

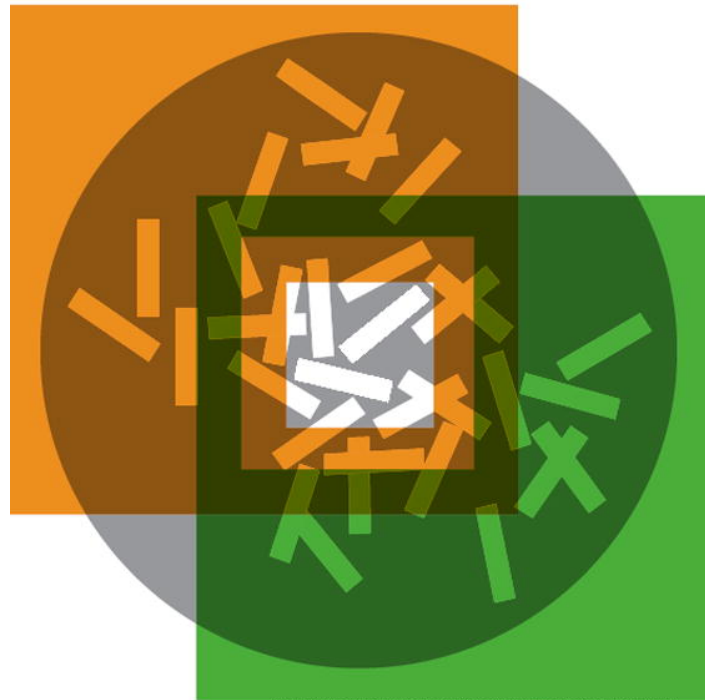
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Dynamics of infections with gastrointestinal parasites and *Dictyocaulus viviparus* in dairy and beef cattle from Costa Rica

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Abstract

A longitudinal survey was carried out to determine and describe the prevalence and intensity of gastrointestinal parasite infections and *Dictyocaulus viviparus* in a dairy and a beef cattle farm of two different ecological zones in Costa Rica. The influence of anthelmintic treatment, age and meteorological factors (rainfall, minimum and maximum temperatures) on gastrointestinal nematodes and *D. viviparus* counts was determined. Calves were subjected to monthly sampling of feces and blood between April 2002 and March 2003. Coprological techniques were used to detect gastrointestinal helminthes, protozoan and *D. viviparus*. Blood samples were analyzed for antibodies to *D. viviparus* by ELISA. The most prevalent gastrointestinal parasites detected on both farms (dairy cattle, A; beef cattle, B) were *Eimeria* spp. (94.7%, 93.7%), Strongylidae (75.0%, 81.4%), *Buxtonella sulcata* (38.0%, 21.6%) and *Strongyloides papillosus* (29.8%, 31.7%), whereas *Moniezia benedeni* (4.8%, 9.1%), *Trichuris* spp. (7.3%, 13.2%), *Toxocara vitulorum* (0.0%, 1.8%) and *Entamoeba bovis* (2.5%, 1.1%) were less prevalent. Mean fecal egg counts (FEC) showed highest values of Strongylidae in April, May and July (>335.3 eggs/g feces) on farm A, and April, May and August (>304.3 eggs/g feces) on farm B. *S. papillosus* presented low FEC throughout the year on farm A, on farm B the highest values were obtained in April (303.0 eggs/g feces). *Trichuris* spp. presented maximum FEC values in May (328.6 eggs/g feces) on farm A and in June (157.5 eggs/g feces) on farm B. Treatment and age had significant influence on infection intensity of Strongylidae (farms A and B), *S. papillosus* (farms A and B) and *Trichuris* spp. (farm A). Rainfall had significant effect on *S. papillosus* (farms A and B) and *Trichuris* spp. (farm B). Maximum temperature showed significant effect on *S. papillosus* (farm A) and *Trichuris* spp. (farms A and B). Minimum temperature had significant influence on Strongylidae (farm A), *S. papillosus* (farms A and B) and *Trichuris* spp. (farm B). *Haemonchus* spp. (57%, 66%) and *Cooperia* spp. (30.0%, 30.7%) were the most prevalent genera identified by coproculture on both farms, in contrast, *Trichostrongylus* spp. and *Oesophagostomum* spp. were less frequent. Patent lungworm infections were low on both farms (10.8%, 1.8%). On farm A, high prevalence of antibodies against *D. viviparus* was determined only at the beginning of the study, in contrast, on farm B the seroprevalence fluctuated throughout the year. Treatment, age and maximum temperature had significant effect on *D. viviparus* counts on farm A, but not on farm B.

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Keywords: Infection dynamics; Gastrointestinal parasites; *Dictyocaulus viviparus*; Dairy and beef cattle farm; Costa Rica

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1. Introduction

Infections with gastrointestinal (GI) parasites and *Dictyocaulus viviparus* include some of the most economically important internal parasites of cattle, which inhabit either the gastrointestinal or respiratory tract, causing parasitic gastroenteritis or bronchitis, respectively. These infections contribute to low productivity in cattle, affecting mainly the health of young grazing cattle (Gibbs and Herd, 1986). The economic losses are associated with severe clinical signs and even more important, with subclinical infections, reduced weight gain, retarded growth and decreased fertility (Perry and Randolph, 1999; Sahoo et al., 2002).

The epidemiology of GI parasites and *D. viviparus* in livestock varied, depending on the local climatic conditions and management practices (including helminth control). These factors largely determine the incidence and severity of various parasitic diseases in a region, being critical issues to be considered in the development of an effective parasite control regime (Gatongi et al., 1997; Thamsborg et al., 1998; Waruiru et al., 2000, 2001).

Despite their importance, studies focusing on the population dynamics of GI parasites and *D. viviparus* in

Costa Rica are lacking. Most of the studies have been conducted to evaluate the anthelmintic efficacy against GI nematodes in bovines. This survey was conducted to provide basic information on the genera or species composition, prevalence and intensity of infections by GI parasites and *D. viviparus*. The effect of anthelmintic treatment, age, rainfall and temperature on nematode counts was determined in a dairy farm and a beef cattle farm from Costa Rica.

