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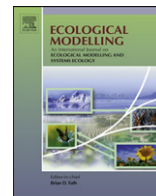
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## Ecological Modelling

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# Estimation of pasture infectivity according to weather conditions through a fuzzy parametrized model for the free-living stage of *Ostertagia ostertagi*

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## ABSTRACT

Gastrointestinal parasitism is one of the diseases that has the highest economic impact on the Argentinian beef production system, rendering it inefficient. In the region of the Humid Pampas, it has been estimated that 22 million dollars are lost annually because of the death of calves and 170 million dollars are lost in sub-clinic costs. A mathematical model with fuzzy parameters was constructed for the analysis of the free-living stages of gastrointestinal parasites, with the purpose of estimating the pool of L3 larvae available for migration to pasture and the levels of infection in pasture at any time of the year under different climatic conditions. The model is formulated in terms of a system of three difference equations. These equations describe the abundance of parasites in each of the successive stages of the population development. The model was calibrated and tested with data gathered through fieldwork carried out in Tandil (37° 19'S, 59° 08' 05"W), province of Buenos Aires (Argentina) and the corresponding weather data. A comparison between model simulations and fieldwork data obtained in other locations achieved satisfactory results.

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## 1. Introduction

In Argentina, cattle feed on pasture during the whole year. *Ostertagia* spp., is predominant in the Pampas region. These parasites cause great weight loss, which can exceed 30 kg per animal per year, and high mortality of calves (Fiel et al., 1994; Steffan et al., 1982; Entrocasso, 1981). From an economic perspective, 1998 estimated costs added up to some 22 million dollar annual loss due to cattle mortality, and 170 million dollars owing to subclinic problems. Therefore, the production system becomes inefficient.

Since the 1960s, the life-cycle of *O. ostertagi* has been studied in a variety of environments. The results have helped understand how processes occur under different environmental conditions. A large number of authors have obtained valuable results on the compound effects of environmental variables such as temperature (Lutzelschwab et al., 2005; Michel et al., 1975), humidity, and rainfall (Onyiah and Arslan, 2005; Stromberg, 1997), and microclimate of dung (Rossanigo and Gruner, 1995; Krecek et al., 1990), as well as biological factors such as hatching times (Young et al., 1980a), length of hatching and length of infective larvae (Gibson, 1981; Pandey, 1972; Rose, 1969), time of development from egg to

infective larva, mortality rates of pre-infective and infective larvae (Pandey, 1972), and conditions for migration and survival in pasture (Almería and Uriarte, 1999; Stromberg, 1997; Al Saqur et al., 1982; Persson, 1974a,b; Rose, 1970; Durie, 1961). It has become clear that the epidemiology of *Ostertagia* changes according to the climate zone. Experts believe that a minimum of five years of fieldwork is necessary in order to obtain a clear understanding of the epidemiology of any cattle area in Argentina (Fiel et al., submitted for publication).

There are numerous investigations on different epidemiological aspects in almost all cattle areas around the world (Williams et al., 1987; Steffan and Fiel, 1986). This has made possible to obtain very interesting and important statistical results.

Lately, cases of resistance to several antihelmintics drugs have been recorded so that the strategies that veterinarians normally use are not always efficient whenever considering cost and benefits. The variability of responses to different environmental factors made relevant the use of modelling tools to help understand the complexity of the dynamics of parasite life cycles. Some parasite models (Louie et al., 2007; Learmount et al., 2006; Ward, 2006a,b; Smith et al., 1986, 1987; Young et al., 1980b) gave very useful insights into the population dynamics of gastrointestinal parasites, becoming interesting complements to field and laboratory research. However, some aspects of the pre-infective cycle have not been taken into account. For example, the effect of rainfall on

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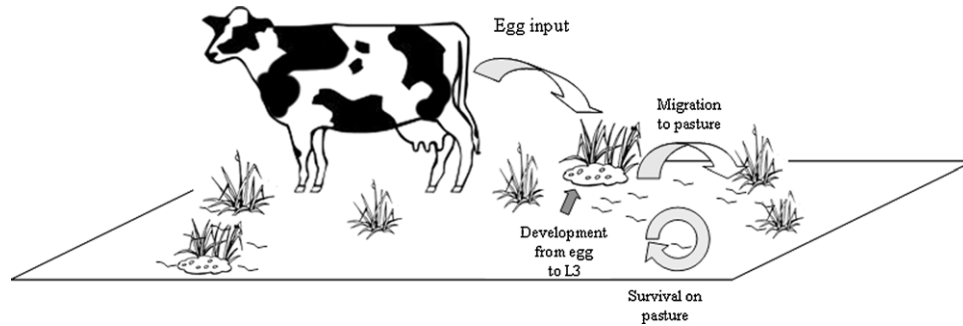


Fig. 1. Conceptual model.

larval pre-infective mortality rate has not been considered so far. Recent models proposed by Ward (2006a,b) and Louie et al. (2007), based on the theory of dynamic systems, are being used as control tools. Nevertheless, it is worth mentioning that the hypotheses for those models were not totally adequate for Argentina.

Here we propose a mathematical model for the free-living stages of *O. ostertagi* adapted to the environmental conditions in the Pampas region of Argentina, where cattle-raising is extensive. The model allows estimating the infectivity levels that the pasture will exhibit, day by day, along the year taking into account the amount of eggs per gram of faecal matter (EPG) produced by the animals in the paddock and the weather conditions recorded in the study area. Hence, it becomes possible to determine the EPG levels that may imply significant risk effects in future pasture infections.

## 2. A brief description of *Ostertagia ostertagi* life-cycle

The life-cycle of *O. ostertagi* is direct, without an intermediary host. There are two distinct stages: the *parasitic stage* (*L4-Adult*) and the *free-living stage* (*Egg-L1-L2-L3*). The free-living stage occurs on the ground, within the dung-pat, and later on the grass. Larvae in *L1* and *L2* stages feed on fungi and bacteria. The infective larva *L3* is ensheathed and does not feed. Following ingestion by cattle, *L3* larvae undergo a process of exsheathment in the rumen before developing to the fourth parasitic stage (*L4*). Afterwards, larvae quickly develop into the adult stage (*L5*).

In general the conditions in the dung-pat are sufficient for their development. The most important environmental factors that affect the free-living stage are air temperature and rainfall. Some

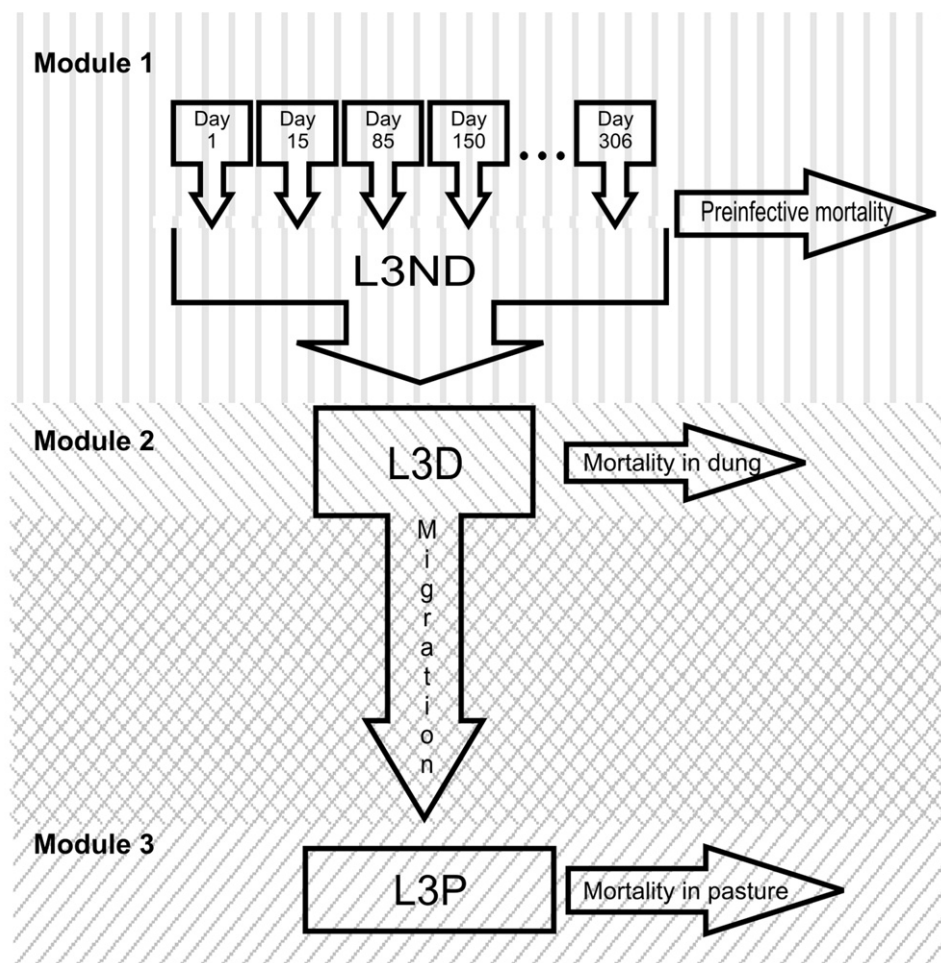


Fig. 2. Diagram of the model.

