EPIDEMIOLOGY OF *OESTRUS OVIS* INFECTION OF SHEEP IN ARGENTINA'S WESTERN PAMPAS

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Summary:

Seasonal population trends and effects of *Oestrus ovis* in naturally infected sheep were studied over 13 months, in the Western of the Pampeana region. At weaning, 140 growing lambs were randomly allocated to two groups: UG, untreated group and TG treated every 4 weeks with closantel (10 mg/kg). Successive *Oestrus* free tracer lambs (TL) by previous treatment ($n = 65$) were slaughtered after 20-30 exposition days for larval counts. Likewise, other group PL of 117 permanent untreated lambs was slaughtered from four to 17 months of age. Weighing and assessment of health signs of UG and TG and blood samples were monthly carried out. The prevalence of infection in permanent group varied from 33 % to 100 %. Mean number of larvae in PL was 6.1 with 3 L1, 1.4 L2 and 1.6 L3 during spring-summer and 17.9 with 16.9 L1, 0.5 L2 and 0.4 L3 during autumn-winter months. In PL the proportions of larvae in each of the different larval stages was similar during spring and summer, but a significant ($P < 0.01$) increase of L1 was detected during autumn and winter. The prevalence in tracer lambs was 100 % during summer time and larvae were absent from 25-May to 25-October. Mean larval burdens of positive TL varied from 6.4 to one *Oestrus* and a significant peak ($P < 0.05$) of larvae was seen from December to March. Since March to November only L1 was recovered from TL. TG group showed a reduction in nasal discharge and in antibody ELISA levels, but no difference was observed in live weight gain between TG and UG. These results show a high prevalence during summer and that the perpetuation of *Oestrus* is ensured by an autumn period of arrested development and the overwintering larvae in the sheep heads.

KEY WORDS : Sheep, *Oestrus ovis*, epidemiology, tracer lambs, Argentina.

MOTS CLÉS : Mouton, *Oestrus ovis*, épidémiologie, agneaux traceurs, Argentine.

Résumé : ÉPIDÉMIOLOGIE DES INFESTATIONS PAR *OESTRUS OVIS* CHEZ LE MOUTON EN ARGENTINE

L'évolution saisonnière et les effets des populations naturelles d'*Oestrus ovis* chez le mouton ont été étudiés pendant une période de 13 mois à l'ouest de la région de la Pampa. Au sevrage, 140 agneaux ont été répartis en deux groupes de façon aléatoire : un groupe non traité (UG) et un groupe traité (TG) toutes les quatre semaines avec du closantel (10 mg/kg). Tout au long de l'étude, des agneaux traceurs (TL) indemnes d'*Oestrus ovis* suite à un traitement préalable ($n = 65$) ont été autopsiés pour un comptage de larves après une période d'exposition de 20 à 30 jours. De plus, un autre groupe de 117 agneaux permanents et non traités (PL) ont été sacrifiés entre l'âge de quatre et 17 mois. Pesée, évaluation des signes cliniques d'oestrose et prises de sang ont été réalisées tous les mois. La prévalence de l'infestation par *Oestrus ovis* chez les agneaux permanents présentait une variation mensuelle de 33 à 100 %. Le nombre moyen de larves était de 6,1 (3 L1, 1,4 L2 et 1,6 L3) pendant le printemps et l'été, et 17,9 (16,9 L1, 0,5 L2 et 0,4 L3) pendant les mois d'automne et d'hiver. Chez les agneaux permanents, les proportions des différents stades larvaires étaient identiques durant le printemps et l'été, mais une augmentation significative ($P < 0,01$) de la proportion des L1 a été enregistrée pendant l'automne et l'hiver. Tous les agneaux traceurs étaient infestés pendant l'été, en revanche, tous étaient indemnes entre le 25 mai et le 25 octobre. Le nombre moyen de larves chez les agneaux traceurs infestés a varié de 1 à 6,4 avec un pic significatif d'infestation entre décembre et mars. De mars à mai et en novembre, seules des L1 ont été retrouvées chez les agneaux traceurs. Le groupe traité a présenté une réduction du jetage nasal et également des taux d'anticorps spécifiques mesurés en ELISA mais aucune différence de gain de poids n'a pu être mise en évidence entre les deux groupes. Ces résultats montrent une forte prévalence des infestations par *Oestrus ovis* en été ainsi qu'une survie de l'espèce pendant l'automne et l'hiver assurée par des larves en hypobiose chez le mouton.