2. Materials and methods

2.1. Study area and meteorological data

The study was performed in a dairy farm (A) and a beef farm (B) from April 2002 to March 2003. The farms were located in two different ecological areas, farm A in Poás and farm B in San Carlos, both in the province of Alajuela, Costa Rica (Fig. 1). Farm A was located on latitude $10^{\circ}16'33''\text{N}$ and longitude $84^{\circ}19'02''\text{W}$, 2500 m above sea level, in a lower montane moist forest ecological area (Holdridge, 1978). Farm B was located on latitude $10^{\circ}41'43''\text{N}$ and longitude $84^{\circ}51'39''\text{W}$, 500 m above sea level, in a tropical moist forest ecological area (Holdridge, 1978).

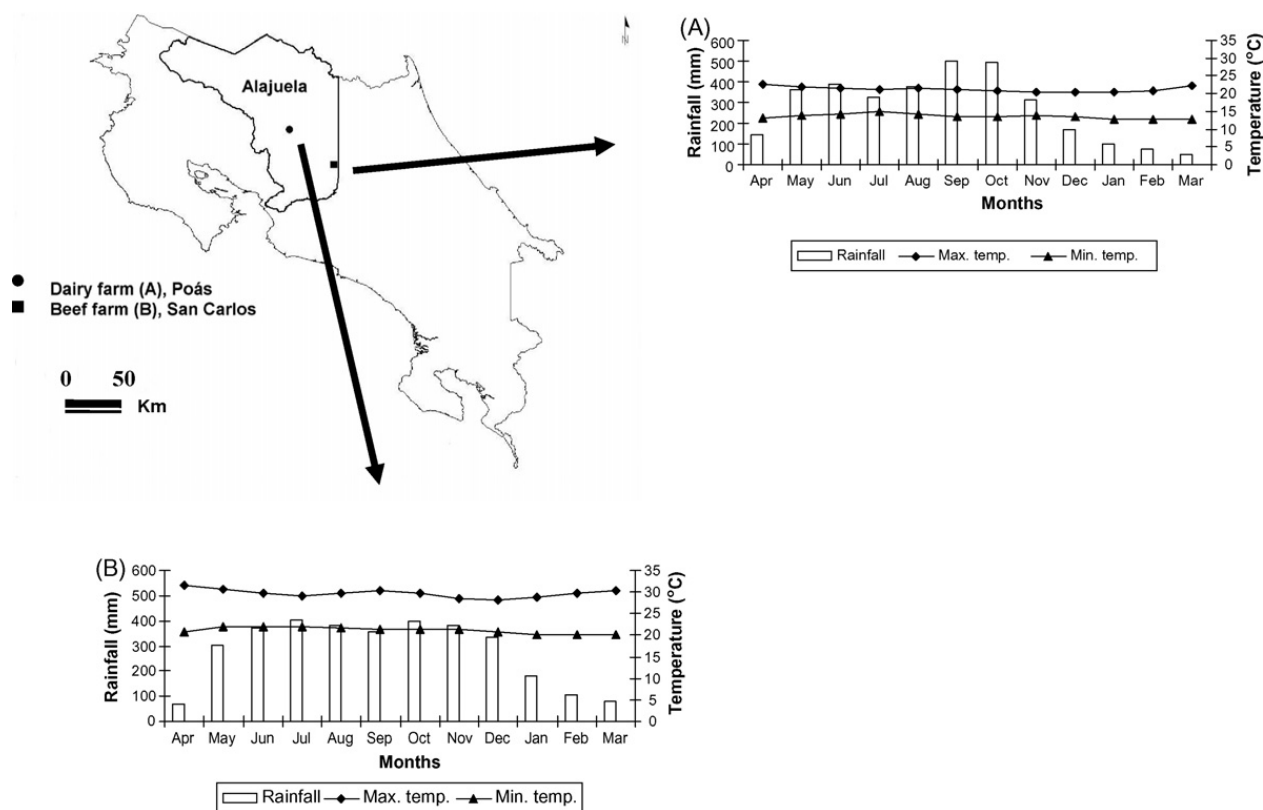


Fig. 1. Map of Costa Rica with the location of the dairy (A) and beef herd (B). Monthly mean rainfall, maximum and minimum temperatures (historical records from 1990 to 2003, National Institute of Meteorology, San José, Costa Rica).

A meteorological station was near each farm, Fraijanes was located 5 km from farm A and Santa Clara 3 km from farm B. The climatic data were obtained from historic records from 1990 to 2003, including the study period (National Institute of Meteorology, San José, Costa Rica). The monthly mean rainfall, maximum and minimum temperatures are shown in Fig. 1.

On farm A the overall annual rainfall was 3333.2 mm (minimum 7.9–maximum 580.7), and mean values of the warmest and coldest months were 22.3 °C (March–April) and 13.1 °C (January–March), respectively. On farm B the overall annual rainfall was 3166.3 mm (minimum 9.1–maximum 583.0), and means of the warmest and coldest months were 31.3 °C (April–May) and 20.1 °C (December–March), respectively. Minimum temperature fluctuated between 12.6 °C and 15.1 °C on farm A and between 20.0 °C and 22.1 °C on farm B. Maximum temperature ranged 20.5–22.6 °C and 28.3–31.6 °C for farms A and B, respectively.

2.2. Animals and management

All animals were in an age range between 3 and 7 months at the beginning of the study and were sampled monthly. The sample size for each farm was chosen using Win Episcope 2.0 software, 95% confidence level (Thrusfield et al., 2001). An expected prevalence of 50% was chosen, to assure the analyses of a maximum sample size, since no previous studies were carried out and the prevalence was unknown. The 37 calves on farm A were Holstein-Friesian and Jersey breeds that were weaned up to 4 days after birth and then fed on milk-replacer. Calves were sent out to pasture between 3 and 4 months of age and were kept separately from adult animals. They rotated every day between 30 available pastures for one full circle. The 45 calves on farm B were Simmental, Brahman, Charolais and crossbreeds that were born on pastures and weaned until 7 months of age. They rotated every 4–5 days between seven available pastures for one full circle. On both farms each pasture remained free for 1 month, until the animals newly returned on it. Sampled animals had access to water from tanks and were supplemented with concentrate and minerals. During the sampling period anthelmintic treatment was given to the animals according to the criteria of the farmers. On farm A, the treatment was done with levamisole (5 mg/kg body weight i.m.) 5–15 days before sampling (May, September and February), whereas on farm B, treatment was done with ivermectin (0.2 mg/kg body weight s.c.) 1–10 days before sampling (August and October) and

with fenbendazole (7.5 mg/kg body weight oral) 1 day after sampling (February).