**Table 1**  
Linguistic variables for pre-infective mortality rate (input).

	Stage	Season [d]	Precipitation [mm]	Mortality
1	L1 (0–0.6)	Summer (–45 to 136)	Drizzle1 (0–2)	Low (0–0.35)
2	L2 (0.3–0.9)	Winter (136–319)	Drizzle2 (2–10)	Moderate (0.2–0.6)
3	L3 (0.7–1)	Spring (228–365)	Rain (5–20)	High (0.5–1)
4		Autumn (45–228)	Downpour1 (10–50)	
5			Downpour2 (50–600)	

well-known facts that relate to temperature are that the eggs never hatch below 4 °C and above 40 °C, and the time needed for hatching reduces as the temperature increases in the range from 10 to 35 °C (Pandey, 1972); and the development time from egg to infective larva L3 can vary from a few days during summer to several weeks in winter (Fiel et al., submitted for publication). On the other hand, rainfall produces high mortality among eggs and pre-infective larvae (L1 and L2) due to “dung washing”. In other words, it removes eggs and pre-infective larvae from the dung and takes them to the pasture where the conditions are not appropriate for continuing their development (Fiel et al., submitted for publication). A larva needs a film of water to be able to migrate outside the dung. Survival depends on whatever energy it accumulated in its intestinal cells and its ability to be eaten by bovine when the larva arrives at the grass. The larva survival time is variable with respect to season of year; it is longer in winter than in summer. The prepatent period is usually twenty-one days but, under certain circumstances, the parasite can arrest its development, prolonging the life cycle for several months.

The free-living stage has been studied under both controlled and field conditions (Fiel et al., submitted for publication; Rossanigo and Gruner, 1995; Gibson, 1981; Young et al., 1980a,b; Pandey, 1972; Rose, 1969). The variability of responses to different environmental factors makes relevant the use of modelling tools to help understand the complexity of the dynamics of parasite life cycle.

### 3. Materials and methods

#### 3.1. Model description

The purpose of the model is to evaluate the contribution to pasture contamination of cattle in a paddock, with varying levels of EPG of *Ostertagia*, taking into account the effect of weather conditions. The model is formulated in terms of a system of three difference equations and parametrized using fuzzy rule based systems (FRBS) (see Appendix A). The model describes the abundance of parasites in each of the successive stages of the population development (Figs. 1 and 2). Three processes are taken into account:

- recruitment of larvae to the L3 population pool available for migration to pasture (Module 1).
- Survival of the L3 larval population in dung waiting for migration to pasture (Module 2).
- Emigration of L3 larvae from dung to pasture and the dynamics of the L3 larval population in the pasture (Module 3).

**Table 2**  
The base of rules for fuzzy parameter pre-infective mortality (input).

IF (stage is L3)/IF (stage is not L3)										
Season/ precipitation	1	2	3	4	5	6	7	8	9	10
1	1	2	1	2	2 × 0.5	2	2	3 × 0.8	2	3
2	1	1 × 0.1	2	1 × 0.5	2	1	3 × 0.5	2 × 0.5	2	2
3	1	1 × 0.5	1	1	1	2 × 0.5	1	2	1	3 × 0.5
4	1	1 × 0.5	1	1	1	2 × 0.5	1	2	1	3 × 0.5

The difference equations are solved with a daily step. The fuzzy parameters are built based on data and information from publications related to the subject and the expertise provided by parasitologist.

#### 3.1.1. Recruitment of larvae to the L3 population pool available for migration to pasture (Module 1)

This module describes the dynamics of eggs and larvae within dung. All the eggs contained in the dung-pats which are produced in during day  $t$  by the herd are considered a cohort corresponding to that julian day of “arrival” as initial time. The cohort is thus named “ $t$ ”.

The only process that affects the cohort “ $t$ ” is mortality, and hence its dynamics is described by the following equation:

$$H(t+a, a) = (1 - \mu_{\text{Pre}}(a, t+a, R(t+a)))H(t+a-1, a-1) \quad \text{if } a \leq \tau(t) \quad (3:1)$$

$$IC \quad H(t, 0)$$

where  $H(t+a, a)$  is the amount of preinfective larvae (eggs, L1 or L2) aged  $a$  of cohort “ $t$ ”,  $\mu_{\text{Pre}}(\cdot)$  is the preinfective mortality rate,  $R(t+a)$  is the amount of mm of rainfall on day  $t+a$ , and  $\tau(t)$  is the average development time from egg to L3 for the cohort. The function  $\tau(t)$  defines the time it takes for cohort  $t$  to reach the L3 stage, estimated by means of the model reported by Chaparro and Canziani (2010).

Eq. (3:1) has the initial condition:

$$H(t, 0, w) = \begin{cases} \frac{N \text{Animal } 6.3 \text{ EPG}(t) \text{PatWt}(w)}{2.20462}, & \text{if the animals are calves} \\ \frac{N \text{Animal } 8.1 \text{ EPG}(t) \text{PatWt}(w)}{2.20462}, & \text{if the animals are cows} \end{cases}$$

where  $N \text{Animal}$  is the amount of animals in the paddock,  $\text{EPG}(t)$  are the eggs per grams of dung on julian day  $t$ ,  $\text{PatWt}$  is the faeces pat weight dependent on the animal's body weight. A cow defecates an average of 8.1 times per day, while a calf may defecate an average of 6.23 times over the same observation period. The actual weight of faeces produced is directly related to the animal's body weight, following the equation:

$$\text{PatWt}(w) = \frac{0.000285(2.20462 \ w)^{1.361}}{2.20462} = 0.0003795 \ w^{1.361}$$

where pat weight ( $\text{PatWt}$ ) and body weight ( $w$ ) are given in kg (Stromberg, 1997).

For example a 200 kg calf would deposit approximately 0.5134 kg of faeces each time it defecates and, assuming it defecates 6.23 times per day, it would deposit on the average 3.2 kg per day. Similarly, a 400 kg cow would produce approximately 10.68 kg per day. Hence, the total egg deposition onto a paddock per day, month or grazing season can be calculated knowing the age (cow or calf), weight, and egg count (EPG) for the animal.



### 3.1.2. Survival of the L3 larval population in dung waiting for migrating to pasture (Module 2)

This module describes the dynamics of the L3 larval population in dung. The population pool grows as larvae from different cohorts complete their development and reach the L3 stage. Losses from the pool are due to migration to pasture and mortality while waiting for the adequate environmental conditions that may allow migration. When rainfall occurs, migration takes place. The proportion of larvae in the pool that migrate to pasture on a given day depends on the time of the year and the amount of precipitation occurring on that particular day. Then the dynamics of this population is described by the following difference equation:

$$L3D(t+1) = (1 - \delta_{DP}(t, R(t)))(1 - \mu_{ID})L3D(t) + L3ND(t+1)$$

where  $L3D(t)$  is the amount of L3 larvae in the dung pool on julian day  $t$ ;  $L3ND(t)$  is the amount of L3 larvae in dung that have completed their development precisely on julian day  $t$ ;  $\mu_{ID}$  is the mortality rate inside dung; and  $\delta_{DP}(\cdot)$  is the migration rate from dung to pasture, dependent on the precipitation  $R(t)$  recorded on julian day  $t$ .

### 3.1.3. Emigration of L3 larvae from dung to pastures and dynamics of the population of L3 larvae in the pasture (Module 3)

The dynamics of the population of L3 larvae in the pasture is described in this third module. L3 larvae that survive and migrate with the help of rainfall are considered a contribution to the larval population in the pasture. Losses are due to mortality in the pasture, which depends on the time of the year and the temperature:

$$L3P(t+1) = (1 - \mu_P(t, T(t)))L3P(t-1) + \delta_{DP}(t+1, R(t+1))L3D(t+1)$$

where  $L3P(t)$  is the amount of larvae in pasture on day  $t$  and  $\mu_P(\cdot)$  is the mortality rate in the pasture.

