difference between results may be explained in part by the fact that in Bagé, the animals were exposed to *O. ovis* infestation for a longer period of time (from one month to one year), while in the present experiment the sheep were exposed for just 28 days. Moreover, other factors may have influenced the *O. ovis* epidemiology, such as the climatic conditions of each region, size of sheep flock and management, which can result in a variable prevalence, as demonstrated by numerous studies conducted in slaughterhouses in different countries

(YILMA; DORCHIES, 1991; ABO-SHEHADA et al., 2000; SCALA et al., 2002; ALCAIDE et al., 2003; ARSLAN et al., 2009; SHOORIJEH et al., 2009).

The mean intensity of infestation reported in this study (16.8) was similar to that previously reported in Sardinia, Italy (SCALA et al., 2001) and Southwestern Spain (ALCAIDE et al., 2003), with mean intensities of 19 and 18.5 larvae per infested head, respectively; but lower than that found in Southwestern France with 24.8 larvae (YILMA; DORCHIES, 1991) and Northeastern Spain with 37.9 (GRACIA et al., 2010). It is noteworthy that a high percentage of first larval instar were found by those authors, especially during the colder months. This was not observed in the present study, where the proportion of different larval instars did not show variation throughout the experimental months.

The respective percentages of L1, L2 and L3 were 46.4%, 31.3% and 22.3%. The presence of different instars indicates that endogenous development and re-infestation with L1 occurred simultaneously. In contrast, in the Southwestern region of France, in temperate weather, L1 predominated at 90.5% of the total burden, while L2 (6.0%) and L3 (3.5%) displayed low proportions (YILMA; DORCHIES, 1991). A greater burden of L1 indicates the occurrence of a period of hypobiosis during the year, when *O. ovis* cease their development, or at least suggests a decrease in the rate of larval maturation. This was not observed in the present research, where synchronous evolution of all larval instars was observed with no evidence of hypobiosis.

Among the climatic factors influencing the oestrid fly activity, temperature, light intensity, and wind are recognized as the most important, but in the case of *O. ovis*, it has been reported that temperature is the main factor in determining fly activity (CEPEDA-PALACIOS; SCHOLL, 2000). During all years of the study, averages of maximum temperatures were between 20.8 °C and 29.8 °C. It was demonstrated that 20 °C was the minimum temperature for fly activity, while optimum temperature appears to range between 26 and 28 °C (CEPEDA-PALACIOS; SCHOLL, 2000). Therefore, the temperature conditions of the present study were optimal for fly activity during all months of the experimental years, except during winter months, especially

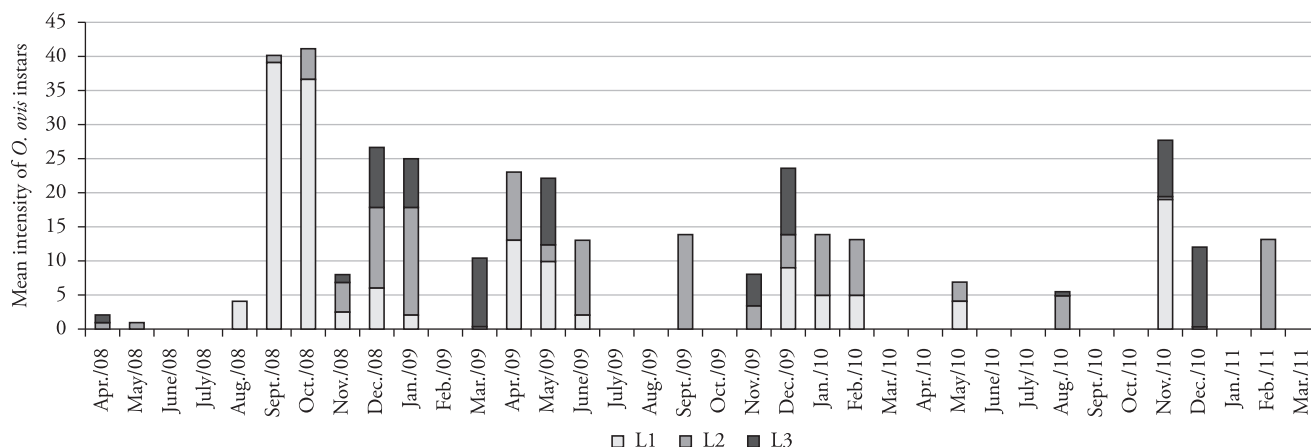


Figure 3. Monthly mean intensity of first larval instar (L1), second larval instar (L2) and third larval instar (L3) of *Oestrus ovis* in tracer sheep from April 2008 until March 2011, Botucatu, São Paulo State.

in July, when regardless of a mean maximum temperature of around 25 °C, the mean of the minimum was always less than 14 °C. Under these climatic conditions, the activity of flies was possibly reduced, which coincided with the lowest mean intensity of infestation in tracer sheep.

Nasal discharges and sneezing were evident in sheep at the farm during the entire study, but only six of the 72 tracer animals had clinical symptoms of oestrosis. The delay between the first infestation and the appearance of clinical signs suggests the possible involvement of a hypersensitivity phenomenon in the modulation of the infection and these first infestations are possibly not associated with clinical signs of overt oestrosis (DORCHIES et al., 1998). Due to the short period between infestation and euthanasia, most of the tracer sheep probably did not have time to develop and display any immune response through sneezing and nasal discharge, which are characteristic clinical signs of parasitism by *O. ovis*.

In conclusion, it is possible to affirm that several annual generations of adult flies may occur in the region. New studies will be necessary to evaluate the impact of oestrosis in sheep production, as well the best strategies of prophylaxis.

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