2.3. Parasitological techniques

Feces and blood samples were collected monthly. A total of 982 rectal fecal samples were subjected to qualitative examination by flotation technique in hyper saturated sugar solution (density 1.3) to detect genera or species of GI parasites of Strongylidae eggs (*Trichostrongylidae*, *Oesophagostomum* spp. and *Chabertia* sp.) and eggs of *Strongyloides papillosus*, *Trichuris* spp. and *Toxocara vitulorum*. Furthermore, the flotation technique allowed to identify cestode eggs (*Moniezia* spp.), cysts (*Buxtonella sulcata* and *Entamoeba bovis*) and oocyst protozoans (*Eimeria* spp.) (Sloss et al., 1995). Fecal egg counts (FEC, eggs per gram feces) were performed only for nematodes following the modified McMaster technique (Sloss et al., 1995). Positive nematode egg feces were processed for coproculture at 27 °C (Kassai, 1999). Identification of Strongylidae infective larvae (L3) was done at genera level (*Haemonchus* spp., *Cooperia* spp., *Trichostrongylus* spp. and *Oesophagostomum* spp.) (Keith, 1952; Bürger and Stoye, 1968; Borgsteede and Hendriks, 1974; Van Wyk et al., 2004) using pooled samples. The percentage of L3 was determined. Fecal first larval stage (L1) of *D. viviparus* was counted (FL1C, larvae per 10 g feces) using the Baermann technique (Kassai, 1999). L1 of *D. viviparus* was identified according to Liébanó et al. (1997). By limited feces quantity, preference was given to McMaster (884 samples analyzed), then to coproculture (884 samples analyzed) and finally to Baermann technique (802 samples analyzed). A total of 1124 serum samples were analyzed by ELISA (Ceditest[®] Lungworm Kit, Lelystad, Netherlands) for the detection of antibodies against *D. viviparus*, according to the instructions provided by the manufacturer (Cornelissen et al., 1997).

2.4. Statistical analysis

Data were analyzed using a Repeated Measures model (Littell et al., 1998) with the MIXED procedure implemented in the SAS software (SAS[®] V.8.0, 1999), because multiple measures of the dependent variables were taken in sequence on the same animal. In this model the dependent variables were FEC and FL1C. FEC of GI nematodes and FL1C of *D. viviparus* were log-transformed [$\ln(\text{FEC or FL1C} + 1)$] to normalize data before analyses. The explanatory variables considered in the model were the categorical variable

anthelmintic treatment and the continuous variables age, rainfall and temperature. A random effect was added to the model assuming an autoregressive covariance structure between multiple measures taken in the same animal (Littell et al., 1998). The effect of the explanatory variables on parasite counts within each farm was assessed with a significance level of $p < 0.05$.

3. Results

Overall prevalences of GI parasites determined on farms A and B are summarized in Table 1. The most prevalent nematodes and protozoan detected on both farms were Strongylidae and *Eimeria* spp. The only cestode found in this investigation was *Moniezia benedeni*. The less prevalent GI parasites were *Trichuris* spp. and *E. bovis* on both farms; *T. vitulorum* was detected only on farm B.

Concurrent GI parasite infections were common showing values of 85% (33 animals) and 83% (38 animals), on farms A and B, respectively. The monthly mean FEC distribution of the most prevalent GI nematodes, in relation with age and anthelmintic treatment, are shown in Figs. 2 and 3. Strongylidae showed highest FEC on farm A in April, May and July and on farm B in April, May and August. FEC were consistently low after July and August on farms A and B, respectively. FEC of *S. papillosus* showed low values throughout the study period on farm A, whereas on farm B high values were detected in April and May. *Trichuris* spp. showed highest

FEC in May on farm A, and in June on farm B. Only on farm B high FEC (4866.7 eggs/g feces) were detected for *T. vitulorum* in April (data not shown).

After levamisole treatment in May, FEC of Strongylidae and *Trichuris* spp. decreased on farm A. On farm B, FEC of Strongylidae, *S. papillosus* and *Trichuris* spp. decreased gradually after ivermectin treatment in August and October. Results of repeated measures analysis are shown in Table 2A and B. Anthelmintic treatment had significant influence on FEC of Strongylidae, *S. papillosus* and *Trichuris* spp. on farm A, but only on Strongylidae and *S. papillosus* on farm B.

Calves with 7–10 months of age (April–July) and 7–11 months of age (April–August) had the highest FEC of GI nematodes on farm A (Fig. 2) and farm B (Fig. 3), respectively. Statistical analysis showed a highly significant effect of age on FEC of Strongylidae, *S. papillosus* and *Trichuris* spp. on farm A, and on FEC of Strongylidae and *S. papillosus* on farm B (Table 2A and B).

On farm A, rainfall, maximum and minimum temperatures had highly significant effect on FEC of *S. papillosus*. Maximum and minimum temperatures presented significant influence on FEC of *Trichuris* spp. and on FEC of Strongylidae, respectively. On farm B, rainfall and minimum temperature had highly significant influence on FEC of *S. papillosus* and *Trichuris* spp., in addition, maximum temperature had significant effect on *Trichuris* spp.

Table 1

Spectrum and overall prevalence of Strongylidae and cestode eggs, protozoan oocysts/cysts and *D. viviparus* first larvae in fecal samples in a dairy (A) and a beef farm (B) during 2002–2003

Parasites	Dairy farm (A) ($n^a = 464$)			Beef farm (B) ($n = 549$)		
	%	Range		%	Range	
		Min.	Max.		Min.	Max.
Nematodes						
Strongylidae	75.0 (348) ^b	30.2	98.0	81.4 (447)	44.7	100.0
<i>Strongyloides papillosus</i>	29.8 (140)	0.0	67.0	31.7 (175)	4.3	77.1
<i>Trichuris</i> spp.	7.7 (61)	0.0	73.2	13.2 (71)	46.3	2.1
<i>Toxocara vitulorum</i>	0.0 (0)			1.8 (10)	0.0	10.6
<i>Dictyocaulus viviparus</i>	10.8 (50)	0.0	51.3	1.8 (9)	2.4	10.3
Cestodes						
<i>Moniezia benedeni</i>	4.8 (22)	0.0	16.7	9.1 (47)	0.0	28.9
Protozoans						
<i>Eimeria</i> spp.	94.7 (440)	75.6	100.0	93.7 (514)	68.9	100.0
<i>Buxtonella sulcata</i>	38.0 (176)	0.0	56.8	21.6 (111)	11.1	38.3
<i>Entamoeba bovis</i>	2.5 (22)	0.0	25.0	1.1 (6)	0.0	12.8

Min.: minimum; Max.: maximum.

