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A cross-sectional survey of gastrointestinal parasites with dispersal stages in feces from Costa Rican dairy calves

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ABSTRACT

A cross-sectional study was carried out to determine the prevalence of gastrointestinal parasites and lungworm nematodes in dairy calves from five different ecoclimatic areas of Costa Rica. Also intensity of infection of nematodes was determined. In order to describe management practices and anthelmintic control, a questionnaire was applied in 73 farms. The influence of area, farm, host (breed, age) and ecological factors (low and high rainfall period) upon eggs per gram feces (epg) of gastrointestinal nematodes (GIN) and first larval stage counts (L1) of *Dictyocaulus viviparus* were investigated. Furthermore, association of host, ecological and management risk factors to the prevalence of gastrointestinal parasites and *D. viviparus* were analyzed. The most prevalent GIN, cestodes and protozoan identified in dairy farms were similar in all areas studied. Strongylidae was the most prevalent parasite group detected, represented mainly by *Haemonchus* spp. and *Cooperia* spp., whereas *Ostertagia* spp. and *Mecistocirrus digitatus* were barely found. The most prevalent protozoan was *Eimeria* spp. The questionnaire applied to producers revealed the following management practices: weaning age of calves 1–4 months (52.1%), semi-confinement of calves upon 5–8 months of age (41.1%), number of paddocks used for calves <10 (57.5%), first deworming of calves at ages ≥15 days (74.70%) and deworming of calves at intervals >60 days (52.1%). Anthelmintic products were changed in 56.1% of the farms at intervals between 13 and 24 months. Although 91.8% of the farms had veterinary assistance, the majority performed parasite control regimes according to the criteria of the producers (66.7%). Common practices were the dispersion of animal feces on the pastures (64.4%) and use of disinfectant in the milking room (63.4%). The analyses of variance showed significant influence ($p < 0.05$) of age, rainfall period, interaction of rainfall period on area (rainfall period × area) and nested effect of farm within area [farm (area)] on epg of Strongylidae; age, area, rainfall period × area and [farm (area)] on epg of *Strongyloides papillosus*; age, rainfall period and farm (area) on epg of *Trichuris* spp.; rainfall period, rainfall period × area and [farm (area)] on L1 of *D. viviparus*. The logistic regression analyses determined area, semi-confinement, management of feces, use of disinfectant in the milking room as risk factors for the presence of Strongylidae, *S. papillosus* and *Trichuris* spp; rainfall, age, paddock numbers for *D. viviparus*; and area, age, veterinary assistance, deworming program, age at first deworming and use of disinfectant in the milking room for *Eimeria* spp. and *Buxtonella sulcata*.

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1. Introduction

In order to design a rational and sustainable control of gastrointestinal parasites in grazing animals, a comprehensive knowledge of the epidemiology of parasites implicated, the interaction of a specific climate with cattle, management systems practiced and the anthelmintic treatments used are a prerequisite (Borgsteede et al., 1998; Barger, 1999; Yazwinski and Tucker, 2006). These epidemiological factors and their influence will allow determining the presentation and impact of parasitic diseases in dairy cattle in a particular region (Jithendran and Bhat, 1999; Wymann et al., 2007). Several studies carried out in tropical and subtropical areas determined climatic conditions, management practices and anthelmintic control as important factors related to gastrointestinal nematodes (GIN), *Dictyocaulus viviparus* and coccidian infections (Rodríguez-Vivas et al., 1996; Vázquez et al., 2004; Keyyu et al., 2005, 2006; Repossi et al., 2006; Wymann et al., 2007; Pfukenyi et al., 2007; Jiménez et al., 2007, 2008; Abebe et al., 2008).

In Costa Rica, specialized milk production systems represent 46% (6408 farms out of 14,355 farms and 138,000 out of 477,446 animals) of the total dairy cattle farms of the country. In 2009, the production of milk contributed with 36.1 million USD (0.87%) to the gross national product of Costa Rica. These specialized dairy farms are located mainly in high regions of the provinces of Alajuela, Heredia and Cartago, and in these provinces 79.9% of the total milk of the country was produced (CORFOGA, 2001; CNPL, 2010).

Previous studies carried out in Costa Rica reported significant association between gastrointestinal nematodes and meteorological factors (rainfall, maximum and minimum temperature) in one dairy and one beef farm of two different geographical areas, as well as significant effects of factors related to farm structure on the seroprevalence of *D. viviparus* (Jiménez et al., 2007, 2008).

This study aimed to determine the prevalence of gastrointestinal parasites and lungworm nematodes in dairy farms located in different ecoclimatic areas, and to analyze the effect of rainfall period on fecal egg counts (epg) of GIN and first larvae counts (L1) of *D. viviparus*. Furthermore, this study intended to describe management practices and anthelmintic treatments carried out in dairy farms of Costa Rica, in order to determine risk factors associated to infections with GIN, *D. viviparus* and gastrointestinal protozoan in these farms.

2. Materials and methods

2.1. Study area and design

An observational cross-sectional study was conducted in 2006–2007 in five different ecoclimatic areas (Poás, San Carlos, Cartago, Tilarán, Alfaro Ruiz), located in the provinces of Alajuela, Heredia and Cartago, where a high number of specialized milk production systems were reported. A total of 73 farms were analyzed. The number of farms analyzed in each area (Poás 11 out of 40, San Carlos 19/90, Cartago 14/60, Tilarán 11/45 and Alfaro Ruiz 18/75) was determined using a stratified random sampling with a 95% confidence level and an expected prevalence of 50%. A correction factor for finite population was applied (Sheaffer et al., 1996).

Farms located in each ecoclimatic area presented similar meteorological and ecological characteristics. Location and altitude of the farms were obtained from CORFOGA (2001). Meteorological data from the different ecoclimatic areas (overall precipitation during low and high rainfall period and temperature) were obtained from the National Meteorological Institute, San Jose, Costa Rica. Low (January–April) and high rainfall period (May–October) comprised the periods with less and most amount of rain, respectively. The life zones present in the different areas studied were characterized according to Holdridge (1978), based on the mean annual biotemperature, annual precipitation and annual potential evapotranspiration (Table 1).

