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Dictyocaulus viviparus seroprevalence and epidemiology in Costa Rican dairy cattle

A.E. Jiménez [a,*], A. Fernández [b], G. Dolz [c], B. Vargas [b], C. Epe [d], T. Schnieder [d]

[a] *Laboratorio de Parasitología, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica, P.O. Box 304-3000, Heredia, Costa Rica*
[b] *Posgrado Regional en Ciencias Veterinarias Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica, P.O. Box 304-3000, Heredia, Costa Rica*
[c] *Programa de Medicina Poblacional, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica, P.O. Box 304-3000, Heredia, Costa Rica*
[d] *Institute for Parasitology, University of Veterinary Medicine, Buenteweg 17, D-30559 Hannover, Germany*

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Abstract

A cross-sectional serological survey of *Dictyocaulus viviparus* was carried out to determine the prevalence of lungworm infections in 28 dairy cattle farms distributed in five selected areas from Costa Rica. The influence of area, farm, host (breed, age and lactation number) and ecological factors (altitude and life zones) on the presence of lungworm infection was analyzed. A subsample of 924 sera collected between September 1998 and July 1999 was processed by ELISA (Ceditest®). A total of 162 (17.5%) animals from 26 (93.0%) farms showed antibodies against *D. viviparus*. The overall seroprevalence detected among areas was Poás 25.0%, Cartago 24.3%, Tilarán 22.0%, Alfaro Ruiz 12.0% and San Carlos 12.1%. Using analysis of variance no significant influence of area and host factors on *D. viviparus* infections was determined, whereas the variable farm within area was highly significant ($p < 0.001$). However, altitude and life zones showed significant association to seropositive animals, when a Chi-square test was applied. In altitudes of 1000–2000 m ($p < 0.001$) and life zones of Lower Montane moist forest and Montane moist forest ($p < 0.001$) *D. viviparus* infections in bovines were significantly higher. The results obtained in this study indicate a high *D. viviparus* seroprevalence in the analyzed farms and that the factors farm, altitude and life zones were significantly related to lungworm infections.

Keywords: Seroprevalence; *Dictyocaulus viviparus*; ELISA; Epidemiology; Dairy cattle; Costa Rica

1. Introduction

Dictyocaulosis in cattle is caused by the nematode *Dictyocaulus viviparus* (Trichostrongyloidea:Dictyocaulidae) whose adult stages inhabit the main stem bronchi and trachea, causing bronchitis and pneumonia. The most common clinical manifestations are coughing, respiratory distress and weight loss (Kassai, 1999; McKeand, 2000). Losses due to bovine dictyocaulosis can be direct (death of calves, mainly during the first year of pasture) or indirect (reduced performance, growth delay and treatment costs) (Berghen et al., 1990; Graham, 1999).

In tropical countries the epidemiological information available on *D. viviparus* infections has been generated

* Corresponding author. Tel.: +506 5624539; fax: +506 2600137.
E-mail addresses: anajimenez@racsa.co.cr, anaj@medvet.una.ac.cr (A.E. Jiménez).

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mainly by using the Baermann technique (Delgado and Fregel, 1977; Sharma and Dhar, 1987; Zurita et al., 1987; Thamsborg et al., 1998; Jithendran and Bhat, 1999; Cardona et al., 2005). No studies have been conducted to determine seroprevalence of *D. viviparus* in dairy cattle farms by means of serological techniques due to that only few commercial tests are available at high costs.

In many temperate countries bovine dictyocaulosis is widespread and disease has been reported (Eddi et al., 1989; Schnieder et al., 1993; Agneessens et al., 2000; Ploeger et al., 2000; Höglund et al., 2004), whereas in tropical areas it remained unknown or little disease is recorded (Thamsborg et al., 1998; Jiménez et al., 2007). In these areas *D. viviparus* infections can be locally or regionally important and the disease should not be disregarded in young animals with respiratory problems or in susceptible adult cattle (David, 1997; Thamsborg et al., 1998; Panuska, 2006; Wapenaar et al., 2007).