The sheep bot fly *Oestrus ovis* (Linné, 1761) has a wide distribution along the temperate and sub-tropical areas of Argentina where sheep and goat are raised. Despite this high frequency in the coun-

try, the pathogenic effects of the bot fly are generally underestimated. The infestation of sheep with *O. ovis* larvae has been recognized since earliest times, but there have been controversies about its deleterious significance. It has been demonstrated that the live weight gain of non infected lambs is better than in those infected (Horak & Snijders, 1974). *O. ovis* causes myiasis in the nasal and sinusal cavities of sheep and goats and disturbs animals at grazing, but besides these local effects, the larvae induce immunomodulation with such consequences as development of abscesses and viral interstitial pneumonia (Dorchies *et al.*, 1995, 1996).

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Several drugs have good and persistent efficacy against the *Oestrus ovis* larvae (Dorchies *et al.*, 1997; Tolosa *et al.*, 2000), however, their frequent use has resulted in the development of resistance in some sheep nematodes. The knowledge of the *Oestrus ovis* epidemiology is very important in order to indicate future rational and integrate control programs and reduce the number of treatments and the risk of creating parasite resistance.

In the Pampeana region of Argentina the sheep bot fly is very frequent, but apart from some isolated or drug efficacy reports, no serious epidemiological studies have been undertaken. Therefore, the aim of the present study was to follow the dynamics of the *Oestrus ovis* infection under natural conditions in slaughtered sheep, tracer lambs and by test detection of antibodies.

MATERIALS AND METHODS

STUDY SITE, ANIMALS AND EXPERIMENTAL DESIGN

The study was carried out at the Agricultural Research Station of Anguil in the Western Pampeana region in the Province of La Pampa, Argentina. A flock of 900 sheep of Pampinta breed (3/4 Ost-Friesian $\times$ 1/4 Corriedale) known to have high natural prevalence of infection by *Oestrus ovis* and generally treated (closantel, 10 mg/kg) once in the year, was used.

Growing weaned ewe lambs from four to 16 months of age were observed from 20 December 1999 to 12 January 2001. The females were allocated at random into two groups of 65 each, TG: closantel (10 mg/kg of live weight) treated group at intervals of 45 days, started at December 20th; UG: untreated group against *Oestrus ovis* naturally infected group. UG was only drenched with levamisole to remove gastrointestinal nematodes in order to establish the effects of nasal bots *per se*.

With the male lambs two types of observations were undertaken: non treated permanent growing wether lambs (PL) and tracer lambs. Permanent grazing lambs (PL) from four to 16 months of age, and randomly selected at the start from the flock were monthly slaughtered to monitor *O. ovis* burdens. Each month, eight growing lambs were observed.

Tracer lambs (TL) of four to nine months of age, treated once with closantel (10 mg/kg) and housed indoors for 50 days under fly-free conditions were utilized. This procedure also eliminated all chances of closantel effect persistence. Batches of five tracers were introduced every month to grazed alongside the flock for 30-40 days. They were then removed from the flock, slaughtered to larval recover after two weeks.

Groups of ewe lambs TG and UG were monthly weighed, examined for nasal discharge and blood sampled (only 20 sheep of each group) for *O. ovis* specific IgG titers determination.