2.2. Animals

In each farm all animals between 4 and 12 months of age were sampled twice during the low and twice during the high rainfall period in intervals of 15 days, and in sampling intervals of 5 months between low and high rainfall period. A total of 2598 animals (626 Poás, 579 San Carlos, 474 Cartago, 436 Tilarán and 483 Alfaro Ruiz) were sampled in both periods (1275 low and 1323 high rainfall period). The calves received anthelmintic treatment according to the criteria of the farmers. The sampling date was chosen at least 1 month before anthelmintic treatment was applied. During fecal sampling, clinical signs in calves were recorded.

2.3. Parasitological techniques

Fecal samples were subjected to qualitative examination by flotation technique in saturated sugar solution (density 1.3) to detect genera or species of gastrointestinal nematodes eggs. Furthermore, the flotation technique allowed to identify cestodes (eggs) and protozoa (cysts and oocysts) (Sloss et al., 1995).

Eggs per gram feces (epg) were determined only for gastrointestinal nematodes, following the modified McMaster technique (Sloss et al., 1995). Animals with >500 epg GIN were considered to have high intensity of infection, whereas animals with <500 epg were classified as having low intensity of GIN infections (Keyyu et al., 2005, 2006). Fecal first stage larvae (L1) of *D. viviparus* were counted (larvae per 10 g feces) using the Baerman technique (Kassai, 1999) and identified according to Liébano et al. (1997). Positive nematode egg feces samples were processed by coproculture at 27 °C for 1 week (Kassai, 1999). Identification of infective larvae (L3) of Strongylidae was done at genera or species level using pooled fecal samples (Keith, 1952; Bürger and Stoye, 1968; Borgsteede and Hendriks, 1974; Van Wyk et al., 2004). Percentage of L3 of Strongylidae was determined.

In order to identify species of coccidia, 73 pooled (mean of 10 animals per farm) fecal samples were mixed with 2% potassium dichromate solution and placed at room temperature for approximately 1 month to allow oocyst sporulation. Prior to examination, oocysts were con-

Table 1
Characterization of dairy farms in five ecoclimatic areas of Costa Rica by meteorological and ecological factors.

Factors	Areas				
	Poás	San Carlos	Cartago	Tilarán	Alfaro Ruiz
Total of farms (*N*)	40	90	60	45	75
Farms analyzed (*n*)	11	19	14	11	18
Mean altitude (min.–max. range) (m)[a]	2227 (2000–2500)	900 (500–2000)	2000 (1500–2500)	786 (1000–1500)	2017 (1500–2500)
Overall low rainfall period (mm)[b]	767.6	268.9	298.1	218.6	243.0
Overall high rainfall period (mm)[b]	1470.9	632.2	515.5	690.6	691.6
Annual mean max. temperature (°C)[b]	21.3	29.7	27.2	26.4	20.4
Annual mean min. temperature (°C)[b]	13.0	21.1	18.1	19.4	13.8
Life zones[c]	LM-wf	LM-rf, T-wf, T-mf, P-wf,	LM-wf, LM-mf, P-mf	LM-wf, LM-rf, T-mf, P-wf	LM-wf, LM-mf

[a] Data from CORFOGA (2001), mm = millimeters, min. = minimum, and max. = maximum.
[b] Data from the National Meteorological Institute, San José, Costa Rica.
[c] Life zones (Holdridge, 1978): LM-wf = Lower Montane wet forest, LM-rf = Lower Montane rain forest, LM-mf = Lower Montane moist forest, T-wf = Tropical wet forest–premontane belt transition, T-mf = Tropical moist forest–perhumid province transition, P-wf = Premontane wet forest, and P-mf = Premontane moist forest basal–belt transition.

centrated with Sheather's solution and identified using morphological keys (Levine and Ivens, 1967; Taylor et al., 2007).

2.4. Questionnaire of management data and anthelmintic treatment

In order to get information about management routines, a questionnaire was applied personally to each producer of the participating farms during 2006, which included: (1) general data of the farm: total area of the farm (ha), total number of animals in the farm, number of animals younger and older than 1 year present in the farm, stocking rate, predominant type of pastures present in the farm (*Pennisetum clandestinum*, *Cynodon nlemfuensis*, *Brachiaria* spp., *Ischaemun ciliare*), breeds (Holstein-Friesian, Jersey, Simmental, Brahman, Charolais, Brown Swiss, Guernsey and cross breeds); (2) management data: length of colostrum feeding (days), substitute food after colostrum feeding (replacer, cow milk, supplement or grass), weaning age (months), confinement of calves (yes/no), age of calves remaining in confinement (months), age of calves remaining in semi-confinement (months), grazing age of calves (months), number of paddocks used for calves, number of paddocks used for adults, time calves remaining in each paddock (days), length of full rotation of calves (days); (3) anthelmintic control: use of deworming program (yes/no), periodic monthly visit of a veterinarian (yes/no), person that recommended deworming (owner, veterinarian, salesman), criteria used to deworm animals, analysis of feces prior deworming (yes/no), age of calves at first deworming process (days), interval between deworming processes (months), type and dose of anthelmintic products used for calves, frequency of change of anthelmintic principle used for calves (<6 months, 7–12 months, 13–24 months), reason for changing deworming product (price, efficacy, resistance, recommendation); (4) additional information: use of dietary supplements (yes/no), type of supplements used, use of feces as installment in the farm (yes/no), methods used to disperse feces on the pastures (gravity method: passive distribution of feces on pastures; aspersion method: application of feces on the pasture in form of pulverized drops by means of irrigation systems; shovel method: dispersion of feces to pastures using a shovel; sawdust method: dispersion of a mixture of feces and sawdust to pastures using a shovel), use of disinfectant in the milking room (yes/no), type of disinfectant used in the milking room. The information of the questionnaires applied to the farmers was included in an Excel database and subjected to statistical analysis.