A previous longitudinal survey has determined the seroprevalence of *D. viviparus* in a dairy and a beef cattle farm in Costa Rica (Jiménez et al., 2007). The objectives of the present study were to investigate in further detail the seroprevalence of *D. viviparus* in five different geographical areas of Costa Rica and to analyze the influence of area, farm, host (age, breed and lactation number) and ecological factors (altitude and life zones) on *D. viviparus* infections in dairy cattle farms.

2. Materials and methods

2.1. Study area

A total of 28 farms distributed in five different geographical areas located at the northwestern part of Costa Rica were studied: Poás, Cartago, Tilarán, Alfaro Ruiz and San Carlos. The location of these farms corresponded to the following life zones (Holdridge, 1978): Lower Montane wet forest (LM-wf), Lower Montane moist forest (LM-mf), Premontane wet forest (P-wf), Premontane moist forest (P-mf), Tropical wet forest (T-wf) and Tropical moist forest (T-mf). The definition of these life zones are based on mean annual temperature, mean total annual rainfall and index humidity (Table 1). A detailed description of altitude, meteorological factors and life zones in each area studied are summarized in Table 2.

2.2. Bovine sera

This study was performed using a reference bank serum of 98 dairy cattle farms in five selected areas of Costa Rica. The serum samples were collected from September 1998 to July 1999 and stored at −20 °C. The number of farms to be analyzed in the present study in each area was determined using stratified random sampling with a 95% confidence level and an expected prevalence of 50% (Sheaffer et al., 1996). A correction

Table 1
Definition of life zones according to Holdridge (1978)

Factor	Lower Montane		Premontane		Tropical	
	Moist forest	Wet forest	Moist forest	Wet forest	Moist forest	Wet forest
Mean annual temperature (°C)	12–17		17–24		24–27	
Mean total annual rainfall (mm)	2000–1400	1850–4000	2200–1200	2000–4000	1950–3000	4000–6000
Index humidity	2.0–4.0	1.0–2.0	2.0–4.0	1.0–2.0	1.0–2.0	0.5–1.0

Table 2
Meteorological and ecological factors of the five selected geographical areas studied for *Dictyocaulus viviparus* seroprevalence

Factors	Areas				
	Poás	Cartago	Tilarán	Alfaro Ruiz	San Carlos
Mean altitude (m) and range	1802.0 (1560–1990)	1786.6 (1480–2070)	776.0 (610–2010)	1543.3 (970–2010)	154.0 (70–270)
Overall rainfall (mm)[a]	3847.5	2289.2	1788.0	4116.0	332.8
Annual mean maximum temperature (°C)[a]	21.5	20.8	25.9	20.4	29.7
Annual mean minimum temperature (°C)[a]	13.3	12.2	20.0	13.8	21.1
Life zone	LM-wf	LM-wf, P-mf, LM-mf	P-wf	LM-wf, P-wf	T-mf, P-wf, T-wf

[a] Data from the National Meteorology Institute, San José, Costa Rica.

factor for finite population was applied. A total of 28 (Poás: 5, Cartago: 3, Tilarán: 5, Alfaro Ruiz: 3 and San Carlos: 12) specialized dairy farms with intensive management were selected. All serum samples of animals in these farms were analyzed. These farms were monitored since 1986 with the Costa Rican livestock information system VAMPP 5.1, Dairy Version (Veterinary Automated Management and Production Control Program) (Noordhuizen and Buurman, 1984), consequently the following data was obtained from each farm: number of animals, farm size, animal identification, age, breed and lactation number.

A total of 924 serum samples from the selected farms available in the bank were analyzed, representing females older than 6 months of age. The number of females in each farm ranged between 19 and 85 animals. The breeds were Holstein-Friesian, Jersey, Guernsey, Brown Swiss and cross breeds. The mean number of animals per farm was 133.8 (minimum 41 and maximum 235), and the stocking rate ranged between 1.23 and 3.28 animals/(ha year).

