ECOLOGY OF THE FREE LIVING STAGES OF CATTLE NEMATODES DURING SUMMER CONTAMINATION IN ARGENTINA WESTERN PAMPAS

SUAREZ V.H.* & LORENZO R.L.*

Summary:

Development, migration and survival of infective larvae (L_3) were studied in the Western Pampeana Region. Faeces of naturally nematode infected cattle were deposited as artificial pats on plots during mid-spring–summer of 1994/1995 and 1995/1996. Since the start and during 1995, the study coincided with a severe drought, rainfalls being 29 % below the 45-year means. The predominant genera recovered were *Cooperia*, *Ostertagia* and *Haemonchus*. Initial and peak recovery of L_3 from pats occurred 8-15 and 15-21 days later respectively. A low percentage of L_3 survived from November (0.3 % L_3) and January (0.06 % L_3) to the following autumn and winter. The mean persistence of larvae detected in pats or herbage was around 200 days from deposition. The migration of L_3 from faecal pats to herbage started 15 to 30 days after deposition according to rainfall occurrence. Maximum herbage recoveries of L_3 from pats deposited in late summer occur during autumn rainfalls. Only, few L_3 were occasionally recovered from soil. Summer conditions were associated with rapid development and translation of L_3 to herbage, but also with low L_3 detection after initial recoveries. Faecal pats deposited from mid-summer were the main source of autumn herbage contamination.

KEY WORDS : cattle, nematode, free-living stages, development and survival, Argentina's Pampas.

Résumé : ÉCOLOGIE DES STADES LIBRES DE STRONGLES DIGESTIFS DES BOVINS AU COURS DE LA CONTAMINATION ESTIVALE DANS LA PAMPA DE L'OUEST DE L'ARGENTINE

Le développement, la migration et la survie des larves infestantes (L_3) ont été étudiés dans la partie ouest de la Pampa Argentine. Des fèces de bovins naturellement infestés ont été déposées sous forme de bouses reconstituées sur des parcelles du milieu, du printemps à l'été en 1994/1995 et 1995/1996. Au début de l'étude et au cours de 1995, une sécheresse exceptionnelle a sévi, les pluies étant de 29 % inférieures à la moyenne des 45 dernières années. Les principaux genres de nématodes rencontrés étaient Cooperia, Ostertagia *et* Haemonchus. *Le début et le pic de récolte de L_3 à l'extérieur des bouses ont eu lieu respectivement 8-15 et 15-21 jours après le dépôt. Un faible pourcentage de L_3 a survécu de novembre (0,3 %) et janvier (0,06 %) jusqu'à l'automne et l'hiver suivant. La persistence moyenne de récolte de L_3 dans les bouses a été de 200 jours après le dépôt. La migration des L_3 des fèces vers l'herbe a commencé 15 à 30 jours après le dépot, en fonction de la pluviométrie. Le maximum de L_3 sur l'herbe à partir des dépôts de fin d'été a été observé lors des pluies d'automne. De faibles taux de récolte de L_3 ont été obtenus occasionnellement à partir d'échantillons de sol. Les conditions estivales ont été associées à un développement et un déplacement rapides des larves des fèces vers l'herbe, mais aussi à des faibles nombres de L_3 récupérées après le début de leur détection. Les fèces déposées à partir du milieu de l'été étaient la source principale de contamination des herbages en automne.*

MOTS CLÉS : *bovin, nématode, stade larvaire libre, développement et survie, Pampa, Argentine.*

INTRODUCTION

Cattle gastrointestinal nematodes are an important economic constraint to stock performance in grazing systems. In the western Pampeana fattening area of Argentina *Ostertagia ostertagi*, *Cooperia oncophora*, *Trichostrongylus axei* and *Haemonchus placei* are the main parasitic species that decreased cattle productivity (Suarez *et al.*, 1990; Suarez *et al.*, 1999). Preventive control of nematode losses by integrated strategies combining anthelmintic treatment with appropriate grazing management implies high epidemiological knowledge. A part of the cycle of these trichostrongylids occurs outside the host and is dependent upon the external environment conditions. The process whereby nematode eggs in faecal pats become infective larvae available for cattle is very important for the understanding of the epidemiology of nematode infection. Therefore, a detailed understanding of the dynamics of larvae populations on the environment is required before management control procedures can be recommended with confidence. In this region infective larvae availability for cattle is highest during autumn (Suarez, 1990). However, the source of these larvae was not investigated and is difficult to estimate because the amount of larvae is the result of conti-