2.5. Statistical analysis

Descriptive statistical analysis was assessed with the general data of the farms (means, maximum and minimum ranges). Mean prevalences and confidence intervals were determined for gastrointestinal parasites, whereas mean epg, minimum and maximum ranges and standard errors were determined for GIN, using the Statistica® V.6.0 package, 2001 (Tulsa, Oklahoma). General management practices and anthelmintic control routines carried out in the participating farms were described.

A univariate analysis of variance (ANOVA) was performed with epg of GIN and L1 of *D. viviparus* using the General Lineal Models procedure implemented in the Statistica software. In the model, the age, breed, area, rainfall period, interaction of rainfall period on area (rainfall period × area) and nested effect of farm within area [farm (area)] were included as fixed variables. The variable rainfall period × area was included in the analysis, in order to consider differences in rainfall patterns between areas. The nested variable [farm (area)] was added in the analysis, to take into account the hierarchical structure of the data and to avoid confusion of main area variable with farm variable within areas. The dependent variables epg of GIN and L1 of *D. viviparus* were log-transformed before analysis to normalize the data. All fixed variables were added simultaneously in the model and statistical significance ($p < 0.05$) was assessed, using marginal (type III) sum of squares. Each variable was corrected respect to all others in the model.

In addition, a logistic regression model was used to analyze the effect of ecological, host and management variables obtained from the questionnaire on infection status (positive/negative) for Strongylidae, *Strongyloides papillosus*, *Trichuris* spp., *Eimeria* spp., *Buxtonella sulcata* and *D. viviparus*. All answers obtained to one question were divided into classes; the model determined the reference class to compare all other categories (classes) of the variable. Information about total mean area, total mean number of animals, mean number of animals younger and older than 1 year, mean stocking rate, breeds, type and dose of anthelmintic used, analysis of feces before deworming and use of dietary supplements was not included in the model. Farm variables and interactions of farms with other variables of the questionnaire could not be estimated, given the complexity of the model and the highly irregular distribution of samples among farms.

A forward stepwise regression approach was followed in building the logistic model. This discriminator function analysis was defined as a built step-by-step. At each step all variables were reviewed and evaluated to determine which one contributed most to the discrimination between groups. This variable was then included in the model, and the process started again. The procedure was controlled by statistical significance achieved with a chi-square test, assuming an exact distribution. The minimum significance level for a variable to enter in the model was 0.05 and the maximum significance to stay in the model was 0.10. At the final step, only variables that were statistically significant remained in the model (Noordhuizen et al., 2001).

3. Results

3.1. General farm data

The total number of females in each farm ranged from 98 to 242 animals. Animals younger than 1 year represented 33.5–57.3% of the total number of animals in the farm. The stocking rate fluctuated between 1.0 and 2.6 animals/ha/year, the predominant pastures were *P. clandestinum* (49.3%), *C. nlemfuensis* (38.3%), *I. ciliare* (7.0%) and *Brachiaria* spp. (5.4%). The predominant breeds were Holstein-Friesian (65.1%), Jersey (19.8%), Brown Swiss (5.9%), Guernsey (2.8%), Simmental (1.9%), Gyr (0.34%), Brahman (0.15%), Charolais (0.11%) and cross breeds (10.2%) (Table 2).

3.2. Prevalence and epg

The most prevalent GIN, cestodes and protozoa identified in the dairy farms were similar in the five ecoclimatic areas analyzed. The Strongylidae group showed mean prevalences between 62.1% and 77.9% in calves (Table 3). The percentage of infective larvae found in fecal culture in the five geographical areas during low and high rainfall period are shown in Fig. 1. The most prevalent genus of GIN was *Haemonchus* spp. in both periods (59.1–66.0% low, 33.4–70.0% high rainfall), followed by *Cooperia* spp. (9.9–29.7% low, 12.9–59.1% high rainfall), *Trichostrongylus* spp. (3.5–12.8% low, 0–15.5% high rainfall) and *Mecistocirrus digitatus* (3.9–11.1% low, 1.9–13.7% high rainfall) (Fig. 1). *Ostertagia* spp. (0–11.1% low, 0–8.3% high rainfall period) and *Oesophagostomum* spp. (0.1–7.2% low, 0–3.3% high rainfall period) were barely found (data not shown). Furthermore, mean prevalences of *S. papillosus* (4.1–20.4%), *Trichuris* spp. (7.8–14.5%), *D. viviparus*

Table 2
General data of the analyzed farms (total means, minimum and maximum ranges).

Characteristics	Areas				
	Poás	San Carlos	Cartago	Tilarán	Alfaro Ruiz
Number of farms	11	19	14	11	18
Area (ha) (min.–max.)	283.8 (52–827)	121.0 (55–400)	106.5 (22–240)	106.1 (40–225)	60.6 (17–143)
Animals	186.2 (72–260)	121.1 (30–400)	241.9 (54–630)	146.6 (56–320)	97.7 (43–160)
Animals <1 year old	47.5 (20–70)	33.8 (8–200)	57.3 (7–180)	39.4 (6–130)	33.5 (11–70)
Animals >1 year old	138.5 (52–205)	84.8 (20–200)	145.1 (70–400)	108.3 (20–249)	62.0 (19–140)
Stocking rate	1.8 (0.5–5.0)	2.6 (1.3–19.7)	1.5 (1.8–2.0)	1.0 (0.56–1.2)	1.0 (0.19–1.64)
Predominant pastures	*Pennisetum clandestinum*	*Cynodon nlemfuensis*, *Brachiaria* spp. and *Ischaemun ciliare*	*Pennisetum clandestinum* and *Cynodon nlemfuensis*	*Pennisetum clandestinum*	*Pennisetum clandestinum*
Breeds[a]	H-F, J, and cross breeds	H-F, J, SM, BS, GY, BR, CH, and cross breeds	H-F, J, BS, and GU	H-F, J, BS, SM, GY, and cross breeds	H-F, J, BS, SM, and cross breeds

[a] H-F = Holstein-Friesian, J = Jersey, SM = Simmental, GY = Gyr, BR = Brahman, CH = Charolais, BS = Brown Swiss, GU = Guemsey, cross breeds = Holstein × Jersey, Holstein × Brahman, Holstein × Simmental, Holstein × Brown Swiss, and Gyr × Holstein × Jersey.