^a Total number of animals examined.

^b Number of positive animals.

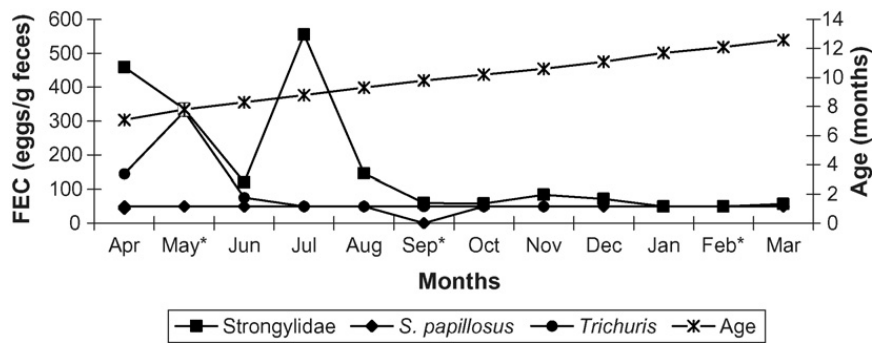


Fig. 2. Monthly geometric means of FEC distribution of GI nematodes in a dairy farm (A) from Poás during 2002–2003. *Anthelmintic treatment.

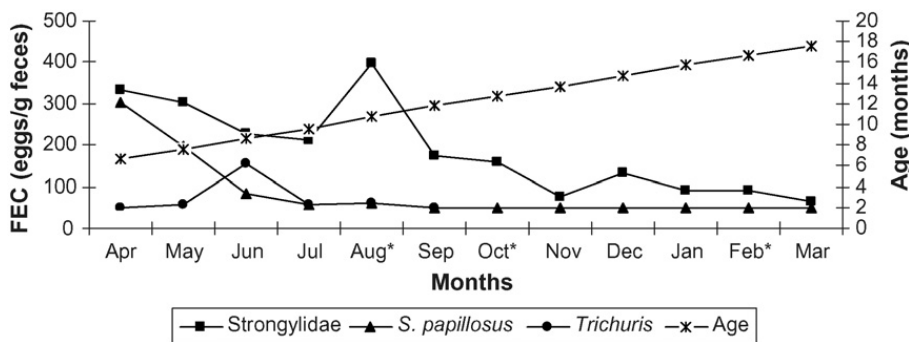


Fig. 3. Monthly geometric means of FEC distribution of GI nematodes in a beef farm (B) from San Carlos during 2002–2003. *Anthelmintic treatment.

The percentage, geometric mean and range of infective strongylidae larvae determined by coproculture of pooled samples during 2002–2003 are presented in Tables 3 and 4. *Haemonchus* spp. and *Cooperia* spp. were identified as the predominant parasites on both farms, whereas the least frequent infective larvae detected throughout the study were *Trichostrongylus* spp. and *Oesophagostomum* spp.

The overall prevalence of patent infections of *D. viviparus* determined on both farms is shown in Table 1. On farm A patent infections were detected at the beginning of the study, gradually diminishing until no larva was detected (Fig. 4). The FLIC of *D. viviparus* presented a median of 15.76 ± 26.98 larvae/10 g feces. Repeated measures analysis showed that anthelmintic treatment had significant influence on FLIC of *D.*

Table 2

Repeated measures analysis of anthelmintic treatment, age, and meteorological factors on FEC of GI nematodes and FLIC of *D. viviparus* in a dairy farm (A) and a beef farm (B)

Variables	Parasites											
	Strongylidae			<i>S. papillosus</i>			<i>Trichuris</i> spp.			<i>D. viviparus</i>		
	<i>F</i>	<i>p</i>	β	<i>F</i>	<i>p</i>	β	<i>F</i>	<i>p</i>	β	<i>F</i>	<i>p</i>	β
A												
Treatment	14.70	<0.0001	-11.799	13.13	<0.0001	-22.941	8.06	0.0004	-7.1313	4.68	0.0098	-2.4760
Age	8.69	0.0034	-0.101	5.27	0.0222	-0.068	4.72	0.0303	-0.0477	33.69	<0.0001	-0.0668
Rainfall	1.58	0.2098	-0.001	11.63	0.0007	-0.002	0.61	0.4370	-0.0008	8.71	0.0034	-0.0008
Maximum temperature	0.17	0.6815	0.070	16.35	<0.0001	0.577	12.34	0.0005	0.375	11.40	0.0008	0.1946
Minimum temperature	28.75	<0.0001	1.065	32.80	<0.0001	0.980	0.13	0.7237	0.0409	0.10	0.7548	-0.0203
B												
Treatment	15.50	<0.0001	-3.892	3.31	0.0374	-9.991	2.75	0.0650	-10.748	0.54	0.5852	-0.5173
Age	25.98	<0.0001	-0.190	32.09	<0.0001	-0.197	0.01	0.9411	-0.0018	0.34	0.5624	-0.0015
Rainfall	0.23	0.6328	-0.001	16.78	<0.0001	-0.008	7.70	0.0057	-0.0048	2.12	0.1463	-0.0002
Maximum temperature	0.05	0.8228	0.037	3.30	0.0701	-0.285	6.79	0.0095	-0.3419	0.31	0.5790	-0.0007
Minimum temperature	1.35	0.2459	0.473	10.59	0.0012	1.175	13.22	0.0003	1.0805	1.62	0.2032	0.0408

Table 3

Percentage, geometric means and range of infective Strongylidae larvae determined by coproculture in a dairy farm during 2002–2003