## 3.2. Parametrization of the model

### 3.2.1. Pre-infective mortality rate

As mentioned earlier, each cohort goes through three stages, from egg to L1, to L2, to L3 within dung. Conditions in dung are sufficient for their development (Fiel et al., 1994). The air temperature determines the length of this period. The eggs never hatch if the temperature is below 4°C or above 40°C. Hatching probability increases as temperature rises from 10 to 35°C (Williams and Bilkovich, 1971; Pandey, 1972). There is evidence of an inverse relationship between development time and temperature both in controlled and in field conditions (Pandey, 1972). Development time also exhibits a very sensitive response to both the order in which the temperature sequence occurs and the amplitude of the temperature range over a given period (Chaparro and Canziani, 2010). Mortality increases when temperature reaches values outside the 10–35°C range (Levine, 1978). Thus, during warm months only a few days will be necessary to reach the L3 stage, whereas several weeks are necessary in cold months, especially in wet and cold winters (Catto, 1982; Durie, 1961).

Consequently, the linguistic variables (membership functions) **Age** ( $H, L1, L2, L3$ ), **Season** (*Summer, Autumn, Winter, Spring*) and **Precipitation** (*Drizzle1, Drizzle2, Rain, Downpour1, Downpour2*) were selected as input variables and **preMortality** (*Low, Moderate, High*) as output variable. The type of membership functions and their parameters are detailed in Table 1. This FRBS appropriately reflects situations that the parasites face in this period. For example:

IF (Age is **not L3**) and (Season is **Spring**) AND (Precipitation is **Rain**) THEN (Mortality is **Moderate**)

**Table 3**

Linguistic variables for migration rate (input).

	Season [day]	Precipitation [mm]	Migration
1	Summer (–45 to 136)	Drizzle1 (0–2)	Low (0–0.1)
2	Winter (136–319)	Drizzle2 (2–10)	Moderate (0.05–0.5)
3	Spring (228–365)	Rain (5–20)	High (0.35–0.6)
4	Autumn (45–228)	Downpour1 (10–50)	
5		Downpour2 (50–600)	

IF (Age is **L2**) and (Season is **Winter**) AND (Precipitation is **Drizzle1**) THEN (Mortality is **Low**)

The complete list of rules is summarized in Table 2.

### 3.2.2. Migration from dung to pasture

As mentioned earlier, larvae need a film of water to be able to migrate out of the dung-pat. This process depends on rainfall and on the time of the year. For example, in summer, a monthly precipitation of more than 50 mm is necessary for *O. ostertagi* to be able to migrate (Fiel et al., 1994; Gordon, 1973) whereas in winter 10 mm are sufficient.

Therefore the linguistic variables (membership function) **Season** (*Summer, Autumn, Winter, Spring*) and **Precipitation** (*Drizzle1, Drizzle2, Rain, Downpour1, Downpour2*) were selected as input variables and **Migration** (*Very-low, Low, Moderate, High*) as output variable. This FRBS appropriately reflects situations that the parasites face in this period. For example:

IF (Season is **Summer**) AND (Precipitation is **Drizzle2**) THEN (Migration is **Very-low**)

IF (Season is **Winter**) AND (Precipitation is **Drizzle2**) THEN (Migration is **Moderate**)

The type of membership functions and their parameters are detailed in Table 3 and the complete list of rules in Table 4.

### 3.2.3. Mortality in pasture

The survival of a L3 larva in the pasture depends on the energy accumulated in its intestinal cells and its ability to be eaten by bovine once in the grass. Temperature and humidity are considered the most important factors in the life cycle of larvae (Levine, 1978). In winter, its energy is slowly exhausted, while in summer the reserves are quickly depleted. Low temperatures, high rainfall and a good coating fodder are associated to periods of high survival (Besier et al., 1993).

The linguistic variables (membership functions) **Season** (*Summer, Autumn, Winter, Spring*), **Temperature** (*Low, Mean-low, Mean-high, High*) and **Precipitation** (*Drizzle1, Drizzle2, Rain, Downpour1, Downpour2*), were selected as input variables and **pastureMortality** (*Low, Moderate, High*) as output. The FRBS was built similarly to that of the pre-infective mortality parameters. The type of membership functions and their parameters are detailed in Table 5 and the complete set of rules in Table 6.

**Table 4**

The base of rules for fuzzy parameter migration (input).

Season/ precipitation	1	2	3	4	5
1	1 × 0.1	1 × 0.5	1	2	3
2	1	2	3 × 0.7	3 × 0.9	3
3	1 × 0.5	1	2	3 × 0.7	3
4	1 × 0.5	1	2	3 × 0.7	3

**Table 5**  
Linguistic variables for rate of mortality in pasture (Input).

	Season [days]	Temperature [°C]	Mortality
1	Summer (–45 to 136)	Low (0–14)	Low (0–0.2)
2	Winter (136–319)	Moderate (12–26)	Moderate (0.1–0.5)
3	Spring (228–365)	High (23–35)	High (0.25–0.7)
4	Autumn (45–228)		

### 3.3. Implementation of the model

The model was implemented using GNU Scilab 5.0. The inputs of the model are:

- weather data: the model includes data on temperature and precipitation in the region of study. Real daily average temperature and daily precipitations data were used in the simulations.
- Initial and final day [day, month, year] of the period in which the animals are in the paddock “scattering” eggs on the pasture. These days are called “the period of study”.
- Number and type (calf or cow) of animals in the paddock.
- Daily number of eggs per grams of dung (EPG) in the period of study.
- Average weight of the animals in the pen.

The simulation runs with these data and creates two graphs which are useful to veterinarians and to managers. One of the graphs describes the dynamics of the L3 larval population available for migrating to pasture (*Module 1*) and the precipitation recorded over the period of the simulation. The second graph describes the infection of pastures on a daily basis (*Module 3*).

### 3.4. Model validation using field data

The epidemiological data used for calibration and validation of the model were provided by Fiel et al. (submitted for publication). A field study was carried on from late 1994 until the beginning of 1998 with the purpose of investigating the free-life stage of gastrointestinal parasites in cattle in the mid-eastern region of the Province of Buenos Aires (Argentina). In particular, the development time from egg to infecting L3 larvae in faeces, infectivity levels in pasture, and survival time of infective L3 larvae on the grass were measured under natural environmental conditions.

A 0.96 hectare clean paddock located in the University Campus (UNCPBA) in Tandil was used for the field work. The paddock was divided into 16 sub-paddocks. Two naturally infected calves contaminated the sub-paddocks with eggs of gastrointestinal parasites. Faecal samples for egg counts and coprocultures were taken weekly from the “contaminating” calves during the grazing period. Faecal egg counts were used to plot the contamination of the paddock. Coprocultures allowed the identification of which nematode species were present in the contamination. On days 1 and 15 of each month, faecal matter of was collected from the paddock. Then weekly samples were taken to the lab from collected faecal matter in order to analyze the development from egg to L3. Simultaneously, grass samples were regularly taken from the paddock over the period of 16 months to assess the infection of pastures as

**Table 6**  
The base of rules for fuzzy parameter mortality in pasture (input).

Season/ temperature	1	2	3
1	1	2	3
2	1	2 × 0.5	3 × 0.5
3	1	1	2 × 0.5
4	1	2 × 0.5	3 × 0.5

well as survival of L3 larvae on pasture (Fiel et al., submitted for publication). It is worth noting that the animals used for the study were transferred to “clean” paddocks in order to avoid reinfection and to determine in a clearer way the seasonal variation of pasture infectivity.

Data from late 1994 until the end of 1995 were used in the calibration of the model, while the validation was performed with data recorded from early 1996 until the end of 1997. Model simulations were set up under the same conditions as those present in the field experiments. In particular, input data were:

- weather data corresponding to the region and period of study, from late 1994 until end of 1997.
- A clean paddock occupied by two infected calves.
- Average weight of calves in the paddock assumed to be 200 kg.
- EPG levels estimated by Fiel et al. (submitted for publication) in the period of study.

Model populations of L3 larvae available for migration and in pasture were monitored for one year. Simulation outputs were processed in order to obtain average L3 densities on the pasture every 15 days, so as to facilitate the comparison to field data.