2.3. Serological analysis

An indirect ELISA (Ceditest® Lungworm kit, Lelystad, Netherlands) for the detection of antibodies against adult *D. viviparus* was used according to the instructions provided by the manufacturer (Cornelissen et al., 1997). This assay has a reported sensitivity of 100% and specificity of 99.2%. The measured optical density (OD) of each tested serum was converted into OD percentage (OD%), using the following formula: $OD\% = (OD_{test\ serum} - OD_{blank}/OD_{positive\ reference\ serum} - OD_{blank}) \times 100$. Test samples with an OD% $\geq$15% were considered positive for *D. viviparus* specific antibodies (Cornelissen et al., 1997). A farm was considered positive, when at least one serum sample was determined positive in ELISA (Höglund et al., 2004).

2.4. Statistical analysis

Descriptive statistics of the seroprevalence by farm (maximum and minimum ranges), area (overall), and age animals (mean and 95% confidence intervals: 95% CI) were performed (Statistica®, V.6.0, 2001; Tulsa, Oklahoma).

The analyzed sera were categorized according to the OD% obtained (negatives: <15%, low positives: 15–25%, medium positives: 26–50% and high positives: $\geq$51%). In each area the OD% of seronegative and seropositive animals were compared against the cut-off (15%) of the serological assay using hypothesis test for one proportion. OD% seropositive animals were also compared against OD% of seronegative animals using hypothesis test for two proportions (Statistica®, V. 6.0, 2001; Tulsa, Oklahoma). Finally, the percentage of seropositive animals in the different categories was determined.

An univariate analysis of variance (ANOVA) was performed using the GLM Procedure implemented in the STATISTICA software (Statistica®, V.6.0, 2001; Tulsa, Oklahoma) to assess the relationships of serological status at cow level and the variables of area and host (age, breed and lactation number). The statistical model was

$$Y = \mu + \text{area} + \text{code farm(area)} + \text{code age} + \text{code breed} + \text{code lactation number} + \text{error}.$$

Y = OD% (log transformed [2(OD% + 10)]); μ = general mean; area = fixed effect of area (1: Poás, 2: Cartago, 3: Tilarán, 4: Alfaro Ruiz, 5: San Carlos); code farm (area) = fixed effect of farm (1–28) nested within area; code age = fixed effect of age (1: 6–23 months, 2: 24–95 months, 3: $\geq$ 96 months); code breed = fixed effect of breed (1: Holstein-Friesian, 2: Jersey, 3: Guernsey, 4: Brown Swiss, 5: cross breed); code lactation number = fixed effect of lactation number (1:0 lactation, 2: 1–2 lactations, 3: >2 lactations); error = random residual effect (NID, 0, σ^2).Altitude (1: <1000 m, 2: 1000–2000 m, 3: > 2000 m) and life zones (Table 2) were not included in the statistical GLM model to avoid confusion with farm and area. In order to infer an association among positive animals and altitude and life zones a Chi-square test (Statistica®, V. 6.0, 2001; Tulsa, Oklahoma) was performed. These parameters were analyzed at cow level without taking into account stratification area.

3. Results

3.1. Seroprevalence

From a total of 28 farms examined with Ceditest®, 26 (93.0%) had at least one seropositive animal (Table 3) and 162 (17.5%) out of 924 sera analyzed yielded positive results. The mean number of animals with *D. viviparus* infection detected in the farms was 5.9 ± 2.5 animals (95% CI: 3.4; 8.4) and the mean age of seropositive animals was 4.0 ± 0.98 years (95% CI: 3.21; 4.91).

The overall seroprevalence of *D. viviparus* among areas was for Poás 25.0%, Cartago 24.3%, Tilarán 22.0%, Alfaro Ruiz 12.0% and San Carlos 12.1%. The seroprevalence at farm level in each area ranged

Table 3
Seroprevalence of *D. viviparus* in 28 dairy farms of five selected areas of Costa Rica determined by Ceditest® ELISA