Table 3
Mean prevalences of nematodes, cestodes and protozoan in calves from five ecoclimatic areas of Costa Rica.

Parasites	Poás ($n = 626$)		San Carlos ($n = 579$)		Cartago ($n = 474$)		Tilarán ($n = 436$)		Alfaro Ruiz ($n = 483$)	
	Mean[a]	95% CI[b]	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Nematodes										
Strongylidae	63.5	59.8, 67.3	62.1	58.2, 66.1	65.8	61.5, 70.1	77.9	74.1, 82.8	73.3	69.3, 77.2
Strongyloides papillosus	4.1	2.1, 5.7	8.1	6.8, 10.3	4.2	2.4, 6.0	20.4	17.6, 24.2	9.3	6.7, 12.9
Trichuris spp.	10.2	7.8, 12.6	8.4	6.8, 10.7	14.5	11.3, 18.7	13.0	9.8, 16.2	7.8	5.4, 10.3
Dictyocaulus viviparus	0.6	0.0, 1.2	0.1	−0.0, 0.6	2.3	0.9, 3.6	0.2	0.2, 0.7	0.0	ND[c]
Cestodes										
Moniezia benedeni	1.7	0.7, 2.7	1.5	0.5, 2.5	1.4	0.0, 2.5	1.0	0.0, 1.8	1.0	0.2, 2.2
Protozoan										
Eimeria spp.	36.9	33.1, 41.9	39.9	36.8, 44.8	47.6	43.2, 52.2	73.6	69.4, 78.7	90.2	88.6, 93.9
Buxtonella sulcata	14.6	12.9, 17.4	14.8	12.9, 18.7	21.5	18.8, 25.2	8.2	5.6, 10.8	3.9	2.2, 5.7

[a] Mean prevalence.
[b] Confidence intervals.
[c] Not determined.

(0–2.3%) and *Moniezia benedeni* (1.0–1.7%) were determined (Table 3).

Eimeria spp. presented prevalences between 36.9% and 90.2%. The more frequently identified species in all areas were *E. bovis*, *E. zuernii* and *E. ellipsoidalis* (data not shown). Another protozoan that was frequently found was *B. sulcata*, with prevalences ranging between 3.9% and 21.5% (Table 3).

The means of epg of Strongylidae, *S. papillosus* and *Trichuris* spp. in the five ecoclimatic areas are shown in Table 4. The mean epg determined for these parasites peaked to 451.0 epg and 348.0 epg, in low and high rainfall period, respectively. A high percentage of calves (90%) presented only low intensity of GIN infections (<500 epg), whereas only 10% of the calves presented high intensity (>500 epg) of Strongylidae, *S. papillosus* and *Tricuhris* spp. infections during low and high rainfall period (data not shown). No clinical signs in calves were recorded during sampling of the feces. *D. viviparus* was rarely found in all areas excepting Alfaro Ruiz. The L1 of *D. viviparus* presented

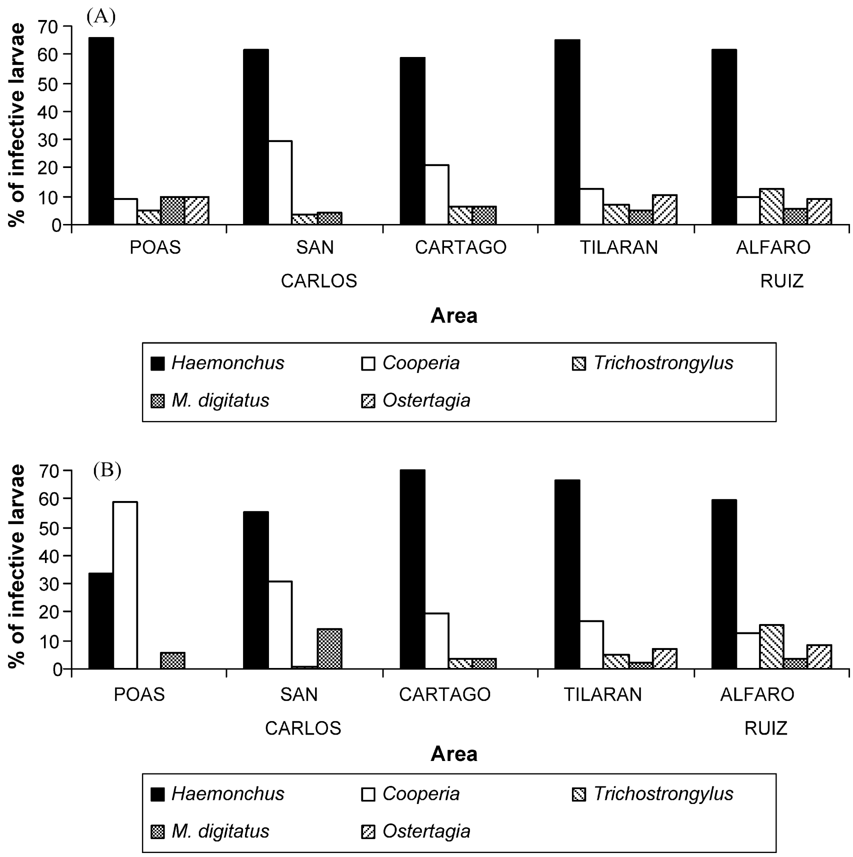

Fig. 1. Percentage of pooled infective larvae after coproculture of Strongylidae determined in five climatic areas of Costa Rica during low (A) and high (B) rainfall period.