The purpose of the model is to analyze the dynamics of the pasture infection. Hence it is not important to accurately estimate the daily abundance of L3 larvae in the pasture but rather to have a reasonable timing of the appearance of L3 larvae, the time of occurrence of the peak of the infection, and how long it takes for the larvae to die out after the cattle is removed from the paddock. The term “reasonable” is subject to the periodicity of the retrieval of field data which was every 15 days as already mentioned.

Simulations over one-year periods were performed for validating the model. Averages of L3 abundance in the pasture were calculated from output data over two-week periods for easier comparison with field data. This was done due to the low temporal resolution of the field experiments as compared to the daily output of the model. For a more detailed analysis of the seasonal dynamics, simulations covering one-semester occupations of the paddocks were performed.

### 3.5. Analysis of the model's response to variations in environmental conditions

Different scenarios were used for evaluating the model's response to variations in temperature and precipitations.

With the purpose of analyzing the effect of changes in temperature, simulations using temperature time series recorded in the cities of Mercedes (annual mean temperature ( $\mu$ ) = 20.24 °C; Standard deviation ( $\sigma$ ) = 5.85 °C, latitude 29° 11' S, longitude 59° 04' O) in the province of Corrientes, Marcos Juárez ( $\mu$  = 17.3 °C;  $\sigma$  = 5.77 °C, latitude 32° 42' S, longitude 62° 06' O) in the province of Cordoba, and Tandil ( $\mu$  = 14 °C;  $\sigma$  = 5.4 °C, latitude 37° 19' S, longitude 59° 08' O) in the province of Buenos Aires, were carried on.

The temperature time series in each of the three locations corresponded to the period 2006–2007. In order to facilitate comparisons and assess the effect of temperature variation, EPG values were fixed and the 1995 rainfall series for Tandil was used in all simulations. In the analysis of the effect of variations in precipitation, temperature data series recorded in Tandil in 1995 were used as input. The objective was to study the response when the seasonality of precipitations was altered.

The 2005 springtime rainfall series, called *SP*, was extracted and four new data series were constructed from it following the rule:

$$Pr\#(i) = \begin{cases} SP(i) & \text{if } i \in [k, k + 90] \\ 0 & \text{otherwise} \end{cases}$$

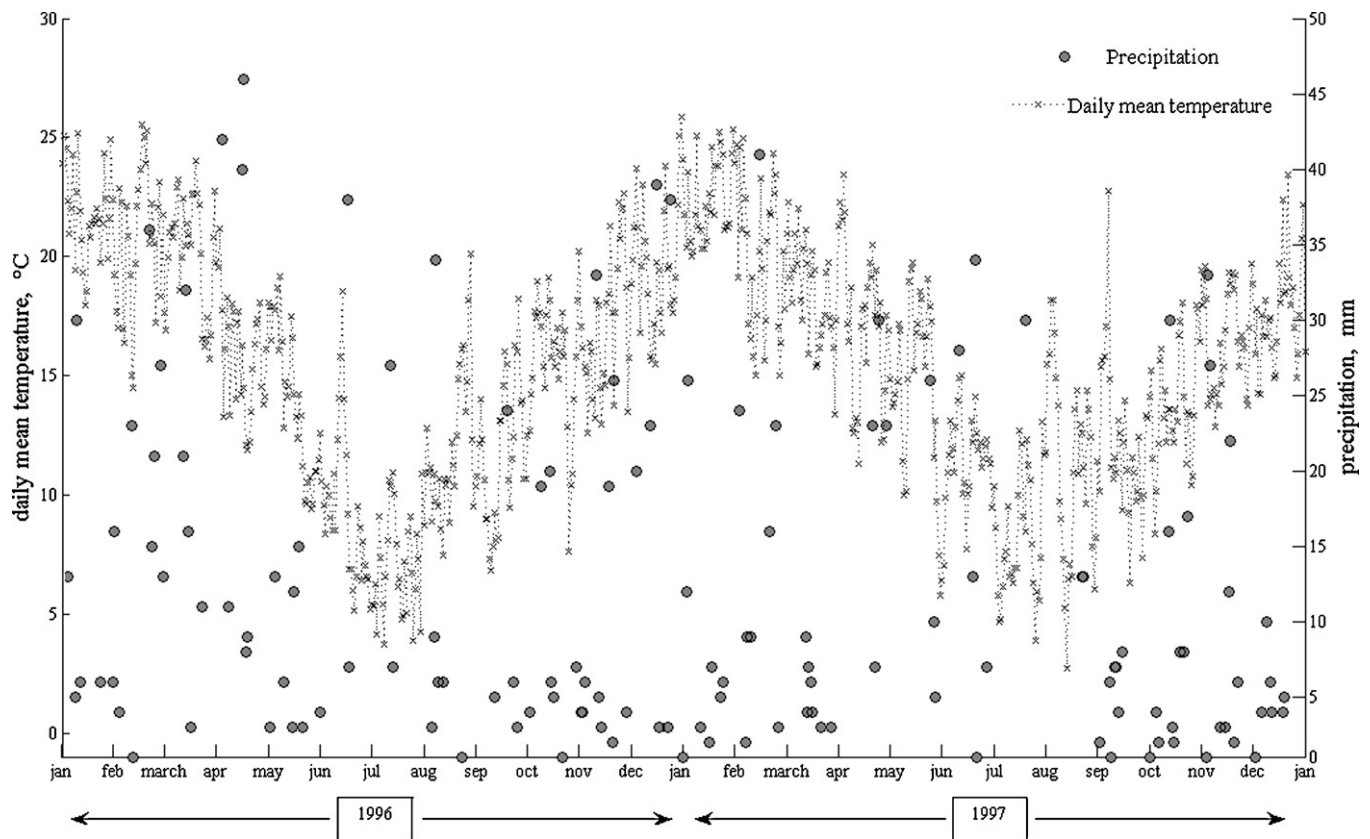


Fig. 3. Meteorological data over the period 1995–1997: daily temperature (°C) and precipitations (mm).

Hence, when  $k=0$ , rainfall seasonality corresponded to summer, when  $k=90$  it corresponded to autumn, when  $k=180$  it corresponded to winter, and finally, when  $k=270$  it corresponded to spring.

#### 4. Numerical results

##### 4.1. Presence of L3 in pasture

Fiel reports that the pasture infectivity during the field experiments showed a clear seasonality during the autumn–winter and the spring field experiments, while a high mortality of larvae in pasture occurred in summer. *Ostertagia* was the predominant genera, with a general survival time of over one year when larvae arrived in the pasture between March and August (Fiel et al., submitted for publication). As mentioned earlier, model simulations were set up under the same conditions as those present in the field experiments.

##### 4.2. Results of computer simulations for the summer–autumn period

Fig. 3 shows the daily mean temperature and precipitation for the two years of the study. The annual mean for Tandil is 13.75 °C and the total precipitation in mm are 455 mm and 433 mm for the period January–June and July–December, respectively.

##### 4.2.1. Period January–June for the 1996 data

In this semester, the mean temperature was equal to the annual mean of Tandil (13.75 °C) while the total precipitation (567 mm) was above the mean for this period (455 mm).

In the simulation, the first L3 larvae corresponding to the January–March cohort (summer) were observed in the pasture on

the first days of January. The abundance levels in pasture were significant since February and the peak of infection took place in mid-May. From then on, it decreased slowly and steadily until its disappearance by the end of September.

The dynamics of the April–June cohort (autumn) was very similar to that of the January–March cohort, the difference being only in timing and numbers. The first L3 appeared at the first half of April and then the population exhibited oscillations but increased until July. The peak of infection took place at the end of June and the levels of infections stayed high until the end of September, after which they decreased until disappearing by the early days of October.

Here again computer simulation results differ depending on the time of the year in which the first day of the simulation was set. The simulated results are shown in Fig. 4(a).