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nuous faecal contamination, larvae development and mortality. In other countries, studies show that dung pats provides a favourable environment for free living stage survival under dry conditions during summer (Durie, 1961; Young & Anderson, 1981). In the eastern Humid Pampeana Region of Argentina, Steffan & Fiel (1986) showed that infective larvae survived in pats or herbage around 5-14 months from deposition. However, the climatic conditions and grass management of the western Pampeana Region differ considerably from those of the humid eastern region and local studies are necessary to understand the ecology of cattle nematode.

As no information was available in this semiarid part of the Pampeana region, the aim of the present study is to determine under natural condition on pasture plots, the contribution made by eggs deposited during mid-spring and summer to larvae availability and to estimate the development, migration and survival of nematode larvae in the faecal pats, herbage and soil.

MATERIALS AND METHODS

Experimental design

The experiment was conducted on the INTA Anguil Experimental Station field at La Pampa province of Argentina from November 1994 until June 1996. In eight trials, dung pats containing gastrointestinal nematode eggs were placed on experimental plots on 30-Nov of 1994, 16-Jan, 28-Feb, 20-Mar, 12-Oct, 12-Dec during 1995, 20-Feb, 19-Mar during 1996. The study was made on a sandy soil covered with lucerne, bromegrass and other natural grasses, which has not been grazed by cattle for at least 10 years.

Experimental rectangular plots of 5 m × 1.5 m of side surrounded with small ditches were made to deposit the experimental faecal pats. Plot herbage was mown to a height of 16-20 cm and subsequently maintained. The faeces were taken from 87 (1994), 155 (1995) and 122 (1996) fattening growing calves from eight to 18 months of age, naturally infected with prevalent gastrointestinal nematodes. These animals kept as donors were representative of the fattening system of the region and reflect the general pattern of the parasites (Suarez, 1990).

Faeces were thoroughly mixed and weighed out into 800 g masses which were shaped into pats of 20 cm in diameter and 8 cm of high. In each trial 30 pats were placed on the plots and spaced 50 cm apart.

At the beginning of each trial, faecal pat samples were examined at eight days after faeces were deposited, then every three days and once the infective larvae was detected in the pat, the faecal pats and surrounded herbage and soil were examined at the 7th, 15th and 30th day and then at monthly intervals. Moreover, another samples were taken during the trial after each rainfall. Faecal pat, herbage or soil sampling of a trial was discontinued after three successive recoveries of zero larvae. For each examination date, six samples of 5 g from different shaped faecal pats were taken at random and the mean value of the larvae recovered per g were calculated. For recovery nematode infective larvae from herbage, the grass samples were taken at random, on a ratio of 25 cm around the faecal pats. Five pinches of grass around each faecal pat were taken until arise 100 samples (around 25 g of grass dry matter). Soil cores of 5 cm were removed near the faecal pats.

Parasitological measurements

At the beginning of each series, estimates of the donor calves contamination were made from three egg counts of mixed faecal pats, according with the method of Roberts & O'Sullivan (1949), and specific infective larval (L_3) differentiation after culture of faecal samples (Suarez, 1997). All faecal pat of 5 g and soil samples of 10 g were subjected to Baermann (1917) modified technique (Suarez, 1997) for 24 hours and examined microscopically to determine number per g and species of infective larvae. Pasture samples were cut to ground level and up to a height of 20 cm. Nematode larvae of these samples were recovered and counted as larvae per dry matter (Suarez, 1997).

Meteorological measurements

Daily rainfall, evaporation, relative humidity, maximum and minimum temperature were recorded from the Department of Meteorology of INTA Anguil, near the experimental side. The faecal water content from each faecal pat sample collection was measured by weighing 10 g before and after drying for 5 h at 100° C.