Table 4
Means of epg of GIN during low and high rainfall period in five climatic areas from Costa Rica.

Area	Low rainfall period			High rainfall period		
	Mean[a]	SE[b]	Min.–Max.[c]	Mean	SE	Min.–Max.
Poás						
Strongylidae	357.0	±64.5	50–12,400	206.6	±28.2	50–2000
S. papillosus	76.3	±24.9	50–500	50	±0.0	50
Trichuris spp.	106.5	±17.2	50–1000	110	±26.5	50–550
San Carlos						
Strongylidae	451.0	±95.6	50–13,550	187	±29.7	50–3300
S. papillosus	69.5	±15.6	50–400	75	±18.3	50–450
Trichuris spp.	50	±0.0	ND[d]	116.6	±35.6	50–800
Cartago						
Strongylidae	95.9	±7.9	50–850	108.5	±10.5	50–1450
S. papillosus	50	±0.0	50–350	78.9	±0.05	50–600
Trichuris spp.	64.1	±19.8	50–400	106.6	±31.2	50–500
Tilarán						
Strongylidae	411.4	±283.9	50–4450	348	±291.6	50–4600
S. papillosus	73.7	±35.1	50–250	64.2	±20.7	50–200
Trichuris spp.	170.3	±327.5	50–9350	115.4	±68.3	50–1200
Alfaro Ruiz						
Strongylidae	247.2	±201.6	50–4250	258.5	±172.1	50–2800
S. papillosus	69.9	±43.8	50–300	81.4	±22.2	50–200
Trichuris spp.	60.0	±21.1	50–100	81.6	±31.0	50–400

[a] Mean epg.
[b] Standard error.
[c] Min.: minimum ranges; Max.: maximum ranges.
[d] Not determined.

a median of 2.5 larvae/10 g feces during low rainfall period, whereas only a median of 1 larva was determined during the high rainfall period.

3.3. Descriptive analyses of management practices and anthelmintic treatments

Producers of the 73 farms answered personally to the questionnaire applied. The length of colostrum feeding was between 1 and 4 days (61.6%), afterwards calves were fed with cow milk (91.8%). The weaning age of calves was 1–4 months (52.1%). Confinement of calves was performed by a total of 64 (87.6%) farms. Calves remained confined 1–4 months (46.6%), afterwards semi-confined 5–8 months (41.1%), and finally sent out to grazing at ages 9–12 months (34.0%). A total of 68 (93.2%) of the farms used specific paddocks for calves. The number of paddocks used for calves was less than 10 (57.5%) and 30–80 for adults (57.5%). The length of full rotation for calves (67.1%) and adults (78.1%) were ≤30 days and the length of rotation in each paddock was ≤1 day for calves (71.2%) and ≤2 days for adults (43.8%).

All farms used deworming programs for GIN without previous feces analyses (100%) and based on the owner's criteria (66.7%) or veterinarian's recommendation (33.3%), although 91.8% of these farms had veterinary assistance. The deworming programs were carried out mainly according to subjective criteria of the owners, based on age of calves, appearance of clinical signs ("sadness of animals", hirsute hair, diarrhoea, nasal secretions or coughing) or change of season (low to high rainfall period). Most of the producers (74.7%) performed the first deworming in calves ≥15 days of age, however a total of 25.3% used to deworm calves less than 15 days of age. The deworming frequency determined for calves was ≥60 days (52.1%), between 30–60 days (28.2%) and ≤30 days (19.7%). The most common products used in calves were Ivermectin (45.8%), Albendazole (35.9%), Doramectin (14.1%), Fenbendazole (2.8%) and Eprinomectin (1.4%). A total of 89.8% of the farms stated to apply the recommended dose of the anthelmintic product as described by the manufacturer and 10.2% of the farms, according to the recommendation of the veterinarian or farmer; however none of the farms weighed the animals' prior treatment. Anthelmintic products were changed in the farms in intervals <6 months (56.2%), between 7 and 12 months (13.7%), 13 and 24 months (19.2%) or were not changed (10.9%). A total of 69.9% of the producers changed the product within the same drug class, whereas 30.1% changed to another drug class. Most of the producers (89.1%) justified the change of the product by costs (34.3%), recommendation of salesman (20.4%) or veterinarian (17.9%), due to low efficacy of the product (17.9%) or as prophylaxis to avoid resistance (9.5%).

Most of the farms provided some type of nutritional supplement to their animals, concentrate and minerals (94.5%) were the most commonly used, whereas 5.5% of the producers supplemented with agro-industrial wastes and probiotics, among others. Bovine feces were used as installment on pastures in 64.4% of the farms. Gravity method was recorded in 50.0% of the cases, aspersion method in 31.0% of the cases, shovel method was determined in 17.2% of the farms and sawdust method in 1.8% of the farms. Disinfection of the milking room was carried out in 68.5% of the farms, whereas 31.5% did not apply any disinfectant. Chlorine (33.3%), carboline (25.0%), soap (22.2%) and iodine (18.7%) were the most commonly disinfectants used by the producers.

Table 5
Results of analysis of variance of host, ecological and farm factors on epg of GIN and L1 of *D. viviparus* in 73 dairy farms from Costa Rica.

Factor	Nematodes							
	Strongylidae		*S. papillosus*		*Trichuris* spp.		*D. viviparus*	
	F[a]	*p*[b]	*F*	*p*	*F*	*p*	*F*	*p*
Age	15.19	<0.001	4.07	0.01	6.67	0.001	0.90	0.89
Breed	1.05	0.38	0.93	0.45	0.44	0.82	0.98	0.98
Area	12.43	0.12	20.06	0.03	5.02	0.49	0.46	0.95
Rainfall period	15.75	<0.001	0.46	0.49	7.47	<0.001	0.00	0.002
Rainfall period x area	9.30	<0.001	8.02	<0.001	0.67	0.61	0.00	<0.001
Farm (area)	6.61	<0.001	7.20	<0.001	5.91	<0.001	0.00	<0.001

[a] ANOVA statistic test, *F*-ratio for hypothesis under test.
[b] Type I error probability for the observed *F*-ratio.