CLINICAL AND PARASITOLOGICAL OBSERVATIONS

Signs of infestation and their clinical severity were measured by the score of nasal discharge (ND). The score was the following: no ND 0, serous ND 1, sero-mucous ND 2; very thick mucous ND 3, muco-purulent ND 4, purulent ND 5.

After slaughter and removal of the skin, heads were removed and cut along their longitudinal axis with an electric saw. Then, larvae were recovered from the mucosa of the nasal septum, nasal passages and sinus. Larvae from each head were counted and identified to first (L1), second (L2) and third (L3) instar according to keys of Zumpt (1965).

SERUM ANALYSIS

Blood samples were analysed in ELISA using L2 crude extracts as antigen. L2 were homogenized into PBS (pH 7.2) at 0.25 g wet weight per ml of buffer, centrifuged at $5,000 \times g$ and filtered through 0.8/0.2 μm Acodisc sieves (Gelman). Protein concentration was determined and L2 crude extract (L2CE) was stored at $-70^{\circ}C$ until use. The ELISA procedure is described in Tabouret *et al.* (2001). Briefly, the L2 crude extracts were used at 2 $\mu g/ml$ in carbonate buffer (pH 9.6), distributed in 96 well plates (Nunc surface, Nunc, Denmark), incubated for one hour at $37^{\circ}C$, then overnight at $4^{\circ}C$. The wells were washed three times with PBST (0.01 M phosphate, 0.15 M sodium chloride, pH 7.2 and 0.1 % Tween 20). The antigen-coated wells were then incubated for 30 minutes with a 10 % skimmed milk solution at $37^{\circ}C$ before blotting dry. Triplicate serum samples diluted 1:200 in PBST were incubated for 60 minutes at $37^{\circ}C$. The plates were washed three times with PBST before addition of a horseradish peroxidase-conjugated donkey anti-sheep IgG (SIGMA A3415) diluted (1:2000) in carbonate buffer (60 minutes of incubation at $37^{\circ}C$). Three final washes with PBST were carried out before addition and incubation at $37^{\circ}C$ of 100 μl per well of the chromogen (2,2'-azino-bis(2-ethylbenzthiazoline-6-sulfonic acid)diammonium). The reaction was stopped after one hour and the optical densities determined with a spectrophotometer by measuring the absorbance at 405 nm. An antibody percentage was calculated for each sample by comparison with positive reference serum (artificially infected sheep) and negative reference serum (young sheep kept indoor to avoid *O. ovis* infections) as follows: % of antibodies = $OD(\text{serum sample}) - OD(\text{negative control}) / OD(\text{positive control}) - OD(\text{negative control})$.

STATISTICAL ANALYSIS

The significance of differences between groups of live weight gain and ELISA parameters were subjected to analysis of variance (SAS, 1988). Differences in seasonal trends of larval instar counts were tested by chi-square. Nasal discharge score comparisons between groups were performed with the non parametric Wilcoxon test with the same statistical program.

RESULTS

PERMANENT WETHER LAMBS

The mean instar larvae burdens recovered from permanent lambs (PL) along the study were presented in Table I. The prevalence of infection of PL varied from 50 % to 100 %, the highest being between January and May and the lowest at late spring (November-December). Mean number of larvae in PL was 6.1 with 3 L1, 1.4 L2 and 1.6 L3 during spring-summer and 17.9 with 16.9 L1, 0.5 L2 and 0.4 L3 during autumn-winter months.

In PL the proportions of larvae in each of the different larval stages was similar during summer (December to February), but a significant (χ^2 53.7; $P < 0.0001$) increase of L1 was detected from late summer to late winter (March to September). Thereafter, since September L2 and L3 larval instars proportions significantly (χ^2 6.6; $P < 0.013$) increased.