Months	<i>Haemonchus</i> spp.			<i>Cooperia</i> spp.			<i>Trichostrongylus</i> spp.			<i>Oesophagostomum</i> spp.		
	%	Mean	Range	%	Mean	Range	%	Mean	Range	%	Mean	Range
1992												
April	78.9	58.7	13–85	18.8	14.0	9–12	1.8	1.3	0–2	0.4	0.3	0–1
May ^a	83.2	39.6	12–68	16.8	8.0	2–12	0.0	0.0		0.0	0.0	
June	72.7	12.0	8–16	9.1	1.5	1–2	18.2	3.0	0–3	0.0	0.0	
July	50.4	27.6	3–60	49.6	34.0	11–60	0.0			0.0	0.0	
August	55.3	26.0	18–34	43.6	10.0	1–37	1.1	^b		0.0	0.0	
September ^a	44.4	^b		44.4	2.0	0–2	11.1	^b		0.0	0.0	
October	33.3	7.0	5–9	66.7	14.0	12–16	0.0			0.0	0.0	
November	56.7	8.5	8–9	30.0	4.5	1–8	3.3	^b		10.0	0.0	
December	73.7	21.0	6–36	22.8	4.3	2–6	5.3	1.0	0–1	0.0	^b	
1993												
January	75.0	1.0	1–2	25.0	^b		0.0	0.0		0.0	0.0	
February ^a	86.0	3.0	2–4	0.0	0.0		0.0	0.0		0.0	0.0	
March	82.3	7.0	1–13	11.7	^b		5.8	^b		0.0	0.0	

^a Anthelmintic treatment.^b *n* = 1.

viviparus on farm A, as well as age, rainfall and maximum temperature (Table 2A). The overall prevalence of antibodies against *D. viviparus* in farm A was 42.1% (range 5.3–78.1). High percentage of positive animals with high titers of antibodies (results not shown) were detected at the beginning of the study, from April to September 2002, then a gradual decrease was determined until December 2002, where another increase was detected (Fig. 4). On farm B, patent

infections were observed from April to July 2002 (Fig. 5), the median of FL1C of *D. viviparus* was 1.75 ± 1.49 larvae/10 g feces. Repeated measures analysis indicated that anthelmintic treatment, age and meteorological factors had no significant effect on FL1C of *D. viviparus* on farm B (Table 2B). The seroprevalence of *D. viviparus* was 34.3% (range 6.2–50.0) with two peaks of high prevalence from June to August 2002 and in January 2003 (Fig. 5).

Table 4

Percentage, geometric means and range of infective Strongylidae larvae determined by coproculture in a beef farm during 2002–2003

Months	<i>Haemonchus</i> spp.			<i>Cooperia</i> spp.			<i>Trichostrongylus</i> spp.			<i>Oesophagostomum</i> spp.		
	%	Mean	Range	%	Mean	Range	%	Mean	Range	%	Mean	Range
1992												
April	51.5	17.5	6–29	30.9	7.0	3–10	17.6	4.0	1–7	0.0	0.0	
May	68.8	26.5	8–36	20.8	5.3	4–6	9.1	2.3	1–3	2.6	^b	
June	66.3	33.0	2–68	22.1	11.0	4–33	6.4	5.0	1–13	5.2	5.7	1–9
July	32.5	3.2	1–6	47.5	3.8	2–7	20.0	2.7	1–6	0.0	^b	
August ^a	56.8	94.2	6–220	34.6	57.3	6–160	8.24	20.5	2–40	2.5	6.2	1–20
September	56.1	12.8	3–34	38.6	22.0	2–23	0.9	^b		4.4	1.6	1–3
October ^a	47.3	13.0	5–21	38.2	10.5	1–20	3.6	1.0	1–2	10.9	3.0	1–5
November	67.5	74.0	1–200	20.4	33.5	2–60	12.1	^b		0.0	0	
December	50.0	14.7	1–28	46.6	13.7	1–31	3.4	1.2	1–2	0.0	0	
1993												
January	66.0	11.5	3–20	29.0	^b	^b	5.7	0.0		0.0	1.0	0–1
February ^a	41.0	3.0	1–5	28.0	2.0	1–5	21.0	1.5	1–2	10.0	^b	
March	79.6	45.6	20–77	5.2	^b	^b	3.5	^b		11.7	^b	

^a Anthelmintic treatment.^b *n* = 1.

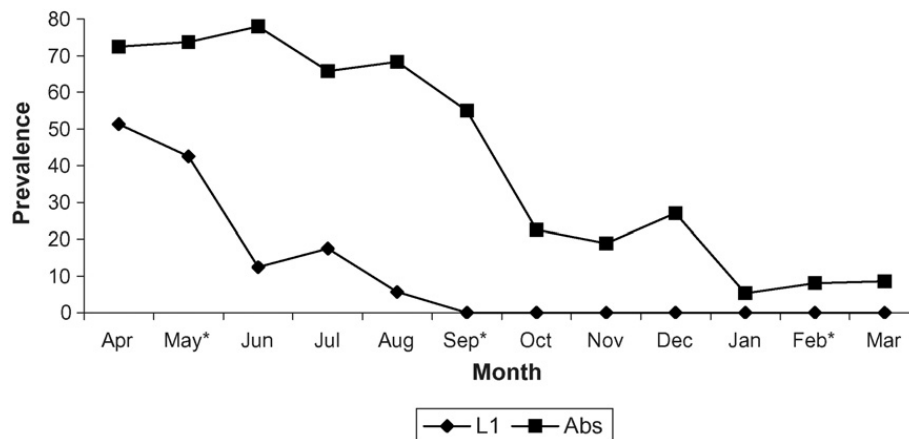


Fig. 4. Prevalence of *D. viviparus* in a dairy cattle farm (A) from Poás during 2002–2003. *Anthelmintic treatment. L1: first larval stage of *D. viviparus*; Abs: antibodies against *D. viviparus*.