3.4. Statistical analysis

The results of the analysis of variance of host, ecological and farm factors on epg of GIN and L1 of *D. viviparus* are shown in Table 5. Age effect was significant on epg of GIN ($p < 0.05$), whereas breed factor was not significant for any nematode analyzed. Area effect was found significant only on epg of *S. papillosus*, whereas rainfall period was significant on epg of all nematodes studied, except for *S. papillosus*. However, the interaction rainfall period × area was significant for all nematodes, except for *Trichuris* spp. and nested [farm(area)] effect was significant for all nematodes (Table 5).

Results of the logistic regression analysis of ecological, host and management risk factors on the prevalence of parasites are shown in Tables 6 and 7. Only statistically significant risk factors are presented.

Significant influence of some areas on the prevalence of Strongylidae, *S. papillosus* and *Trichuris* spp. was determined. Tilarán presented the highest risk for having bovines infected with *S. papillosus* (OR = 2.92), and Alfaro Ruiz for having bovines infected with Strongylidae and *Trichuris* spp. In contrast, animals in farms from San Carlos and Poás showed a low probability to be infected with *S. papillosus* (OR = 0.42 and OR = 0.27, respectively) and Strongylidae (OR = 0.45 and OR = 0.18, respectively),

Table 6
Results of logistic regression analysis of ecological, host and management risk factors associated on the prevalence of GIN and *D. viviparus* in 73 dairy farms of Costa Rica (only significant factors are presented).

Parasites	Variable	Reference	Classes	Positive	Total	OR[a]	95% CI[b]	
							LL[c]	UL[d]
Strongylidae	Area	Alfaro Ruiz	San Carlos	380	579	0.45	0.22	0.92
			Poás	398	625	0.18	0.10	0.33
	Semi-confinement	1–4 months age	9–12 months age	487	705	0.28	0.12	0.61
			>12 months age	256	360	0.19	0.10	0.40
			No confinement	249	363	0.42	0.15	0.42
	Management of feces	Aspersion	Gravity	651	870	5.68	3.47	9.34
			Sawdust	58	128	0.20	0.10	0.43
			Shovel	18	23	7.82	2.26	27.08
	Use of disinfectant	No	Yes	1029	1457	1.33	1.01	1.76
S. papillosus	Area	Alfaro Ruiz	Tilarán	90	436	2.92	1.39	6.13
			San Carlos	48	565	0.42	0.19	0.95
			Poás	27	619	0.27	0.12	0.61
	Semi-confinement	1–4 months age	5–8 months age	27	686	0.39	0.22	0.68
			>12 months age	33	358	0.37	0.21	0.64
	Management of feces	Aspersion	Gravity	61	417	0.26	0.08	0.82
	Use of disinfectant	No	Yes	103	1437	0.57	0.37	0.86
Trichuris spp.	Area	Alfaro Ruiz	San Carlos	50	565	0.38	0.16	0.89
	Semi-confinement	1–4 months age	No confinement	138	1421	0.05	0.00	0.63
	Management of feces	Aspersion	Gravity	45	352	2.30	1.17	4.48
			Sawdust	6	128	0.28	0.10	0.84
	Use of disinfectant	No	Yes	166	1437	1.76	1.15	2.71
D. viviparus	Rainfall period	High	Low	28	1275	4.18	1.41	12.38
	Age	4–7 months age	>8 months age	4	1266	0.20	0.07	0.60
	Paddock numbers	<10	No separation calves from adults	8	56	7.76	3.06	19.73
			>10 paddocks	9	1153	0.71	0.31	1.63

[a] Odds ratio.
[b] Confidence interval.
[c] Lower limit.
[d] Upper limit.

Table 7
Results of logistic regression analysis of ecological, host and management risk factors associated on the prevalence of protozoan in 73 dairy farms of Costa Rica (only significant factors are presented).

Parasites	Variable	Reference	Classes	Positive	Total	OR[a]	95% CI[b]	
							LL[c]	UL[d]
Eimeria spp.	Area	Alfaro Ruiz	Tilarán	321	436	0.37	0.32	0.59
			San Carlos	231	579	0.11	0.07	0.18
			Poás	231	626	0.07	0.04	0.10
			Cartago	226	474	0.11	0.07	0.18
	Age	4–7 months	>8 months	809	1274	1.67	1.38	2.01
	Veterinary assistance	Yes	No	1109	2453	4.7	2.50	9.09
	Use of deworming program	No	Yes	998	1656	2.12	1.62	2.79
	Age at first deworming	15–30 days	<15 days	557	1030	1.43	1.07	1.91
			>30 days	456	907	0.71	0.52	0.96
	Use of disinfectant	No	Yes	753	1457	1.31	1.07	1.76
B. sulcata	Area	Alfaro Ruiz	San Carlos	86	579	3.77	1.59	8.94
			Poás	92	626	10.65	5.35	21.17
			Cartago	102	474	8.72	3.41	22.27
	Age	4–7 months	>8 months	178	1266	1.88	1.45	2.45
	Veterinary assistance	Yes	No	2130	2443	5.20	2.77	10.00
	Use of deworming program	No	Yes	248	1656	1.84	1.26	2.70
	Age at first deworming	15–30 days	>30 days	86	900	0.41	0.27	0.60
	Use of disinfectant	Yes	No	195	1467	1.58	1.18	2.17

[a] Odds ratio.
[b] Confidence interval.
[c] Lower limit.
[d] Upper limit.

and farms from San Carlos with *Trichuris* spp. (OR = 0.38) (Table 6). Semi-confined animals of 1–4 months age showed higher risk to be infected with Strongylidae, *S. papillosus* and *Trichuris* spp., than animals of the same age without strict confinement or elder animals (Table 6). Another risk factor determined as statistically significant on the prevalence of Strongylidae, *S. papillosus* and *Trichuris* spp. in the logistic regression analysis was the use of feces as installment in the farms. The gravity and the shovel method presented the highest risk for having bovines infected with Strongylidae (OR = 5.68 and OR = 7.82, respectively), whereas the use of the gravity method showed higher risk on the prevalence of *Trichuris* spp. (OR = 2.30) and lower risk on the prevalence of *S. papillosus* (OR = 0.26) (Table 6). The use of disinfectant in the milking room was determined to have increased odds on the prevalence of Strongylidae (1.33 times) and *Trichuris* spp. (1.76 times) compared with not using any disinfectant. However, not using disinfectant in the milking room represented higher risk on the prevalence of *S. papillosus* (Table 6).