TRACER LAMBS

The data of the larval recovery from tracer lambs are showed in Figure 1. The prevalence in tracer lambs was 100 % during summer time, whereas larvae were absent from 25-May to 25-October. Mean *Oestrus* burdens of positive TL varied from 6.4 to one larvae. A significant peak (χ^2 16.5; $P < 0.001$) of larvae was seen from December to March, but a different proportion of larval instars was registered from February, when the number of L2 and L3 decreased (χ^2 22.7; $P < 0.001$) significantly. From March to May and then since 25-October to 1-December only L1 was recovered from TL.

Fig. 1 – Mean monthly larval burden of the three *Oestrus ovis* instars recovered from tracer lambs.

NASAL DISCHARGE

The arithmetic means of nasal discharge scores of both groups along the trial are showed in Figure 2. The number of ewe lambs without nasal discharges of the UG was always lower than those of the TG group. The nature of the discharge founded in the latter group was mainly serous, whereas the frequency of sero-mucous and purulent nasal discharges was high in the UG group. Since February to the middle of the summer, the nasal discharges mean scores of UG were always higher ($P < 0.001$) than those registered in the TG group.

ANTIBODY (ELISA LEVELS)

No significant differences in ELISA values of both groups were observed at the start of the blood samplings at the middle of the summer (09-Feb-00). Thereafter, significant increases ($P < 0.0001$) of ELISA values of UG towards the autumn were registered. Subsequently, at the end of the autumn (13-Jun-00) UG values decreased. At spring, significant differences ($P < 0.0001$) between groups remained and UG showed the highest ($P < 0.0001$) values until the end of the trial in summer (12-Jan-01), whereas ELISA values of treated TG remained low. Mean ELISA values, expressed as the percentage of the optical density values of the standard positive serum, are given in Figure 3.

Month	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.
Prev.	85	100	86	100	100	100	78	80	79	83	83	50	67	100
L1	3	3,8	4.5	14.5	28	21.1	18,1	11.7	10.7	9.1	2.6	0.5	2	5.7
L2	1.1	0.9	1	0.8	0.8	0.3	0.4	0.6	0.8	1	1	1	1,7	4
L3	1.5	1.9	2	0.18	0.1	0.5	0.2	0.3	0.7	1.6	0.5	2.1	1.2	3.3
Total	5.5	6.6	7.5	16.2	28.9	22.0	18.8	12.7	12.2	11.6	4.1	3.6	4.8	13.0

Table I. – Monthly prevalences (Prev.) of permanent lambs (PL) infected with *Oestrus ovis* and mean *Oestrus ovis* larval and total burdens.

Fig. 2 – Mean scores of nasal discharge observed on ewe lambs groups. TG: closantel treated group; UG: untreated naturally infected group.

Fig. 3 – ELISA values (expressed as a percentage of antibodies compared to a positive reference serum) observed on ewe lambs groups. TG: closantel treated group; UG: untreated naturally infected group.

WEIGHT GAIN

No difference was observed in live weight gains between both groups during the whole study.

DISCUSSION

The mean prevalence (84.07 %) of *Oestrus ovis* infections found in the Pampeana region are similar to those observed in the central region of Argentina (Tolosa *et al.*, 2000), South of Brazil (Sardá Ribeiro *et al.*, 1990) and in Senegal (Pangui *et al.*, 1988), but higher than those observed in the Mediterranean zone of Morocco, France or Sicily that were

around 33.2-69.2 % (Pandey & Ouhelli, 1984; Yilma & Dorchie, 1991; Caracappa *et al.*, 2000; Dorchie *et al.*, 2001). These results show that the Western Pampeana region of Argentina presents favourable conditions for the development and the survival of the bot fly.

The study with permanent lambs showed a progressive increase in the number of L1 since March (late summer) to April, which prevail until September (late winter). This L1 accumulation can be ascribed to several factors. Firstly, L1 instar larvae arrest or retard their development during this period as shown in the tracer lambs, although the presence of few L2 and L3 larvae shows that some of L1 larvae continue to develop to maturity. Secondly, it is possible that the acquisition of immunity by growing lambs during autumn arrived to diminish the larval growth. During winter, dead L3 larvae were recovered from several slaughtered lambs. The lowest prevalences of *O. ovis* infections are observed in spring because L1 larvae resume their development. In summer, the intensities of infections are minimum due to the rapid development of newly deposited L1.

