Veterinary Parasitology: Regional Studies and Reports 9 (2017) 115–121

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

Original Article

Environmental factors associated with *Dictyocaulus viviparus* and *Fasciola hepatica* prevalence in dairy herds from Costa Rica

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ARTICLE INFO

Keywords:
Prevalence
Milk
Dictyocaulus viviparus
Fasciola hepatica

ABSTRACT

Dictyocaulosis and fasciolosis are parasitic diseases that cause considerable economic losses for owners of farm animals worldwide, with special relevance on fasciolosis because it is an emerging zoonosis. Indirect diagnosis of these diseases through analyses of bulk milk tank samples has allowed carrying out large-scale prevalence studies, while the use of geographical information systems has helped to visualize and determine those variables that affect distribution of these pathogens. This study is intended to describe the spatial distribution of *Dictyocaulus viviparus* and *Fasciola hepatica* in dairy herds from Costa Rica, as well as their associated environmental factors. Bulk milk tank samples from 526 dairy herds in the three most important dairy regions of Costa Rica were analyzed using enzyme immunoassays. Results from the farms were subjected to spatial analyses using Holdridge's life zones, relief and soil type environmental layers. Of the total bulk milk tank samples analyzed, 3.8% (n = 20) and 3.6% (n = 19) were positive for *D. viviparus* and *F. hepatica*, respectively. Moran's I analysis revealed the existence of potential cluster (Moran's I = 1.789, z = 12.726 $p < 0.05$) for *D. viviparus*. Consequently, Getis-Ord General G analysis showed that the spatial distribution of positive farms in the dataset was clustered (Observed General G = 0.015, variance = 0.000001, z = 12.823, $p < 0.05$). No significant positive spatial autocorrelation (Moran's I = 0.038, z = 0.286, $p > 0.0.5$) was observed for *F. hepatica*. Furthermore, a significant difference was detected in the spatial locations of both parasites (latitude $p < 0.05$, longitude $p < 0.05$), and about the spatial distribution of both *D. viviparus* negative and positive farms (latitude $p < 0.05$, longitude $p < 0.05$), as well as in *F. hepatica* negative and positive farms regarding on latitude ($p < 0.05$), but not on longitude ($p > 0.05$). In the case of environmental factors, significant differences were found for *D. viviparus* and *F. hepatica* with respect to types of soil, precipitation, altitudinal belts, life zones, biotemperature, and elevation.

1. Introduction

Bovine dictyocaulosis and fasciolosis are parasitic diseases that cause considerable economic losses in bovine herds (Wapenaar, 2011; Dank et al., 2015; Radfar et al., 2015; Rojas, 2015), and are caused by the *Dictyocaulus viviparus* nematode and the *Fasciola hepatica* digenea trematode; both parasites affect a wide range of domestic and wild animals (Issia et al., 2009; Dracz and Lima, 2014; Pyziel et al., 2015). Additionally, fasciolosis is well known as an emerging zoonotic disease (Olsen et al., 2015).

There are only a few reports on the economic losses caused by dictyocaulosis, estimations made in the United Kingdom in 2007, based on two dairy herds, indicate an average loss of US $22,852.60 per farm (Holzhauer et al., 2011). On the other hand, in Costa Rica (2015), an outbreak of dictyocaulosis in 28 cows caused losses in milk production close to US $16,225.00 (unpublished data). Regarding fasciolosis, Rojas (2015) estimated annual economic losses for US $67,313.00 from viscera confiscations in class A slaughterhouses in Costa Rica. These losses are the result of milk and weight reduction, developmental delays and fertility-related problems, as well as the discarding of a great amount of livers affected in slaughterhouses (Das Chagas et al., 2011; Howell et al., 2015).

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http://dx.doi.org/10.1016/j.vprsr.2017.06.006
Received 26 September 2016; Received in revised form 31 May 2017; Accepted 13 June 2017
Available online 15 June 2017

Worldwide, several studies have used geographical information systems (GIS) to determine the variables that affect distribution of dictyocaulosis and fasciolosis, as well as their relationship with environmental factors and management practices (Bennema et al., 2011; Kuerpick et al., 2013a; Schunn et al., 2013).

Different enzyme-linked immunosorbent assays (ELISAs) have been developed for serological diagnosis of *D. viviparus* and *F. hepatica* in bovines, using recombinant, somatic and excretory-secretory (ES) antigens (Schnieder, 1992; Cornelissen et al., 1997; Salimi-Bejestani et al., 2005; Kuerpick et al., 2013b; Schunn et al., 2012), as well as coproantigens to diagnose *F. hepatica* (Kajugu et al., 2015).

Bulk milk tank ELISA's have made possible to detect infections resulting from the exposure of dairy cattle to both agents, and they have been considered as highly useful diagnostic tools in large-scale prevalence studies or in epidemiological surveillance systems for monitoring herd health programs, given their quickness of use, low cost and high specificity (Sekiya et al., 2013). From 2005 to date, the use of ELISA on milk tank has been implemented in the diagnosis of *D. viviparus* and *F. hepatica* (Salimi-Bejestani et al., 2005; Bennema et al., 2009; Bloemhoff et al., 2015; Howell et al., 2015).