Farm numbers	Poás %[a] (a/b)[b]	Cartago % (a/b)	Tilarán % (a/b)	Alfaro Ruiz % (a/b)	San Carlos % (a/b)
1	24.2 (8/33)	4.0 (1/26)	26.5(9/34)	15.6 (5/32)	8.8 (3/34)
2	20.6 (7/34)	29.7(11/37)	41.4 (12/29)	14.7 (5/34)	5.7 (2/35)
3	15.0 (6/40)	32.5(13/40)	28.6 (10/35)	0.0 (0/19)	10.0 (3/30)
4	25.0 (8/32)		8.6 (3/35)		8.8 (3/34)
5	44.4 (12/27)		8.6 (3/35)		10.0 (3/30)
6					5.9 (2/34)
7					65.7 (23/35)
8					5.4 (2/37)
9					6.4 (2/31)
10					3.1 (1/32)
11					0.0 (0/35)
12					14.3 (5/35)
Overall	25.0 (41/166)	24.3 (25/103)	22.0 (37/168)	12.0 (10/85)	12.1 (49/402)

[a] Percentage of prevalence.
[b] Number of positive animals/total of animals examined.

between 15.0 and 44.4% in Poás, 4.0 and 32.5% in Cartago, 8.6 and 41.4% in Tilarán, 0.0 and 5.6% in Alfaro Ruiz, and 0.0 and 65.7% in San Carlos (Table 3).

3.2. Analysis of OD%

Mean of OD% of positive (posmean) and negative (negmean) sera and respective confidence intervals determined in each area were the following: Poás: $27.2\%_{\text{posmean}}$ (95% CI: 23.0, 31.3), $5.8\%_{\text{negmean}}$ (95% CI: 5.2, 6.5); Cartago $33.6\%_{\text{posmean}}$ (95% CI: 25.5, 41.6), $4.7\%_{\text{negmean}}$ (95% CI: 3.8, 5.6); Tilarán $28.5\%_{\text{posmean}}$ (95% CI: 24.2, 32.8), $6.3\%_{\text{negmean}}$ (95% CI: 5.6, 6.9); Alfaro Ruiz $43.1\%_{\text{posmean}}$ (95% CI: 17.7, 68.4), $3.3\%_{\text{negmean}}$ (95% CI: 2.6, 4.0) and San Carlos $22.5\%_{\text{posmean}}$ (95% CI: 20.1, 25.1), $5.0\%_{\text{negmean}}$ (95% CI: 4.7, 5.4) (Fig. 1). Significant differences ($p < 0.001$) were determined between seropositive and seronegative groups when compared to the cut-off line. Significant differences ($p < 0.001$) were also determined between seropositive and seronegative groups. Seropositive animals were found ranging as following in the different categories of OD%: 30.0–73.4% low, 28.5–48.0% medium and 0–30.0% high positives. In the areas analyzed 51% (mean) of the positive animals were low positives.

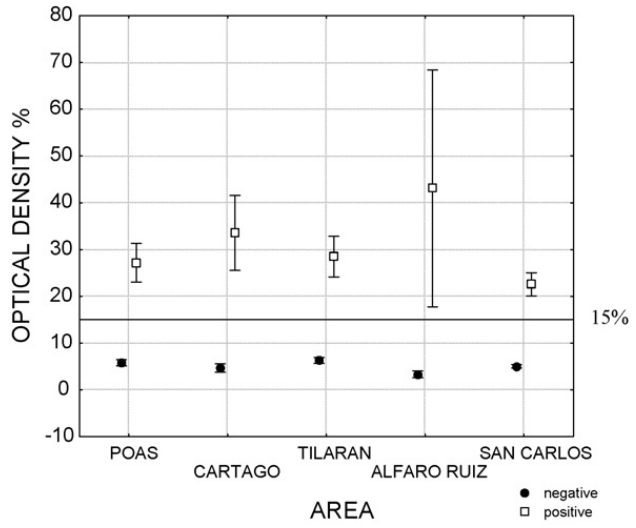

Fig. 1. Optical density percentage of seropositive and seronegative dairy cattle to *Dictyocaulus viviparus* in five selected areas from Costa Rica determined with Ceditest®.