The work with tracer lambs indicated that only since November to early March all or at least some of the larvae deposited were able to develop to the third instar. Contrarily, in March, April and May tracers showed residual contaminations with only first instars and evidenced that *Oestrus* was not able to normally develop in 20 days and retarded its evolution during autumn months. All these data suggest the existence of a period of hypobiosis during the autumn and the winter. The same situation was reported by Yilma & Dorchie (1991) and Dorchie *et al.* (2001) in the French Piémont pyrénéen where all larvae present in

the animals during autumn-winter were L1 instar larvae. The study results under different regions show the *Oestrus ovis* adaptation to different situations. *Oestrus ovis* L1 larvae cease their development before the winter in the temperate regions or before the dry season in the sahelian regions (Dorchies *et al.*, 1995). In the south-mediterranean countries no hypobiosis period could be proved because the larval proportions are similar throughout the year (Kilani *et al.*, 1986; Caracappa *et al.*, 2000).

The high nasal discharge scores observed in the treated group and the lack of differences with the UG from the beginning of the trial until February in the middle of the summer could be due to the first treatment data. This treatment was made at the start of the summer with a flock exposed to a high bot fly activity and further nasal chronic inflammations. More than two months free from infection could be necessary for the mucosa to recover and decrease the level of nasal discharge of the flock.

The increase of the ELISA values of the growing lambs from summer towards the beginning of the autumn closely followed the L1 instar larvae accumulation and the presence of the subsequent development larvae of *Oestrus ovis* during the past summer. Thereafter towards late autumn, the levels of ELISA declined in accord with the lack of fly activity and L1 depositions. Detection of infected animals by ELISA is difficult for winter samples, resulting in false negatives. Hypobiosis of L1 larvae leads to the loss of metabolic and migratory activities and hence a decreased of antigen stimulation. Finally, the sharp rise of antibodies noted in November (spring) paralleled the start of bot fly activity, despite of the low larvae prevalence. The development of *O. ovis* larvae in late spring and summer dramatically increased the IgG response as previously shown in Sardinia (Scala *et al.*, 2001). This could be due to the L2 and L3 capacity to produce higher quantities of antigenic proteins related to the increased size of larval secretory organs (Tabouret *et al.*, 2001).

The larvae recovered from the heads and the other parameters obtained show that the first generation of bot flies hatch around last October-November (spring) from the resume development to third instar larvae of the overwintering first instar larvae in the sheep head. In spring the climatic conditions became favorable for the new pupae and a second fly generation is produced around late December at the start of the summer. During summer the generation time takes around 50-60 days when the highest prevalence was detected. From late March onwards the temperature goes down when the last fourth fly generation is sometime able to hatch. Then from late May the conditions became unfavorable for the adult flies and the pupation and no new infection occurred from late autumn until spring.

Despite the treatments of the TG ewe lambs, no live weight gain responses were obtained in the present trial. Conversely, Horak & Snijders (1974) in South Africa demonstrated in the rafoxanide treated group a reduction in the nasal discharge and an increased gain in weight under similar consecutive study designs. Apparently this parasite causes nasal infections, induces immunomodulation and hypersensitivity reactions that predispose to chronic respiratory pathologies that disturb the future performance of adult sheep (Dorchies *et al.*, 1995).

These results show a high prevalence during summer and that the perpetuation of *Oestrus ovis* is ensured by an autumn period of arrested development and the over wintering larvae in the sheep heads. The first generation appears in November and up to four generations may occur during the favourable period. However, these results emphasise the need for further investigations in order to advance in the knowledge of the epidemiology and the productive impact of the bot fly under a variety of conditions, and particularly on long-term evaluations.

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