##### 4.2.2. Period January–June for the 1997 data

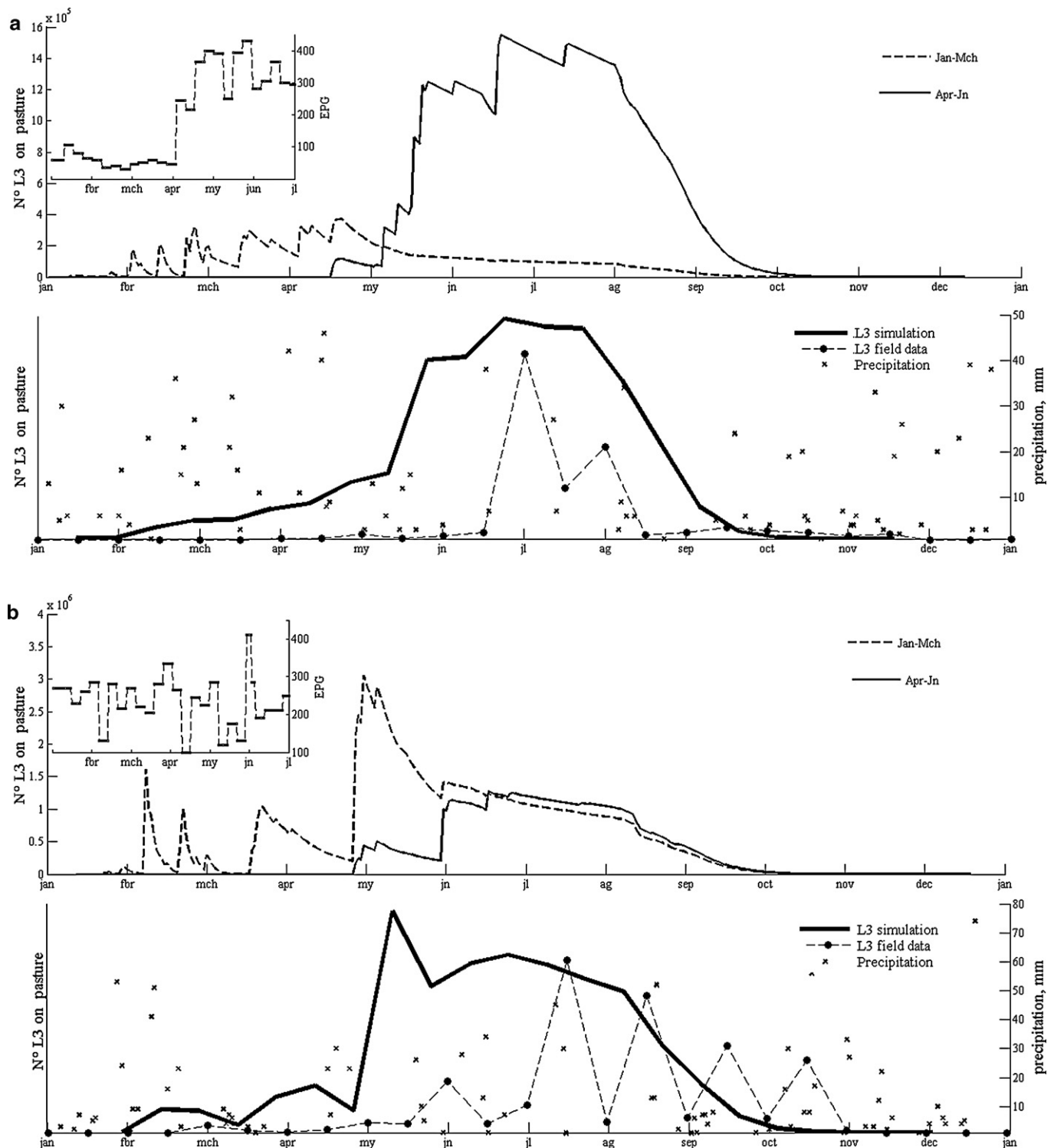
In this year, the mean temperature coincided with the annual mean for Tandil (13.75 °C) and the total precipitation (500 mm) was above the mean for this period (455 mm).

The first L3 larvae in the pasture were observed in January at low-moderate levels, but survived only a few days in the pasture. The peak of infection was recorded during May, and from then on the population decreased steadily until disappearing from pasture in October. The April–June cohort (autumn) showed a peak of infection in June–July and then decreased with some oscillations until disappearing by mid-October. The simulated results are presented in Fig. 4(b).

##### 4.3. Results of computer simulations for the winter–spring period

##### 4.3.1. Period July–December for the 1996 data

In this semester, the mean temperature coincided with the annual mean for Tandil (13.75 °C) and the total precipitation



**Fig. 4.** (a) (i) Simulation output corresponding to the 1996 summer–autumn season showing the January–March (---) and the April–June cohorts (—). The upper window shows the EPG daily values used in the simulation for the semester January–June. (ii) Simulation output averaged over two-week periods (—) and field data (●), also showing precipitations recorded over the study period (×). (b) Same as above for the 1997 summer–autumn season.

(466 mm) was roughly equal to the mean for this second period (433 mm).

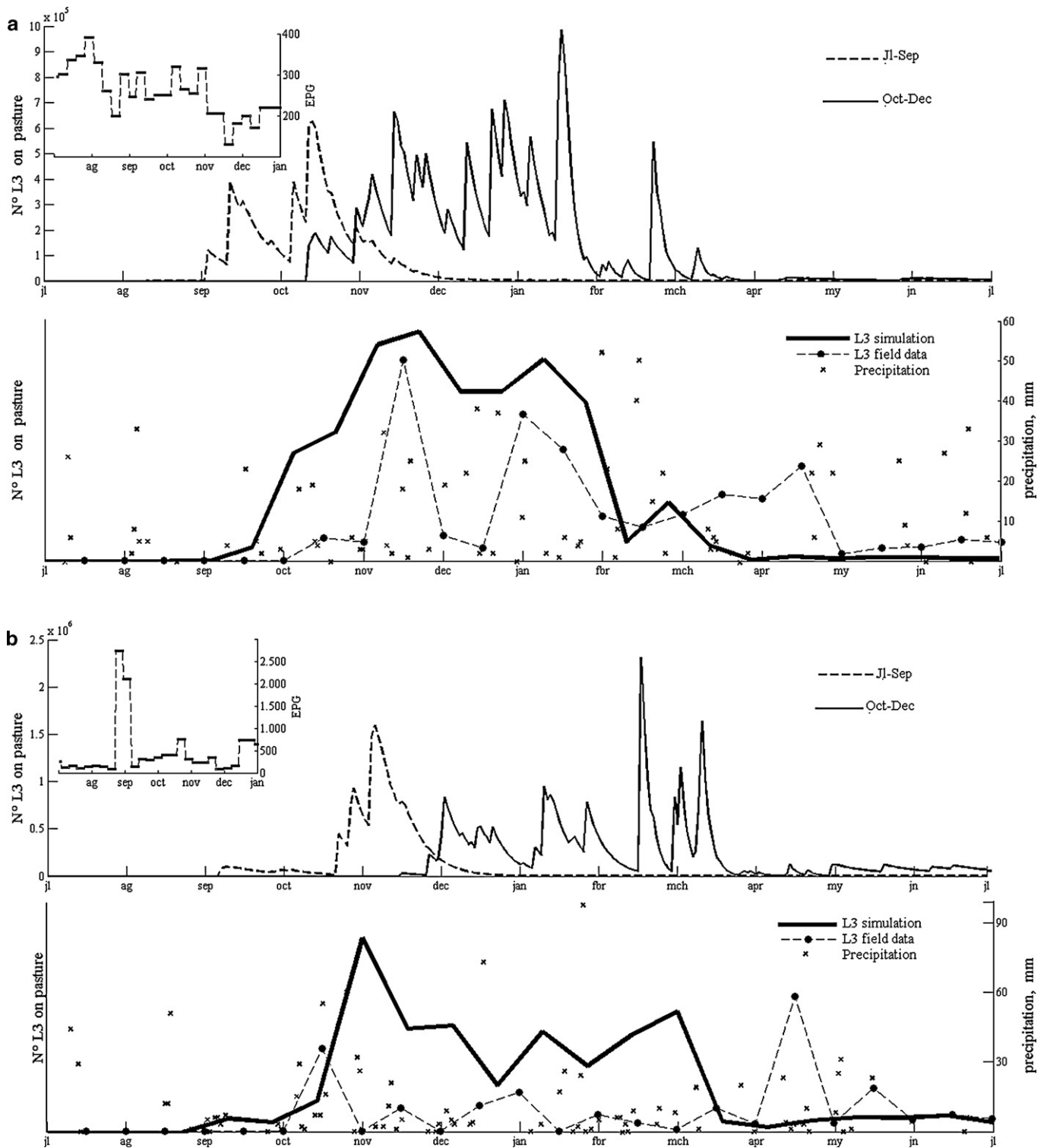
The first L3 larvae corresponding to the July–September cohort (winter) were observed in the pasture on the first days of September. The maximum abundance of larvae took place in November, sustaining significant levels until the early days of December. The October–December cohort (spring) exhibited a sequence of peaks of infection, maintaining moderate-high levels, from December until

mid-January. Afterwards, the population decreased until disappearing by mid-March. The simulated results are shown in Fig. 5(a).