RESULTS

The annual rainfall in this region ranged between 650 and 700 mm; the highest incidence occurs from October (spring) to April (autumn). The mean maximum summer temperature reaches 30°C and the mean winter minimum temperature falls to 0°C. At the end of 1994 and during 1995, the study coincided with a severe drought, as rainfalls were 29 % below the 45-year means (Fig. 1), and environmental relative humidity was 19.7 % below the 26-year means. From the spring of 1994 to the end of 1995 the temperatures and the evaporation rates exceeded average values, with 2° C and 19.8 % above the last 35-years average, fundamentally during the springs. The faecal pats usually lost 23 to 50 % of their weight within the first 8-12 days after the deposition. After two months of environment exposure, the weight loss of the pats was 56 to 77 %.

The level of nematode infections of donor cattle were very low and faecal counts were below 75 epg. Faecal samples egg counts exceeded the 130 epg only during 1995 March and October.

Genera of infective larvae

The predominant genera recovered from culture of faecal samples from the donor cattle were *Cooperia* (43 %) and *Ostertagia* (30 %) during the spring (Table I) and *Haemonchus* (54 %) and *Ostertagia* (23 %) during the summer months (Table II). *Trichostrongylus*, and *Oesophagostomum* were the other genera recovered.

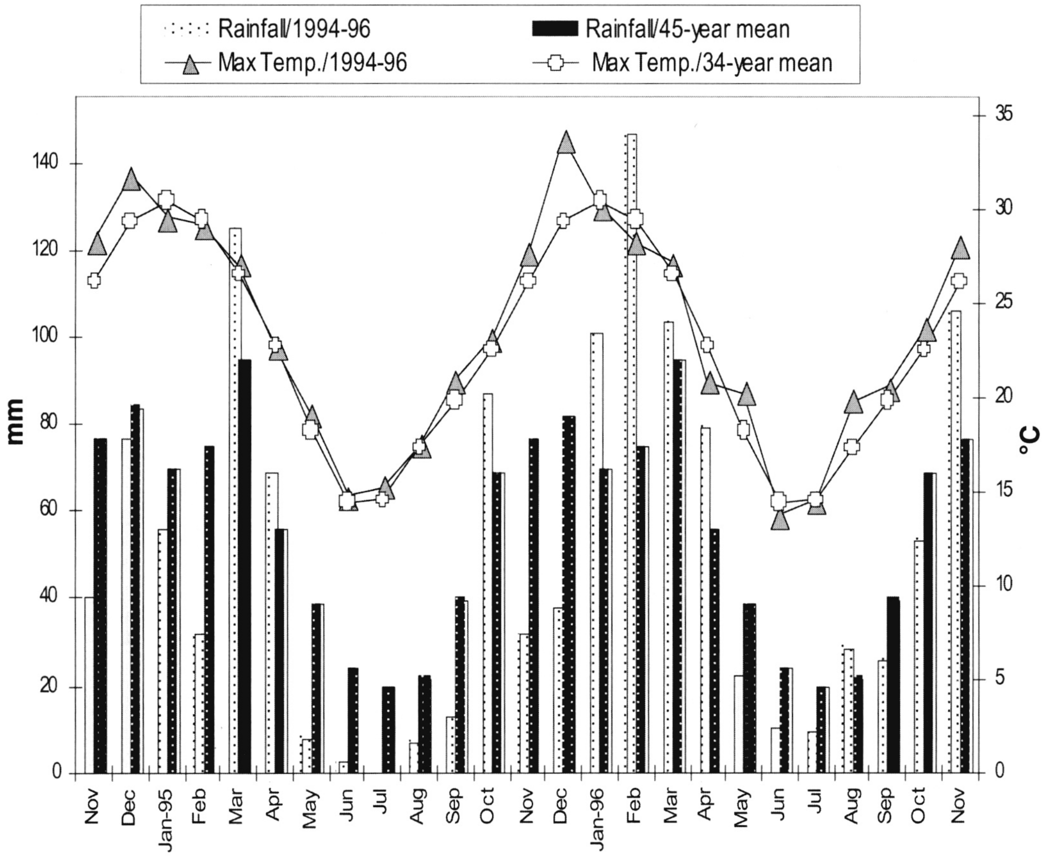

Fig. 1. – Mean monthly maximum temperature and rainfall compared with 34-year average and 45 year average respectively.