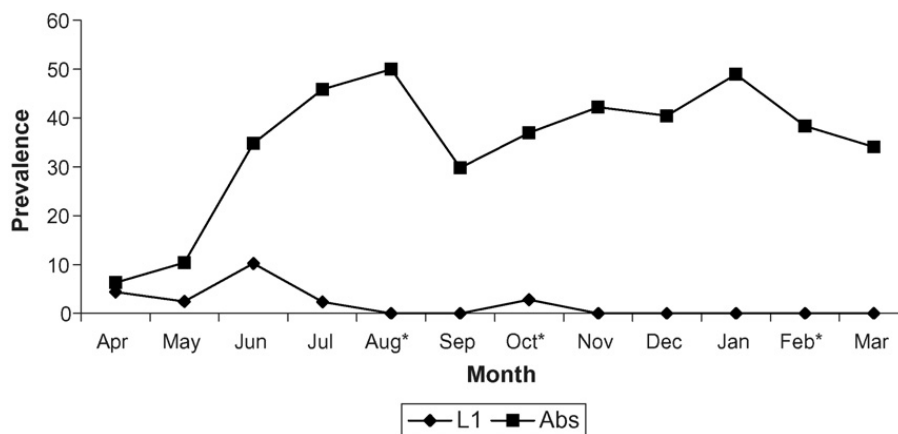


Fig. 5. Prevalence of *D. viviparus* in a beef cattle farm (B) from San Carlos during 2002–2003. *Anthelmintic treatment. L1: first larval stage of *D. viviparus*; Abs: antibodies against *D. viviparus*.

4. Discussion

The most prevalent GI nematode group found in both farms were Strongylidae, which is in accordance with small scale studies carried out in Venezuela (Morales et al., 2001), Colombia (Choperena et al., 2005), Cuba (Socca et al., 2003; Socca, 2005) and Mexico (Vázquez et al., 2004). Morphological differentiation of L3 Strongylidae by coproculture showed that *Haemonchus* spp. and *Cooperia* spp. were the most prevalent species, whereas *Trichostrongylus* spp. and *Oesophagostomum* spp., were the less prevalent detected throughout the study on both farms. These results agree with Fernández (2006), who reported *Haemonchus* spp. and *Cooperia* spp. as the most prevalent parasites in 14 dairy farms investigated in the province of Cartago, Costa Rica, and with reports in other tropical countries (Vázquez et al., 2004; Socca, 2005). The high prevalence of *Haemonchus* spp. in bovine livestock of tropical countries could be due to its great reproductive capacity and

adaptability to different climatic conditions (Vázquez et al., 2004; Socca, 2005). *Cooperia* spp. was the second predominant Strongylidae species detected, this was expected, since it is considered a cosmopolitan species, showing only mild pathogenicity and a lower biotic potential than *Haemonchus* spp. (Urquhart et al., 1996). In future investigations it is mandatory to determine the species and the importance of *Haemonchus* and *Cooperia* infections in bovines from Costa Rica.

The prevalence of *M. benedeni* showed low values on both farms. The low proportion of calves infected, could be due to the opportunity of exposure to the intermediate hosts, the free-living soil mites on the pasture (Cox and Todd, 1962). Although FEC of this parasite was not quantified, it is important to indicate that the farmers did not use any anticestodial drug during the investigation.

Eimeria spp. was the most prevalent protozoan detected in both farms, and the prevalence was similar or lower to the reported in Mexico (Quiróz and Casillas,

1969; Domínguez et al., 1993; Rodríguez et al., 2001). The high prevalence observed in each farm might be related to the fact, that the farmers do not use anticoccidial drugs, and consequently, pastures are contaminated throughout the year, exposing cattle continuously to sporulated oocysts (Matjila and Penzhorn, 2002). *E. bovis*, *E. zuernii* and *E. ellipsoidalis* are species reported in Costa Rica (Ortiz and Ruiz, 1961), but in the present investigation the determination of the species of *Eimeria* were not an objective proposed. However, Pérez et al. (1998) associated diarrhea with coccidiosis (species were not determined) of calves in Costa Rica.

The parasitic infections found on both farms were plurispecific with animals frequently being infected with three or more different parasite genera. This finding is in accordance with studies carried out in Venezuela by Moreno et al. (1996) and Morales et al. (2001). Recently, Wymann et al. (2007) reported that calves (4–13 months) can be parasited by 2–8 parasite types, similar to the findings in the present study.

The highly significant influence of anthelmintic treatment on FEC of Strongylidae, *S. papillosus* and *Trichuris* spp. on farm A, and of Strongylidae and *S. papillosus* on farm B, is in accordance with the reduction of FEC of these parasites, when levamisole, ivermectin or fenbendazole was used. However, at least the required dosage for an effective treatment with levamisole was not correct, since no second application was done 21 days post-treatment. A subdosification and consequently reinfection could have masked the efficacy of the drugs to control *Haemonchus* spp.

Although significant influence of anthelmintic treatment was detected on both farms, an acquired resistance of the calves could not be ruled out, since an effect of age on FEC of *S. papillosus* (farms A and B), Strongylidae (farms A and B) and *Trichuris* spp. (farm A) was also determined (Lima, 1998; Waruiru et al., 2000). Finally, other management practices (“dose and move” system, biological control, pasture type, supplemental feeding), and host factors (sex, genetic, immunity, growth performance, grazing behaviour) factors could have influenced the infection intensity of GI nematodes but were not investigated in the present study (Forbes et al., 2000; Waruiru et al., 2000, 2001; Gasbarre et al., 2001; Dinander et al., 2003; Charlier et al., 2005; Keuyu et al., 2005, 2006).

Rainfall and maximum temperature did not show effect on FEC of Strongylidae on both farms, however, this variable was influenced by minimum temperature on farm A. This could be a restrictive factor for the development of Strongylidae, since the minimum

temperature (13–15 °C) in farm A was outside the optimum limit (20–35 °C) for the development of this group of parasites (Ciordia and Bizzell, 1963; Domínguez et al., 1993).