#### 4.3.2. Period July–December for the 1997 data

In this semester, the mean temperature was equal to the annual mean of Tandil (13.75 °C) and the total precipitation (618 mm) was considerably higher than the mean for this second period (433 mm).

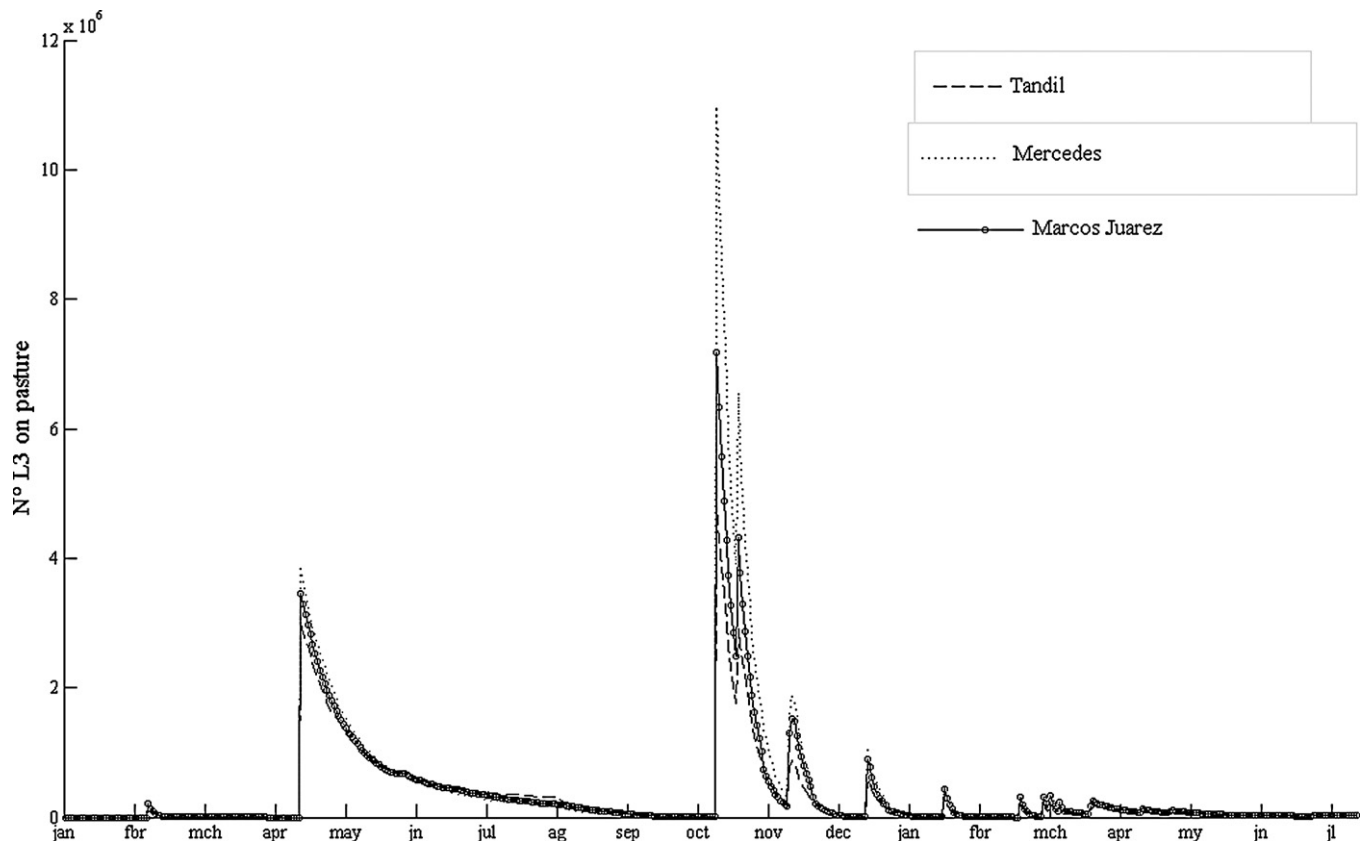




**Fig. 5.** (a) (i) Simulation output corresponding to the 1996 winter-spring season showing the July–September cohorts (---) and the October–December cohorts (—). The upper window shows the EPG daily values used in the simulation for the semester July–December. (ii) Simulation output averaged over two-week periods (—) and field data (●), also showing precipitations recorded over the study period (×). (b) Same as above for the 1997 winter-spring season.

The July–September cohort (winter) did not exhibit significant presence in pasture from July to October. The maximum abundance of larvae took place in November, then the population decreased steadily until disappearing by the end of December. The first L3 larvae in the October–December cohort (spring) appeared in mid-November but survived only a few days in pas-

ture. The maximum abundance of larvae was reached by the end of February. Finally L3 larvae exhibited a minimal level from April to July. It is worth noting the effect of heavy rainfall on mortality during the L1 and L2 stages and the impact in the dynamics of pasture infection. The simulated results are shown in Fig. 5(b).



**Fig. 6.** Response to variations in temperature. The time series corresponding to: Mercedes, province of Corrientes (···, annual mean temperature ( $\mu$ ) = 20.24 °C, standard deviation ( $\sigma$ ) = 5.85 °C), Marcos Juarez, province of Cordoba (○,  $\mu$  = 17.3 °C,  $\sigma$  = 5.77 °C), and Tandil, province of Buenos Aires (---,  $\mu$  = 13.75 °C,  $\sigma$  = 5.4 °C). Precipitation data are those corresponding to Tandil for better comparison.

#### 4.4. Analysis of the model's response to variations in environmental conditions

The dynamics of pasture infection did not vary significantly between simulations with different temperature series (Fig. 6).

Precipitations were set to occur in one season (winter, spring summer or autumn). The results of the various simulations show significant differences. These differences were noted both in the values of the peak of infection and the length of infection in pasture.

When spring was rainy, L3 larvae were present in pasture during all spring and summer. If the rainy season was in winter then the presence of L3 in pasture began on the first days of winter, due to the summer–autumn L3 cohorts waiting to migrate combined to high survival rates within dung pat and pasture because of low temperatures. Rainfall in summer and high temperatures caused the L3 in pasture to be present only a few days in January and February. However, in March the infection peaked, and these L3 survived on pasture until next spring because of the temperature decrease in autumn. Rainfall in autumn was most beneficial to the population, since the L3 arrived in the pasture in autumn and survived until the end of next spring. The simulated results are presented in Fig. 7.

Also, the model allows simulating worm control. We can consider the effect of an anthelmintic suppression treatment on the series of EPG values by, for example, supposing that when the anthelmintic is most effective, the EPG count falls almost to 0. As an example, we can consider a simulation using weather conditions corresponding to Tandil (2005 data series) and a theoretical EPG curve. The cattle were treated two times, once with ivermectin and the second with moxidectin (controls faecal eggs for five weeks) in midsummer (February 20th) and in midwinter (August 1st).

Due to the fact that the prepatent period is three weeks, we added this time to “control time”. Therefore the total time of control was eight weeks by application. As a result of the simulation, it was observed that pasture infection was strongly affected. With only these two treatments the population of L3 on pasture was reduced to almost 0 during autumn and winter. Results are shown in Fig. 8. Strategic deworming maximizes resources spent on controlling *O. ostertagi* in cattle on grass. The model can be a tool for generating new strategies for deworming.

#### 5. Discussion

Understanding the epidemiological dynamics is very important when attempting an effective and efficient control of the disease that causes large economic losses. Accomplishing the characterization of the dynamics requires a lot of effort. A minimum of four years of data collections is required to develop a complete study of the disease.

The model here constructed allows to visualize possible effects on the pastures in relation to the values of average EPG in the roundup. Clearly, the number of animals in the paddock affects the amount of eggs that are contributed to the system.

This model is built based on the data on egg-to-L3 development time, pasture infectivity levels over time, and L3 survival on pasture provided by parasitologists. Parasitologists' expertise is fundamental for setting the linguistic variables and the inference rules for the different fuzzy parameters that are being used in the model. Consistent and detailed data sets obtained over the period 1994–1997 have been used for the calibration and corroboration of the model.