Oct./Nov./Dec.	***Ostertagia***		***Cooperia***		**Trichostro.***		***Haemonchus***		**Oesophag.***	
Faecal culture	30		43		13		8		6	
	FPL_3	HL_3	FPL_3	HL_3	FPL_3	HL_3	FPL_3	HL_3	FPL_3	HL_3
November	40		48		10		0		2	
December	30	45	58	35	1	5	11	15	0	0
January	72	70	22	15	5	5	0	10	1	0
February	42	42	36	36	2	1	12	13	8	8
March	20	33	80	33	0	0	0	34	0	0
April	15	23	62	50	0	0	23	27	0	0
May	22	8	78	64	0	0	0	28	0	0
June	0	0	100	100	0	0	0	0	0	0

* Trichostro.: *Trichostrongylus*; Oesophag.: *Oesophagostomum*.

Table I. – Mean percentage of L_3 genera recovered from initial culture of faecal samples from the donor cattle and from faecal pats (FPL_3) deposited in October, November and December. Mean percentage of L_3 genera recovered from herbage from the same month trials ($H\ L_3$).

Oct./Nov./Dec.	*Ostertagia*		*Cooperia*		Trichostro.*		*Haemonchus*		Oesophag.*	
Faecal culture	23		15		6		54		2	
	FPL_3	HL_3	FPL_3	HL_3	FPL_3	HL_3	FPL_3	HL_3	FPL_3	HL_3
February	33	38	10	10	10	14	44	38	3	0
March	26	22	8	12	18	28	48	38	0	0
April	25	36	15	27	20	8	40	25	0	4
May	30	34	13	14	12	17	45	35	0	0
June	50	15	33	29	10	36	7	18	0	2
July	50	43	30	25	15	15	5	17	0	0
August	43	55	29	22	20	11	8	12	0	0
September	75	60	0	25	25	15	0	0	0	0

* Trichostro.: *Trichostrongylus*; Oesophag.: *Oesophagostomum*.

Table II. – Mean percentage of L_3 genera recovered from initial culture of faecal samples from donor cattle and from faecal pats (FPL_3) deposited in January, February and March. Mean percentage of L_3 genera recovered from herbage from the same month trials (H L_3).

In general, *Cooperia* was the most prevalent genus in faecal pats and herbage from spring deposits at the end of the summer (Table I). In summer deposits of faecal pats and herbage *Haemonchus* was the most prevalent genus until mid-autumn, then followed by *Ostertagia* until the end of the winter (Table II).

Development to infective larvae

In general, the development of eggs to infective larvae was rapid and took no more than two weeks. The eggs disappeared from the pats within 15 days. In most of the trials, the peak recoveries of larvae from faecal pats were obtained immediately within 15-21 days. However, in the trials begun from late spring to early summer (Nov., Dec., Jan.) the time for development to L_3 was reduced to 8-10 days.

Survival of infective larvae

The recovery of infective larvae in each trial from faecal pats, herbage and soil are indicated in Figures 2 and 3. A summary of the average number of L_3 per g recovered from faecal pats and the percentage of L_3 recovered, expressed as a proportion of the initial number of eggs estimated to be in each faecal pat deposition trial are given in Table III. Low numbers of L_3 survived during the spring-summer high temperatures and drought of 1994-1995. A low percentage of L_3 survived from November/94 (0.5 % L_3) and January/95 (0.06 % L_3) respectively to the following autumn and winter (Fig. 2). Larvae in faecal pats deposited in October-1995 survived until July 1996, during 283 days (Fig. 3). This was the maximal length of time during which L_3 were recovered from the dried faecal pats. From the faecal pats deposited in late summer, recovered L_3 persisted until late winter – early spring.