The prevalence of patent infections of *D. viviparus* reported in this study was lower than the prevalence found in dairy calves from Colombia (Cardona et al., 2005) and represents the first report in Costa Rica. On farm A, patent infections of *D. viviparus* were detected only at the beginning of the study, diminishing gradually, until no larva was detected, whereas on farm B, patent infection within the herd were low throughout the study period. However, the serological prevalence of *D. viviparus* in farms A and B showed different patterns: in farm A the detection of animals with antibodies against *D. viviparus* decreased during the study, and in farm B, the prevalence remained above 30% throughout the study. These results suggest, that probably no further contamination of the pastures occurred with L1 in farm A and consequently the host immune response declined. The immunity against lungworms develops rapidly and disappears within 6–12 months, if no booster infection occurs (Mitchel et al., 1965). On farm B the animals might have been reinfected constantly, reinforcing their immune response (Wassall, 1991). The gradual decrease of the larval output together with the decrease of serum antibodies on farm A seem to indicate that treatment with levamisole was effective against *D. viviparus*. On farm B, the serum antibodies fluctuated throughout the year, indicating that at least the ivermectin and fenbendazole treatments did not show any effect on the antibody course of *D. viviparus*. This can be linked to suboptimal frequency of the treatment, rather than to poor effectiveness of the drug, as it was confirmed by the observation that anthelmintic treatment showed no significant ($p > 0.05$) effect on FL1C.

The results obtained on both farms confirm unpublished data suggesting that clinical cases of Dictyocaulosis are diagnosed mainly in highlands (Poás) and are scarce in lowlands (San Carlos). In the present study a significant effect of rainfall and maximum temperature was determined on FL1C of *D. viviparus* on farm A, which is in accordance with the findings of Thamsborg et al. (1998), indicating that tropical areas of high altitude (from 1000 to 2000 m) allows the development and survival of free-living stages of *D. viviparus*, particularly during the rainy period.

The present study determined that Strongylidae group were the most predominant parasites, represented mainly by *Haemonchus* spp. and *Cooperia* spp., and

that anthelmintic treatment, age and meteorological factors seem to play an important role in the population dynamics of GI nematodes and *D. viviparus* in Costa Rica.

Taking into account that *Haemonchus* spp. and *Cooperia* spp. were present throughout this investigation, future studies are required to evaluate the economic impact of these species in the bovine productivity and to evaluate the deworming practices.

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References

- Borgsteede, F.H.M., Hendriks, J., 1974. Identification of infective larvae of gastrointestinal nematodes in cattle. *Tijdschr. Diergeneesk.* 99, 103–113.
- Bürger, H.J., Stoye, M., 1968. Parasitologische Diagnostik (Teil II). Elzählung und Larvendifferenzierung. *Therapogen Praxidients.* 3, 5–21.
- Cardona, E., Montoya, M., Ospina, J., 2005. Prevalencia de *Dictyocaulus viviparus* en un hato lechero del municipio de Don Matías Antioquia. *Rev. Col. Cienc. Pec.* 18, 385.
- Charlier, J., Claerebout, E., De Muelenaere, E., Vercruyse, J., 2005. Associations between dairy herd management factors and bulk tank milk antibody levels against *Ostertagia ostertagi*. *Vet. Parasitol.* 133, 91–100.
- Choperena, M., Cardona, E., Quijano, J., López, G., 2005. Caracterización de nematodos gastrointestinales de vacunos que llegan a la central ganadera de Medellín. *Rev. Col. Cienc. Pec.* 18, 384.
- Ciardia, H., Bizzell, W.E., 1963. The effects of various constant temperatures on the development of free living stages of some nematode parasites of cattle. *J. Parasitol.* 49, 60–63.
- Cornelissen, J.W.W., Borgsteede, F.H.M., Milligen, F.J.v., 1997. Evaluation of an ELISA for the routine diagnosis of *Dictyocaulus viviparus* infections in cattle. *Vet. Parasitol.* 70, 153–164.
- Cox, D.D., Todd, A.C., 1962. Survey of gastrointestinal parasitism in Wisconsin Dairy Cattle. *J. Am. Vet. Med. Assoc.* 141, 706–709.
- Dinander, S.O., Hoglund, J., Uggla, A., Sporndly, E., Waller, P.J., 2003. Evaluation of gastro-intestinal nematode parasite control strategies for first-season grazing cattle in Sweden. *Vet. Parasitol.* 111, 193–209.
- Domínguez, J.L., Rodríguez, R.I., Honhold, N., 1993. Epizootiología de los parásitos gastrointestinales en bovinos del estado de Yucatán. *Vet. Méx.* 24, 189–193.
- Fernández, A., 2006. Parásitos gastrointestinales y *Dictyocaulus viviparus* en bovinos de fincas lecheras de Costa Rica [Gastrointestinal parasites and *Dictyocaulus viviparus* in dairy cattle farm in Costa Rica]. M.Sc. Thesis. Posgrado Regional en Ciencias Veterinarias Tropicales. Universidad Nacional, Heredia, Costa Rica, pp. 10–36.
- Forbes, A.B., Huckle, C.A., Gibb, M.J., Rook, A.J., Nuthall, R., 2000. Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake and growth in young grazing cattle. *Vet. Parasitol.* 90, 111–118.
- Gasbarre, L.C., Leighton, E.A., Sonstegard, T., 2001. Role of the bovine immune system and genome in resistance to gastrointestinal nematodes. *Vet. Parasitol.* 98, 51–64.
- Gatongi, P.M., Scott, M.E., Ranjan, S., Gathuma, J.M., Munyua, W.K., Cheruiyot, H., Prichard, R.K., 1997. Effects of the three nematode anthelmintic treatment regimes on flock performance of sheep and goats under extensive management in semi-arid Kenya. *Vet. Parasitol.* 68, 323–336.
- Gibbs, H.C., Herd, R.P., 1986. Nematodiasis in cattle. Importance, species involved, immunity and resistance. *Vet. Clin. N. Am. Food. Anim. Pract.* 2, 211–244.
- Holdridge, L.R., 1978. Ecología basada en zonas de vida. Centro Científico Tropical, San José, Costa Rica, p. 216.
- Kassai, T., 1999. *Veterinary Helminthology*. Butterworth-Heinemann, Oxford, p. 260.
- Keith, R.K., 1952. The differentiation of the infective larvae of some common nematode parasites of cattle. *Aust. J. Zool.* 1, 223–235.
- Keuyu, J.D., Kyvsgaard, N.C., Monrad, J., Kassukum, A.A., 2005. Epidemiology of gastrointestinal nematodes in cattle on traditional, small-scale and large-scale dairy farms in Iringa District, Tanzania. *Vet. Parasitol.* 127, 285–294.
- Keuyu, J.D., Kassuku, A.A., Msalilwa, L.P., Monrad, J., Kyvsgaard, N.C., 2006. Cross-sectional prevalence of helminth infections in cattle on traditional, small-scale and large-scale dairy farms in Iringa District, Tanzania. *Vet. Res. Commun.* 30, 45–55.
- Liébano, E., López, M.E., Vázquez, V., 1997. Identificación morfológica de los estadios inmaduros de *Dictyocaulus viviparus* de un aislado de Hueytamalco, Puebla. *Vet. Méx.* 28, 251–254.
- Lima, W.S., 1998. Seasonal infection pattern of gastrointestinal nematodes of beef cattle in Minas Gerais State-Brazil. *Vet. Parasitol.* 74, 203–214.
- Littell, R.C., Henry, P.R., Ammerman, C.B., 1998. Statistical analysis of repeated measures data using SAS procedures. *J. Anim. Sci.* 76, 1216–1231.
- Matjila, P.T., Penzhorn, B.L., 2002. Occurrence and diversity of bovine coccidian at three localities in South Africa. *Vet. Parasitol.* 104, 93–102.
- Mitchel, J.F., Mackenzie, A., Bracewell, C.D., Cornwell, R.L., Elliot, J., Herberth, C.N., Holman, H.H., Sinclair, I.J.B., 1965. Duration of the acquired resistance of calves to infection with *Dictyocaulus viviparus*. *Res. Vet. Sci.* 6, 344–395.
- Morales, G., Pino, L., Sandoval, E., Moreno, L., Balestrini, D., 2001. Dinámica de los niveles de infección por estrongilidos digestivos en bovinos a pastoreo. *Parasitol. Día.* 25, 115–120.