It is necessary to emphasize that the field study did not consider the stocking rate. A higher number of animals causes the more

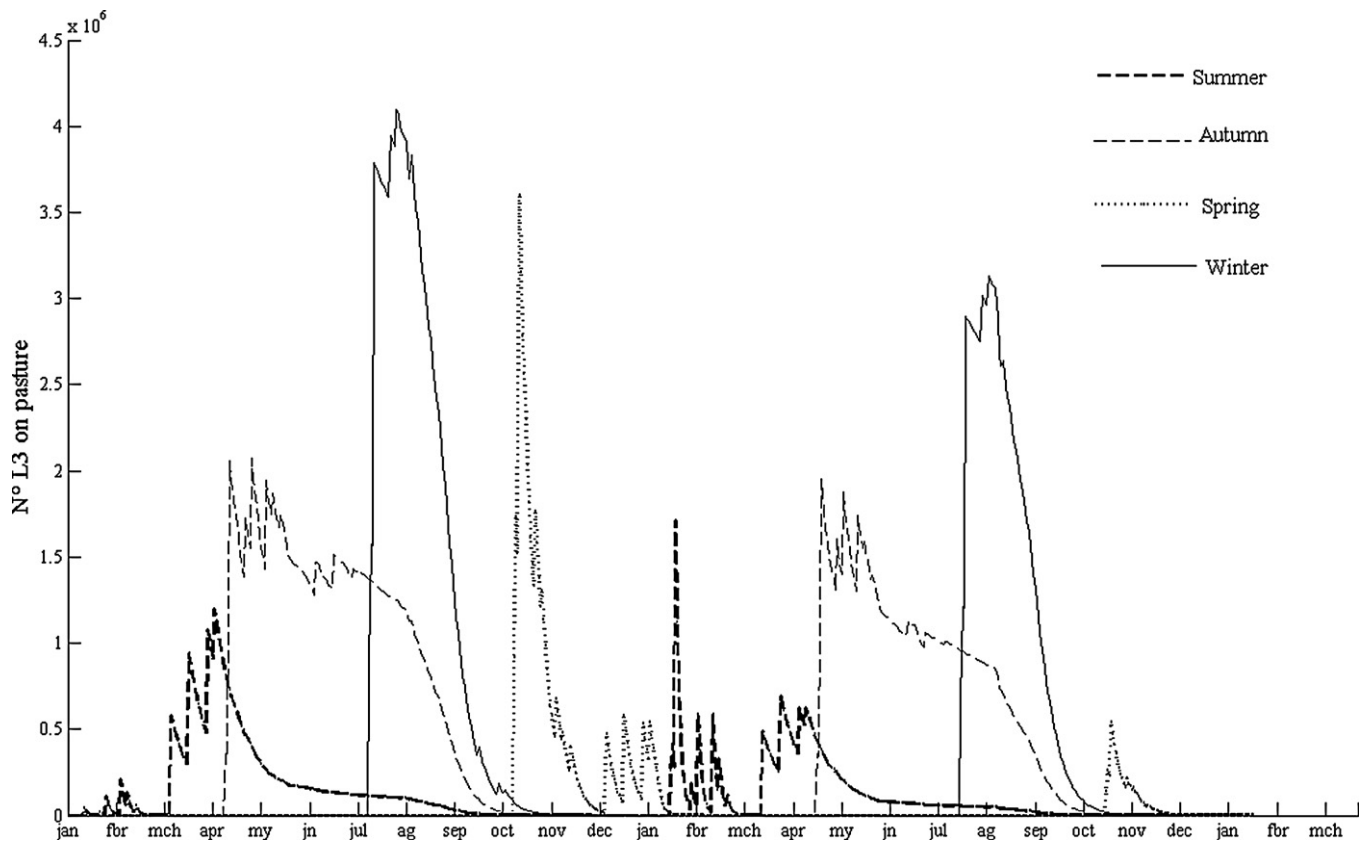


Fig. 7. Response to variations in the seasonality of precipitations: (---) summer; (---) autumn; (—) winter; (···) spring. Precipitations were allowed only during the corresponding trimester. Temperature data are those recorded in Tandil in 1995.

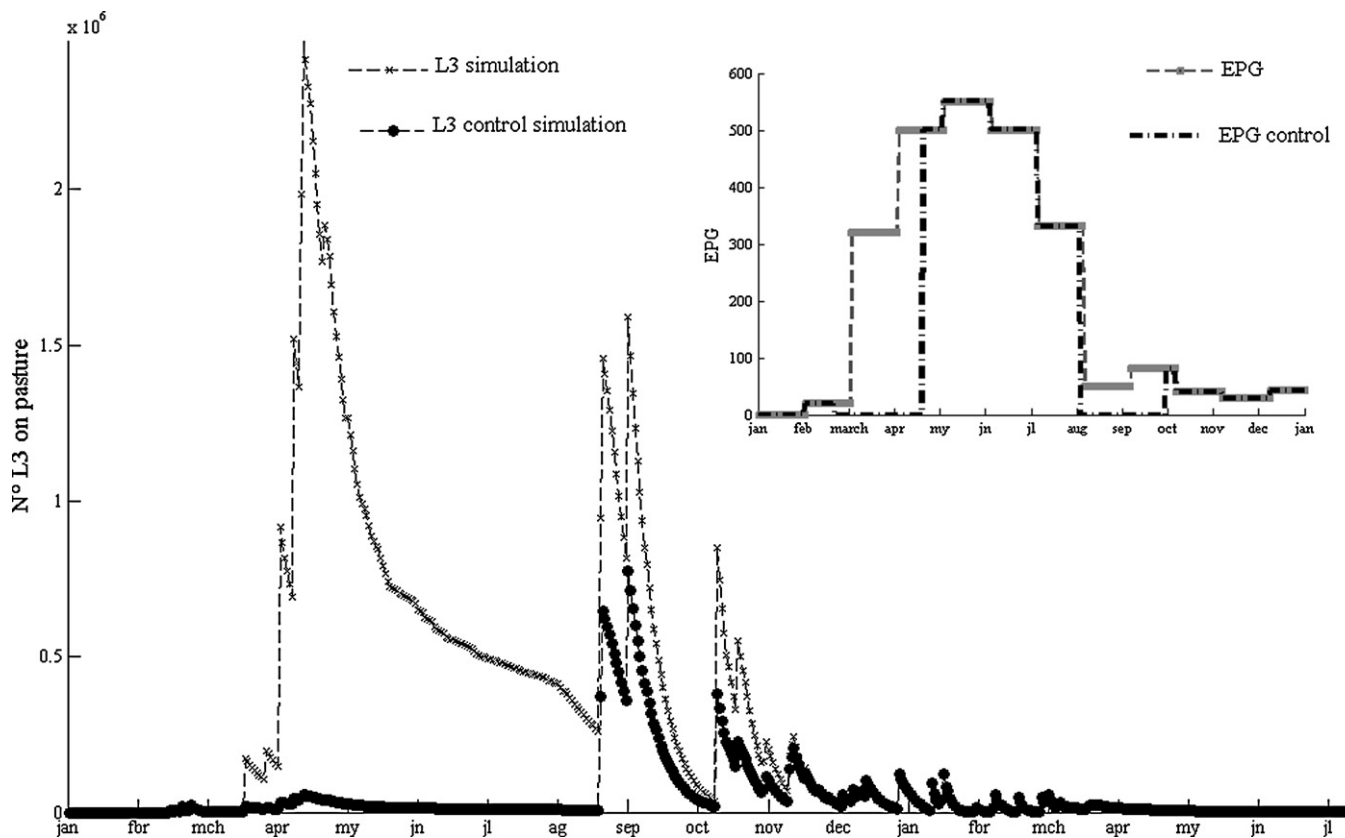


Fig. 8. Simulation of a possible strategy of parasite control. Weather conditions corresponding to Tandil, 2005 and a theoretical EPG curve were used. The cattle were treated in midsummer and in midwinter. The curves show the effect on L3 on pasture without parasite control (x) and with parasite control (o).

**Table 7**

Beginning of pasture infection obtained from field trials and estimated by the model from Tandil's precipitation and temperature data.

Semester (year)	Beginning of infection	
	Field	Simulation
1 (1996)	April 1996	January 1996
2 (1996)	October 1996	September 1996
1 (1997)	March 1997	February 1997
2 (1997)	October 1997	September 1997

**Table 8**

Peak of pasture infection obtained from field trials and estimated by the model from Tandil's precipitation and temperature data.