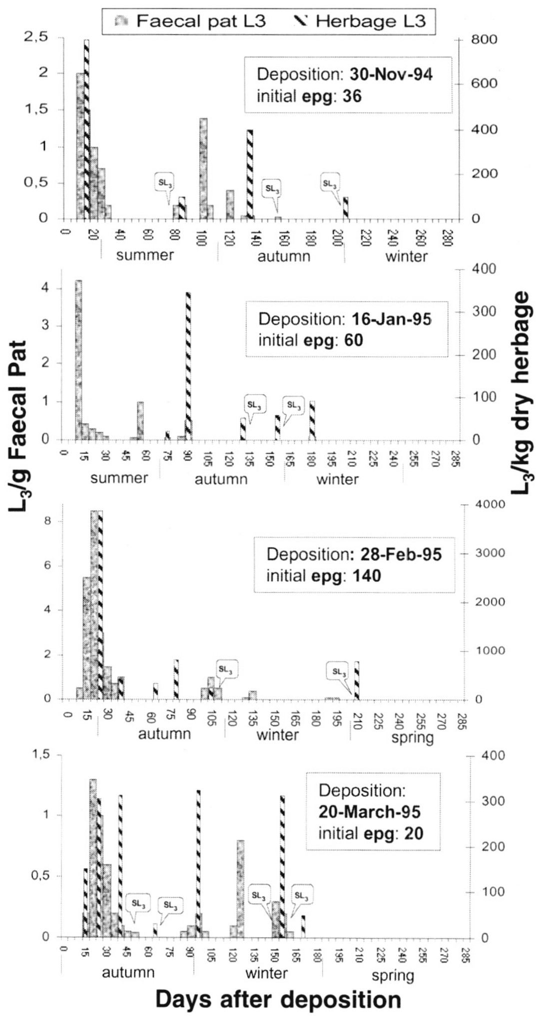

Fig. 2. – Concentration of infective larvae in faecal pats (L_3 per g of dry faecal pat) and herbage (L_3 per kg of dry matter) resulting from pats deposited in November 1994, January, February and March 1995. Detection of L_3 from soil samples (SL_3) and initial epg of the faecal deposition is also indicated.

Faecal pat deposition	Initial epg	Percentage of L_3 Period of days following the faecal pat deposition 8-20 d	21-50 d	51-100 d	101-150 d	151-200 d	> 201 d
30-Nov-94	36	1.98	0.68	0.90	0.18	0.011	
		5.5 %	1.9 %	2.5 %	0.5 %	0.03 %	
16-Jan-95	60	4.20	0.96	0.48	0.04	0.012	
		7 %	1.6 %	0.8 %	0.06 %	0.02 %	
28-Feb-95	140	8.40	2.94	1.54	0.98	0.42	0.10
		6 %	2.1 %	1.1 %	0.7 %	0.3 %	0.07 %
20-Mar-95	20	1.30	0.01	0.30	0.80	0.20	
		6.5 %	5 %	1.5 %	4 %	1 %	
12-Oct-95	130	4.16	6.63	0.91	1.17	0.60	0.20
		3.2 %	5.1 %	0.7 %	0.9 %	0.46 %	0.15 %
12-Dec-95	40	2.88	1.00	0.88	0.05		
		7.2 %	2.5 %	2.2 %	0.12 %		
20-Feb-96	22	1.30	0.40	0.11	0.29	0.20	
		5.9 %	1.8 %	0.5 %	1.3 %	0.9 %	
19-Mar-96	75	6.32	7.35	2.10	0.60	0.06	
		8.3 %	9.8 %	2.8 %	0.8 %	0.08 %	

Table III. – Average number of L_3 per g recovered from faecal pats during each period, and its percentage expressed as a proportion of the initial number of eggs (epg) estimated to be in each faecal pat deposition trial.