Risk factors determined as statistically significant in the logistic regression model on the prevalence of *D. viviparus* were rainfall period, age and paddock numbers (Table 6). Low rainfall period represented more risk to acquire infections with *D. viviparus* (OR = 4.18), also calves with ages 4–7 months showed higher risk to become infected with this parasite. An increased odds ratio in calves rotating in paddocks, that were also used by adult animals, was also determined (OR = 7.76) (Table 6).

For protozoan area, age, veterinary assistance, deworming programs, age at first deworming and use of disinfectant in the milking room were determined as risk factors in the regression model (Table 7). Cattle located in Alfaro Ruiz and Tilarán (OR = 0.37 times) showed increased risk to be infected with *Eimeria* spp., compared to cattle located in San Carlos, Poás and Cartago, whereas cattle located in farms from Poás (OR = 10.65), Cartago (OR = 8.72) and San Carlos (OR = 3.77) showed a higher risk for *B. sulcata* infections, respect to cattle from Alfaro Ruiz. Animals older than 8 months of age showed an increased risk of infection with *Eimeria* spp. (OR = 1.67 times) and *B. sulcata* (OR = 1.88 times), compared to younger animals. Not having veterinary assistance in the farms (OR = 4.70, OR = 5.20), not using deworming programs for protozoans (OR = 2.12, OR = 1.84), deworming calves the first time with ages <15 days and 15–30 days showed risk factors, that animals were infected with *Eimeria* spp. (OR = 1.43) and *B. sulcata*, respectively. Using disinfectant in the milking room represented a risk factor on the prevalence of *Eimeria* spp. (OR = 1.31), whereas not using disinfectant represented a risk factor for *B. sulcata* infections (OR = 1.58) (Table 7).

4. Discussion

For the first time the prevalence of nematodes and protozoan in calves of 73 representative dairy farms from five ecoclimatic areas of Costa Rica was determined. In all areas *Haemonchus* and *Cooperia* were the most prevalent nematode genera found during low and high rainfall period, confirming previous results obtained by Jiménez et al. (2007). *Oesophagostomum*, *Ostertagia* and *M. digitatus* were identified in low percentages in these farms. To our knowledge, *Ostertagia* and *M. digitatus* are reported for the first time in Costa Rica.

Eimeria spp. was the most prevalent protozoan genus detected in all farms, the most frequently species determined were *E. bovis*, *E. zuernii* and *E. auburnensis*, in agreement with other reports throughout tropical areas

(Molina and Montero, 1987; Rodríguez-Vivas et al., 1996; Daugschies and Najdrowski, 2005; Abebe et al., 2008). Another protozoan species found in all farms of different climatic areas was *B. sulcata*, a ciliate that has been studied less worldwide (Tomczuk et al., 2005) and was barely reported in Costa Rica (Velázquez, 1983; Jiménez et al., 2007).

The analysis of mean epg of GIN in calves during low and high rainfall period determined only a low percentage of animals (<10%) with high intensity infections (>500 epg). Although no clinical signs were recorded during feces sampling in calves in the present study, strategic treatment might be especially important in these young animals (Keyyu et al., 2005, 2006).

The analysis of management practices and anthelmintic treatments revealed that although most of the farms had veterinary assistance, producers performed parasite control regimes based mainly on subjective criteria at different time intervals and without analyzing fecal samples prior to deworming. This finding is in accordance with a previous report, describing that only 2% of the producers dewormed following fecal analyses and to the advice of veterinarians (Alvarez and Hernández, 2006). However, for a rational use of anthelmintics, parasitological diagnosis on feces and grass, establishment of grazing management strategies and biological control is mandatory, in order to implement adequate integrated control programs of parasites (De Graaf et al., 1995; Kassai, 1999; FAO, 2003; Luna et al., 2006; Ballweber, 2006). Another common practice determined in the surveyed farms was the change of anthelmintic products in variable periods of time, justified mainly by costs, without considering the active principle of the drug, furthermore, performing subjective measurements of weight, promoting the use of wrong doses (sub- or over-dosing). All these factors could contribute to develop parasite resistance to anthelmintic products as reported elsewhere (Guglielmone et al., 2004; Repossi et al., 2006).

The analysis of variance and regression analysis determined area as a major risk factor for Strongylidae (*Haemonchus* spp.), *S. papillosus* and *Trichuris* spp. infections. The area factor is mainly influenced by weather (temperature) and season (rainfall period). Although the temperature factor was not evaluated in the present study, a significant effect of rainfall on *S. papillosus*, of minimum temperature on Strongylidae and *S. papillosus*, and a significant effect of maximum temperature on *S. papillosus* and *Trichuris* spp. was determined previously (Jiménez et al., 2007).

The higher risk of semi-confined animals of 1–4 months of age, to be infected with Strongylidae, *S. papillosus* and *Trichuris* spp., can be explained by lack of immunity of calves the first time they had contact with contaminated pastures (Domínguez et al., 1993), by the high environmental resistance of infective eggs of *Trichuris* spp., due to immune suppression related to *Haemonchus* spp., *O. ostertagi* and *Eimeria* spp. infections, as reported elsewhere (Snider et al., 1986; Daugschies and Najdrowski, 2005; Yazwinski and Tucker, 2006).