Semester (year)	Peak of infection	
	Field	Simulation
1 (1996)	July 1996	July 1996
2 (1996)	November–December 1996	November–December 1996
1 (1997)	July–August 1997	May–June 1997
2 (1997)	April–May 1998	November 1997

trampling of dung pats and therefore a higher level of mechanical damage that affects the survival of L3 larvae (more larval death and reduced amounts of grass). We believe this aspect will be important when constructing a model for the parasitic stage.

The results of simulations suggest different implications about the dynamics of infection subject to the variation of meteorological conditions. It should be noted that the model was analyzed over a wide range of different situations and the resulting infection patterns varied accordingly. The model reflects that the impact of the rainy season on the preinfective (within dung-pat) and infective (in pasture) populations is substantial (Fig. 7). The hypothesis that the rainy season is the most important factor regulating pasture infection (Fiel et al., submitted for publication) has been corroborated using this model. On the other hand, the variation on temperatures series result in minimal impact, affecting only the infection level attained.

The simulations adequately mimic the dynamics of the pasture infection (Figs. 4 and 5). This accurate representation obtained from the model is reflected in the correspondence between the model's output and the field experiment data in several key aspects which are necessary information for a diagnosis of the disease:

- Estimation of the time required for the first L3 larvae to appear in the pasture under different climatic conditions and in different seasons (Table 7).
- Estimation of the Julian day in which the peak infection is expected (Table 8).
- Estimation of the duration of the infection (Table 9).

The estimation of the level of pasture infection in the field is influenced by various factors that can induce poor assessments. Among the factors that may facilitate or hinder the gathering of samples it is possible to list: type of pasture, weather conditions, the time of the year in which samples are taken, and the time of

day (Fiel et al., 1994), as well as the laboratory techniques used in processing the samples. Not only these factors can induce variability in the estimates of L3 larval densities in pastures, but also the way in which the grass is collected or the larvae are recuperated in the laboratory may reduce the counts to any figure between 20 and 60% of the larvae actually present (Ferreira et al., 2003). We believe that the difficulty in obtaining accurate density estimations of larvae actually present in pasture may be the cause of the difference in the density values obtained in the simulations as compared to those estimated from field experiments, which are always lower. However, methods for counting eggs in dung seem to be quite reliable.

Regarding L3 larvae in dung ready to migrate to pasture, it is not yet possible to evaluate the results obtained from the simulations because no data on this matter has been recorded during field experiments. It would be interesting to have this information from future field assessments in order to fully corroborate the model and indirectly evaluate the goodness of the pasture infection estimates. However, model estimates of L3 larvae in dung seem reasonable and can be indirectly evaluated as good given that they are source to the infection levels.

With respect to the rate of migration of L3 from dung to pasture, we believe that it is very important to determine this parameter with an experimental design that is appropriate for the problem, because in the experimental field work migration rates were not taken into account.

As stated earlier, the focus of this model is on the dynamics of the infection, so it is not important at this stage that the model does not yield the value of abundance of L3 on pasture with precision. However, we believe that if given higher precision in field estimates of migration and survival rates on pasture, the model will accurately compute this abundance.

These results encouraged us to test the model with field data from other locations in Argentina and the outcome was very satisfactory. One clear result was that the differences in the infection pattern were observed as response to the precipitation pattern in each location and basically no variation was observed regarding the respective temperature patterns.

The model is able to evaluate possible scenarios that a meat production system could face, giving a rapid answer about the infection dynamics. The scenarios may be as diverse as wished, including simulating worm control. We can consider the effect of an anthelmintic suppression treatment on the series of EPG values by, for example, supposing that when the anthelmintic is most effective, the EPG count falls almost to 0. Therefore it is possible analyze the impact of different strategies of parasite control, simulating the efficiency and/or delay of various drugs on the dynamics of pasture infection. For example, it is possible to generate a set of simulations by varying the time of year of the application of a medical treatment with drugs. The results of a simulation of one possible strategy of parasite control is given in Fig. 8. As seen earlier, the model can be a tool for generating new strategies for deworming, which can have an important economic impact if taking into account the area of the cattle production region.

## 6. Conclusions

The model presented in this paper is a first attempt at modelling the potential effect of environmental conditions on the dynamics of an *O. ostertagi* larval population. The most important features of this model are the simplicity of its formulation and its adaptability to a range of weather conditions. It is expressed in terms of three difference equations and their fuzzy parameters. The results of simulations illustrate the potential of using the fuzzy modelling approach, which incorporates expert knowledge that is not

**Table 9**

End of pasture infection obtained from field trials and estimated by the model from Tandil's precipitation and temperature data.

Semester (year)	End of infection	
	Field	Simulation
1 (1996)	End of November 1996	Mid-October 1996
2 (1996)	End of July 1997	End of July 1997
1 (1997)	End of October 1997	End of September 1997
2 (1997)	End of July 1998	End of July 1998



straightforwardly quantifiable and that couldn't have been possible to include if using classic parameterization methods. The inclusion of knowledge gained through experience along many years of research is a very valuable aspect of this modelling approach. The model synthesises current quantifiable and non quantifiable knowledge and helps identify voids in the information necessary for better understanding the role of factors involved in the life-cycle of *Ostertagia* spp. As such, it helps organize further research.

#### Appendix A. Mathematical modelling with fuzzy rule-based systems (FRBS): a brief introduction

The modelling of non quantifiable concepts by means of fuzzy sets allows giving mathematical meaning to natural language statements. Imprecision and uncertainty are inherent to human thinking and behaviour. We usually perceive what surrounds us, even what is precise, in a fuzzy way and it is this ability to summarise information into classes (fuzzy sets) that separates human intelligence from machine intelligence (Zadeh, 1965). Using fuzzy set theory allows us deal with the vagueness and uncertainty associated with real data. Basically, fuzzy inference system (FIS, Klir and Yuan, 1995) is formed by four components: (a) the *input processor*, which translates non quantifiable or quantifiable inputs into fuzzy sets of their respective universes; (b) the *fuzzy rule base*, consisting of a collection of fuzzy IF-THEN rules, which is a key knowledge-encoding component of fuzzy rule-based systems; (c) the *fuzzy inference engine*, performing approximate reasoning by using the compositional rule of inference; and (d) the *defuzzifier*, which assigns a real (or crisp) number representing the corresponding fuzzy set answer.

First, it is necessary to partition the universe into possible classes (fuzzy set) to build the model. In formal terms a fuzzy set normal  $A$  defined in a discursive universe  $X$  is a set of pairs  $(x, \mu_A(x))$  where  $x$  belongs to  $X$  and  $\mu_A(x)$  is a number in the interval  $[0,1]$  representing the degree of membership of  $x$  in  $A$ . The knowledge and data are used to select variables and to partition the universe. This fuzzy partition, instead of an exact partition, and the fuzzification comprise the process of transforming the data into degrees of membership. The rules comprise different situations and they are defined as conditional statements involving linguistic variables, and values determined by fuzzy sets. Expert knowledge is represented by a set of fuzzy rules, which are of the form IF *this* THEN *that*. Rules make associations between input and output fuzzy sets. They relate one event or process to another event or process, for example:

“IF **ground humidity** is *dry* THEN **irrigation** is *much*”.

Here the linguistic variables are **ground humidity** (input) and **irrigation** (output), and the fuzzy set are *dry* and *much*.

In order to obtain a single value for output corresponding to an input, we need to transform the fuzzy set  $F$  into a real number  $D(F)$ . Such transformation is called *defuzzification procedure*. This procedure is performed in a *defuzzifier* block. Some frequent defuzzification procedures are *centroid defuzzification*.

$$D(F) = \frac{\int x\mu_F(x)dx}{\int \mu_F(x)dx},$$

and center-of-maximum defuzzification

$$D(F) = \frac{\inf\{x : \mu_F(x) = \max_z \mu_F(z)\} + \sup\{x : \mu_F(x) = \max_z \mu_F(z)\}}{2}$$

where  $\mu_F(x)$  is the membership function corresponding to fuzzy set  $F$  (Nguyen and Walker, 1997).

Designing a fuzzy system (choice of membership functions, construction of the rules, choice the defuzzification procedure, etc.)

might depend on the particular problem at hand. However, it is possible to make these choices “optimal” if we have some performance criteria. A design is only useful if it leads to a good representation of input–output relation.

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