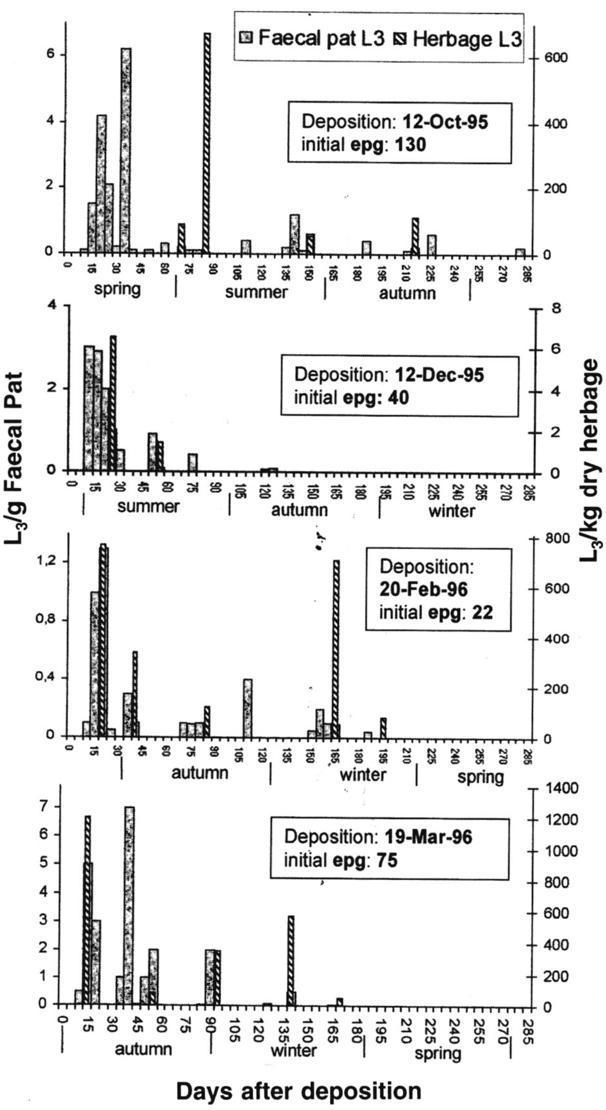

Fig. 3. – Concentration of infective larvae in faecal pats (L_3 per g of dry faecal pat) and herbage (L_3 per kg of dry matter) resulting from pats deposited in October, December 1995 and February, March 1996. Initial epg of the faecal deposition is also indicated.

MIGRATION OF INFECTIVE LARVAE

Larvae recovery from the surrounding herbage varied according to the different weather conditions. The migration of L_3 from faecal pats to herbage started 15 to 30 days after deposition according to rainfall occurrence. During spring and summer when faecal pats dried up, L_3 could be recovered from the herbage only after rainfalls. The results of larvae recovered from the herbage of the trials are show in Figures 2 and 3. Generally, maximum herbage recoveries of L_3 from pats deposited in late summer occur during autumn rainfalls. The maximum number of larvae from herbage (3,800 L_3/dry matter) was obtained for the 23rd day of March 1995 trial deposit. Contrarily, negligible L_3 herbage counts (9 L_3/dry matter) were obtained from pats deposited in January 1996.

Infective larvae from soil were only occasionally recovered from faecal pats deposited during 1994/1995. The number of L_3 detected from 10 g of soil, in general did not exceed 50-100 L_3 and were always recovered after rainfalls.

DISCUSSION

Genera recovered reflect the general pattern of faunistic nematode composition of cattle herds in this region, where the prevalent species were *Ostertagia ostertagi*, *Cooperia oncophora*, *Haemonchus placei*, *Trichostrongylus axei* and *Oesophagostomum radiatum* (Suarez, 1990). In trials begun in spring (Oct., Nov., Dec.), *Cooperia* and *Ostertagia* were the prevalent genera, but the former persisted in the plots for long time until mid-autumn. The lower survival of *Ostertagia*, suggests that this genus was more susceptible to summer higher temperatures (Pandey, 1974) than *Cooperia* (Table I). *Cooperia* showed the highest persistence, according with Rose (1963), that reported a survival time for *C. oncophora* of two years. During trials begun in summer (Jan., Feb., Mar.), the development and survival of *Haemonchus* was better until mid-autumn during the first three months from deposition. It seems to be more susceptible to cold weather conditions than *Ostertagia*, *Trichostrongylus* and *Cooperia* that persisted in more numbers. *Ostertagia* was prevalent from mid-autumn until late winter (Table II).