The use of feces as installment by the gravity and paddock method was confirmed to represent a source of contamination of Strongylidae and *Trichuris* spp. for calves at pasture, particularly for those animals subjected to full rotations less than 15 days. The use of muck collectors or sedimentation tanks, to ferment feces at least 1 month, before it is used as installment, is recommended (Luna et al., 2006; Repossi et al., 2006). In the present study only few producers in San Carlos (36%), Poás (18%) and Tilarán (9%) used sedimentation tanks. The lower risk determined on the prevalence of *S. papillosus* using the gravity method could be explained by the higher susceptibility of the infective larvae to extreme weather conditions (Urquhart et al., 1996).

The use of disinfectant in the milking room was determined as a risk factor for Strongylidae and *Trichuris* spp. infections, and no use of disinfectant as a risk factor for *S. papillosus*. The application of disinfectant in the milking room probably induces the farmers to believe, that all types of infectious agents will be controlled, neglecting other methods of parasite control. The most commonly disinfectants used by the producers in the milking rooms are well known to prevent fungal, viral and bacterial infections, however, to control effectively Strongylidae and *Trichuris* spp., anthelmintic treatment of animals and correct management of pastures has to be carried out (Yazwinski and Tucker, 2006). For *S. papillosus*, the use of 10% copper sulphate, 5% formalin, 1% iodine or boiled water has been recommended (Cordero and Rojo, 2002).

D. viviparus was rarely found in the present study (prevalence 0–2.3%), probably due to the prevailing climatic conditions in Costa Rica. Low rainfall period was determined as a risk factor to acquire *D. viviparus* infections, in agreement with previously reports (Jiménez et al., 2007, 2008). Also calves 4–7 months age had higher risk to become infected with *D. viviparus*, probably due to the lack of acquired immunity or due to limited previous exposure (Eysker, 1994; Panuska, 2006). The importance of raising calves in separated pastures, which were not used previously by adult animals, or control schemes involving rotational grazing for 36–40 days period, should be recommended, to prevent *D. viviparus* infections (Eysker, 1994).

A significant association of climatic area with *Eimeria* spp. infections was determined in agreement with previous reports (Abebe et al., 2008), however no significant association of rainfall with *Eimeria* spp. infections was found in this survey, in contrast with other studies (Rodríguez-Vivas et al., 1996; Abebe et al., 2008).

Cattle located in Alfaro Ruiz and Tilarán showed an increased risk to be infected with *Eimeria* spp., in accordance with studies carried out by Pérez et al. (1998) and Oviedo et al. (1987), who reported these parasites more frequently in healthy calves from Tilarán than in calves with diarrhoea from Poás and Irazú (Cartago).

In contrast, cattle located in farms from Poás and Cartago showed a higher risk for *B. sulcata* infections, which could be explained by changes in the protein content of the pasture during low and high rainfall period, favoring the proliferation of this parasite due to changes in the ruminal pH (Fox and Jacobs, 1986; Hong and Youn, 1995). This is in agreement with recent studies, which determined metabolic acidosis in cattle of Poás caused by ingestion of high quantities of protein present in *P. clandestinum* in the high rainfall period (Murillo, 2009, Personal Communication).

In the present study, animals older than 8 months of age showed an increased risk of infection with *Eimeria* spp. and *B. sulcata* compared to younger animals, although no clinical signs were recorded, probably due to endemic stability of *Eimeria* spp. and lack of pathogenic effect of *B. sulcata*, in accordance with other reports (Daugschies and Najdrowski, 2005; Tomczuk et al., 2005; Abebe et al., 2008).

Other risk factors determined for *Eimeria* spp. infections were not having veterinary assistance, not performing deworming programs and age of the calves at first deworming, possibly due to the fact, that producers dewormed without previous fecal analysis and that none anticcocidial drug was applied, evidencing a missing parasite control regime for *Eimeria* spp. infections. The use of amprolium or decoquinate is recommended to prevent coccidiosis caused by *E. bovis* and *E. zuernii* and should be recommended to the producers in Costa Rica (Bowman et al., 2003).

The increased probability of *Eimeria* spp. infections in farms that used disinfectant in the milking room could be explained, that no cresol-based compounds were used as disinfectant to inactivate *Eimeria* oocysts (Daugschies and Najdrowski, 2005). In at least 72% of the analyzed farms in Alfaro Ruiz and Tilarán, hygienic measures were missing and the facilities were deficient, since fecal contamination of food was recorded. Both areas showed highly significant differences ($p < 0.002$) in the mean prevalences of *Eimeria* spp. compared to other areas (data not shown). Taking into account that coccidiosis appears mainly in confined young animals with little hygiene (Matjila and Penzhorn, 2002), corrective and preventive measures should be implemented in these farms to diminish the contamination levels.

Also farms without veterinary assistance, not using disinfectant in the milking room, not using deworming programs and deworming calves at ages 15–30 days had higher risk to be infected with *B. sulcata*. No references were found in the literature that supported these findings, since *B. sulcata* is considered a commensal, however, it is important to include this parasite in the differential diagnosis of calves presenting diarrhoea (Tomczuk et al., 2005; Göz et al., 2006).

In conclusion, *Haemonchus* spp. and *Eimeria* spp. were found to be the most widely distributed parasites in all ecoclimatic areas analyzed in the present study. Furthermore, the applied questionnaire determined risk factors associated to incorrect management practices and anthelmintic control schemes, pointing out that corrective measures, for example, rotation of drug class, avoiding subjective measurement of weight and use of coccidiostates should be implemented by the producers to prevent and control GIN and *Eimeria* spp. in dairy cattle. Although this work was of cross-sectional type with a limited number of sampling within a period rainfall, the prevalence of gastrointestinal parasites and *D. viviparus* determined were in agreement with previous reports (Jiménez et al., 2007), however, a study of population dynamics selecting target farms in all areas is recommended to confirm the presented results.

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