- Moreno, L., Pino, L., Morales, G., Surumay, W., 1996. Análisis de la comunidad de los nematodos del orden Strongylida parásitos de bovinos en relación con la edad. *Vet. Trop.* 21, 3–11.
- Ortiz, G., Ruiz, A., 1961. Eimerias de ganado bovino. *Rev. Biol. Trop.* 9, 215–218.
- Pérez, E., Kummeling, A., Janssen, M.M., Jiménez, C., Alvarado, R., Caballero, M., Donado, P., Dwinger, R.H., 1998. Infectious agents associated with diarrhoea of calves in the canton Tilarán, Costa Rica. *Prev. Vet. Med.* 33, 195–205.
- Perry, B.D., Randolph, T.F., 1999. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. *Vet. Parasitol.* 84, 145–168.
- Quiróz, H., Casillas, M.A., 1969. Coccidias de ganado bovino identificadas en México. *Téc. Pecu. Méx.* 17, 19–22.
- Rodríguez, R.I., Cob, L.A., Domínguez, J.L., 2001. Frecuencia de parásitos gastrointestinales en animales domésticos diagnosticados en Yucatán, México. *Rev. Biomed.* 12, 19–25.
- Sahoo, N., Mohanty, T.N., Samal, S., 2002. Prevalence of gastrointestinal helminthic infection among grazing and stall-fed cattle in a rainfed district of Orissa. *J. Vet. Parasitol.* 16, 61–62.
- SAS, 1999. Statistical Analysis System Institute Inc. Version 8.0. Cary, NC, USA.
- Sloss, M., Kemp, R., Zajac, A.M., 1995. *Veterinary Clinical Parasitology*. Iowa State University Press/Ames, United States, p. 198.
- Socca, M., 2005. Los nematodos gastrointestinales de los bovinos jóvenes, comportamiento en los sistemas silvopastoriles cubanos [Gastrointestinal nematodes in calves, behavior in silvopastoral systems]. Ph.D. Thesis. Universidad Agraria de la Habana, Cuba, pp. 8–35.
- Socca, M., Simón, L., Socca, M., García, E., 2003. Las nematodosis gastrointestinales de los bovinos jóvenes en sistemas silvopastoriles comerciales. I Empresa Pecuaria El Cangre. Pastos y Forrajes 26, 1–6.
- Thamsborg, S.M., Boa, M.E., Makundi, A.E., Kassuku, A., 1998. Lungworm infection (*Dictyocaulus viviparus*) on dairy farms in tropical highlands of Tanzania. *Trop. Anim. Health. Prod.* 30, 93–96.
- Thrusfield, M., Ortega, C., De Blas, I., Noordhuizen, J.P., Frankena, K., 2001. WIN Episcope 2.0: improved epidemiological software for veterinary medicine. *Vet. Rec.* 148, 567–572.
- Urquhart, G.M., Amour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., 1996. *Veterinary Parasitology*. Blackwell Science, London, p. 307.
- Van Wyk, J.A., Cabaret, J., Michael, L.M., 2004. Morphological identification of nematodes larvae of small ruminants and cattle simplified. *Vet. Parasitol.* 119, 277–306.
- Vázquez, V.M., Flores, J., Valencia, C.S., Herrera, D., Palacios, A., Liébano, E., Peleastre, A., 2004. Frecuencia de nematodos gastroentéricos en bovinos de tres áreas de clima subtropical húmedo de México. *Téc. Pecu. Méx.* 42, 237–245.
- Waruiru, R.M., Kyvsgaard, N.C., Thamsborg, S.M., Nansen, P., Bøgh, H.O., Gathuma, W.K., Gathuma, J.M., 2000. *Vet. Res. Commun.* 24, 39–53.
- Waruiru, R.M., Thamsborg, S.M., Nansen, P., Kyvsgaard, N.C., Bøgh, H.O., Munyua, W.K., Gathuma, J.M., 2001. The epidemiology of gastrointestinal nematodes of dairy cattle in Central Kenya. *Trop. Anim. Health. Prod.* 3, 173–187.
- Wassall, D.A., 1991. Use of an ELISA for serodiagnosis of parasitic bronchitis in cattle. *Vet. Rec.* 129, 353–355.
- Wymann, M.M., Bonfoh, B., Traore, K., Tembely, S., Zinsstag, J., 2007. Species diversity and acquisition of gastrointestinal parasites in calves aged 0–13 months in periurban livestock production in Mali. *Vet. Parasitol.* 143, 67–73.