3.3. Relationships between area, farm, host, ecological factors and D. viviparus infections

The analysis of variance determined no significant influence of area and host factors (age, breed and lactation number) on *D. viviparus* infection, whereas the variable farm within areas was highly significant ($p < 0.001$). Lungworm infections were also significantly associated to altitude ($x^2 = 13.48$, d.f. = 2 and $p < 0.001$) and life zones of moist forest ($x^2 = 30.42$, d.f. = 2 and $p < 0.001$). Farms located at altitudes of 1000–2000 m (24.5%) and in Lower Montane moist forest (30%) and Premontane moist forest (32.5%) life zones showed higher percentages of lungworm infections.

4. Discussion

The present study demonstrate that *D. viviparus* infections are widespread in the farms analyzed in the

northwestern region of Costa Rica, since 93.0% (26) of the farms showed at least one seropositive animal in ELISA. This value is considerably higher than results reported in Sweden (44%), Netherlands (41%, 73% and 75%) and Germany (39.9%) (Boon et al., 1986; Schnieder et al., 1993; Cornelissen et al., 1997; Ploeger et al., 2000; Höglund et al., 2004). The higher lungworm seroprevalence found in Costa Rica might be due to differences in weather conditions, sampling date, serological test used, stocking rate and other factors (Boon et al., 1986; Schnieder et al., 1993; Ploeger et al., 2000).

The overall seroprevalence was 17.5% (data not shown) fluctuating within farms from 3.1 to 65.7% which is in agreement with previous findings. Seroprevalences of 42.1% (range 5.3–78.1) and 34.3% (range 5.0–62.0) to *D. viviparus* were reported in young dairy and beef cattle from Costa Rica subjected to a longitudinal study (Jiménez et al., 2007).

The Ceditest ELISA used in the present study have high sensitivity and specificity compared to Baermann technique (Höglund et al., 2004). Although, false positive results were often reported with ELISA, that could influence the statistical analysis of factors associated to *D. viviparus* infections, it was demonstrated in the present study that no overlapping among infected and non-infected animals occurred, and that the cut-off line was adequate for its use in serum samples of Costa Rica. However, the overall seroprevalence only reflects the number of *D. viviparus* ''carriers'' and the number of adult cattle with a recent primary infection (Agneessens et al., 2000).

When the ANOVA model was used, the variability found at farm level had more impact than variability among areas, consequently the variable area was not significant, confirming others studies reported, that lungworm infections are influenced by management practices in the farms rather than areas or regions where the farms are located (Boon et al., 1986; Schnieder et al., 1993; Höglund et al., 2004; Wapenaar et al., 2007). Management practices that could differ among farms are for example: date of beginning and length of grazing period, prevalence of carrier animals, anthelmintic control measures, structure of the herd at time of sampling and others (Boon et al., 1986; Schnieder et al., 1993; Agneessens et al., 2000; Ploeger et al., 2000; Borgsteede et al., 2000; Wapenaar et al., 2007). Recently, a questionnaire applied in these areas showed that management practices and use of anthelmintics varied greatly among farms (unpublished data).

The altitude was found to have a significant relationship to *D. viviparus* infections where high percentage of seropositive animals were recorded at middle altitudes (1000–2000 m). These results are in accordance with data from Thamsborg et al. (1998) and Jiménez et al. (2007). Significant influence of life zones and *D. viviparus* infections was demonstrated in the present study. The LM-mf and P-mf life zones showed higher percentage of seropositive animals, in contrast to the other life zones analyzed, and may be due to differences in temperature, rainfall and humidity among these zones (Holdridge, 1978). Also other reports have related the occurrence of *D. viviparus* with amount of rainfall and low temperature (Eddi et al., 1989; Jiménez et al., 2007).

Further surveys are required to examine *D. viviparus* seroprevalence in areas with semi-intensive or extensive cattle farms and to update information in the areas analyzed. To identify management practices that influence lungworm infections a survey using coprological and serological testing, slaughter surveillance and recording of clinical signs has to be carried out.

In conclusion this study determined that *D. viviparus* infections were present in all areas and in a vast majority of farms sampled. Area and host factors appear to have no influence on *D. viviparus* infections, whereas the factors farm, altitude and life zones seems to be related.

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