Eggs developed to infective larvae in the faecal pats deposited in all plots. The rapid rates of development found, where preinfective stages persisted up to about 17 days can be attributed to rapid drying and progressive aeration of the pat and to high maximum mean temperatures of about 28-33° C. Working with *O. Ostertagi*, Pandey (1974) and Young & Anderson (1981) saw that under conditions where oxygen was not limiting and at temperatures over 20° C, eggs or preinfective larvae die or develop to the infective stage within 7-8 days. Conversely, during winter months the authors (V.H. Suarez, unpublished data 1999) reported a delayed development of 15-45 days when the minimum mean air temperature was below 4° C and the pats have a slower rate of drying. Rossanigo & Gruner (1994) show that temperature was the most important factor in the development rate of the cattle nematode species, because there is sufficient moisture in freshly faeces.

In this experiment the rain was necessary for L_3 detection on the herbage, and no migration of infective larvae occurred in its absence during spring and summer periods. These observations coincide with those of Williams & Bilkovick (1973) in Louisiana. In agreement with our results, Pandey (1974) reported that when weather was dry, pats became hard, dry and crusty and therefore soon after L_3 development no migration could occur. Few experiments have indicated that infective larvae are dispersed by rain splash droplets (Gronvold, 1984). Young & Anderson (1981) and Durie (1961) found that rainfalls up to 20 and 25 mm are necessary to allow migration from pats. In our case larvae were detected from herbage after falls of 12-20 mm.

In the trials started during the spring months (Oct., Nov., Dec.), maximum larval herbage recoveries coincided with the first rainfalls on the newly exposed faecal pats. Despite this important and immediate larvae translation to the herbage, a small number of L_3 persisted during the 1994-1995 drought period until the autumn within the faecal pats. According with reports of Goldberg (1970), high temperatures and evaporation rates were probably responsible for the death of most larvae on herbage. Barger *et al.*, (1984), observed that larval survival in faecal pats was enhanced by dry periods as may possibly happen in this study with spring faecal depositions. Our results showed that low numbers of infective larvae from spring contamination survived the summer in faecal pats and could be available in the herbage after the autumn rains. Contrarily of those preceding mentioned reports, negligible number of L_3 was recovered from pats deposited during December 1995 in late spring (Fig. 3), that were exposed to successive rainfall periods alternated with high temperatures during 1996 summer. This absence of repetitive results show the irregularity weather conditions during summer in this region. Probably during summer, larvae that were translated in waves by the rain died as the successive moisture of the pat or the surrounding herbage subsequently dried. During dry summer periods, no L_3 were recovered from pats in absence of rains until the re-establishment of moist conditions. Perhaps, L_3 became desiccated and non motile during dry conditions and survived in an anhydrobiosis state (Todd *et al.*, 1977, Demeure *et al.*, 1979) in the central part of the pat, and it is unlikely that such L_3 would be recovered using the techniques employed in this study. Then with moist conditions, L_3 would be re-hydrated, motility restored and the larvae were then be able to be recovered with Baermann techniques.

The largest L_3 recoveries from herbage were obtained early in the autumn from pats deposited during late summer after abundant rainfalls. This autumn high level of infective larvae, not necessarily associated with rainfall, coincided with previous results in this region (Suarez, 1990), about contamination patterns, where L_3 availability for weaned calves was high during autumn.

The irregular rate of L_3 recovery from soil samples during the trials and the fact that, only from 1994-1995 period, L_3 were detected, make results not clear to be interpreted. Gruner *et al.* (1982) showed that active migrations through the soil is favoured according to the amount of soil water content. Therefore, the recovery of L_3 after rainfalls may be favoured by the increase of soil moisture. Borgsteede & Boogaard (1983) in the same line with our observations, found very few larvae in the soil and concluded that survival in the soil was negligible.

In conclusion, results show that eggs deposited from February to March make the most important herbage infestation since late summer to autumn and provide the source of infections for weaned calves or yearling fattening cattle. Likewise, the possibility of considerable infections occurring in late spring-summer would also be likely as in periods of frequent heavy rainfall associated with young weaned calves or high stoking rates on permanent pastures. The mean persistence of larvae detected in pats or herbage was around 200 days from deposition. In general, spring-summer condition was associated with rapid development and translation of larvae to herbage, but also with low pat or herbage larvae detection after initial recoveries.

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