In Costa Rica, only a few studies have been carried out on the prevalence of *D. viviparus* in dairy cattle (Jiménez et al., 2007; Jiménez et al., 2008; Jiménez et al., 2010); likewise, studies with bovines and humans have emphasized the economic and zoonotic importance of fasciolosis in the counties of Turrialba and Pococí (Mora et al., 1980; Arroyo et al., 1981; Chang and Cartín, 1983; Alpízar et al., 2013). No studies have been carried out on bulk milk tank samples involving herds of all areas of our country using immunodiagnostic tests for *D. viviparus* and *F. hepatica* for epidemiological surveillance, nor geographic information systems (GIS) has been used for such purposes. Therefore, the objective of the present study was to describe the spatial distribution of *D. viviparus* and *F. hepatica* in dairy herds from Costa Rica, as well as associated environmental factors.

2. Material and methods

2.1. Study population and type of study

A cross-sectional study was carried out in a total of 526 specialized dairy farms, distributed in three regions of Costa Rica: Chorotega (101), Central (160) and North Huetar (265). They are considered specialized dairy farms, because they have mostly Holstein and Jersey cows, use a milking room with milking machines, pasture rotation, concentrated feed and nutritional supplements, artificial insemination, recording systems, and veterinary and other technical assistance.

2.2. Milk samples

Bulk milk tank samples were collected from each farm only once during October 2008. In farms with less than 50 dairy cows, the sample was taken at the end of the milking process; in other farms, samples were taken when 50 cows had been milked. The bulk milk tank mixer was activated for 3 min before the sample was taken. Milk samples were collected in boric acid 10%.

2.3. Serological analysis

Once samples were in the laboratory, they were centrifuged at 2000 r.p.m. for 15 min, fat was removed and milk was then sampled and stored at − 20 °C until assayed. Serum samples were analyzed at the Parasitology Institute of the Tierärztliche Hochschule in Hannover, Germany, using the ELISA technique to detect *D. viviparus* and *F. hepatica* antibodies, using the major sperm protein recombinant and excretor-secretor products as antigens, respectively (Charlier et al., 2007; Von Holtum et al., 2008; Bennema et al., 2009; Fiedor et al., 2009).

2.4. Spatial data management

A database of *D. viviparus* and *F. hepatica* positive and negative farms was created, and all the farms were georeferenced at the location of the dairy facilities, using a Garmin model 12 XL GPS. The points representing the farms were overlaid in environmental layers obtained from the Costa Rican Digital Atlas (2014), using the Spatial Join command of Arc Gis 10.2. (ESRI, 2013). The environmental layers used were type of soils, relief (altitude) and Holdridge's life zones (Holdridge, 1978), all at a scale of 1:200.000. Life zone, annual precipitation (mm), biotemperature (°C) and altitudinal belts variables were used from Holdridge's life zones layer.

2.5. Statistical analysis

The global herd level prevalence for each parasite was calculated, as well as for co-infection and it was performed a comparison of percentages by region. Differences between positive and negative farms about longitude and latitude averages, between and among parasites were assessed by one-way ANOVA and a post-hoc Bonferroni test. Besides, a Chi^2 test for multiple percentages comparison was performed. In this study, Moran's I was used as the measure of spatial autocorrelation for each species and Getis-Ord General G statistic as the measure of clustering of positive/negative farms. For all tests, a *p*-value of 0.05 was established as critical threshold of statistical significance.

3. Results

3.1. Serology

Out of the total (526) of bulk milk tank samples analyzed, a 3.9% (20) and a 3.7% (19) were positive for *D. viviparus* and *F. hepatica*, respectively.

In the specific case of *D. viviparus*, frequencies by region showed that there were four positive farms in the Central region (4/161, 2.48%), three positive farms in the North Huetar region (3/265, 1.13%), while the Chorotega region showed the greatest percentage of positive farms (13/101, 12.87%) ($p < 0.05$).

On the other hand, prevalence of *F. hepatica* did not show statistical differences between regions, although the Central region had a prevalence of 5.60% (9/161), which is slightly more than twice the prevalence found in the North Huetar region (2.27%, 6/265); while the prevalence in the Chorotega region was 3.96% (4/101) ($p > 0.05$).

Antibodies against both parasites were detected only in one of the 38 positive farms located in the Central region.

3.2. Spatial occurrence of infected herds

The spatial distribution of infective herds that were positive for *F. hepatica* and *D. viviparus* are shown in Fig. 1. A significant difference was observed in terms of the spatial location of both species in latitude ($p < 0.05$) and longitude ($p < 0.05$).

Significant positive spatial autocorrelation (Moran's $I = 1.789$, $z = 12.726$ $p < 0.05$) was observed for *D. viviparus*, revealing the existence of potential cluster. Getis-Ord General G analysis showed that the spatial distribution of positive farms in the dataset was clustered (Observed General $G = 0.015$, variance = 0.000001, $z = 12.823$, $p < 0.05$). No significant positive spatial autocorrelation (Moran's $I = 0.038$, $z = 0.286$, $p > 0.05$) was observed for *F. hepatica*.

Regarding *D. viviparus*, the spatial distribution of negative and positive farms was significantly different (Fig. 2): for latitude $p < 0.05$ and 0for longitude $p < 0.05$. Significant differences, with higher percentage of positive farms were found in the annual precipitation, specifically in the categories of 2000 to 4000 mm, as well as in the basal altitudinal belt, in the life zone variable of Moist Forest, and in the category of > 24 °C of biotemperature variable (Table 1).

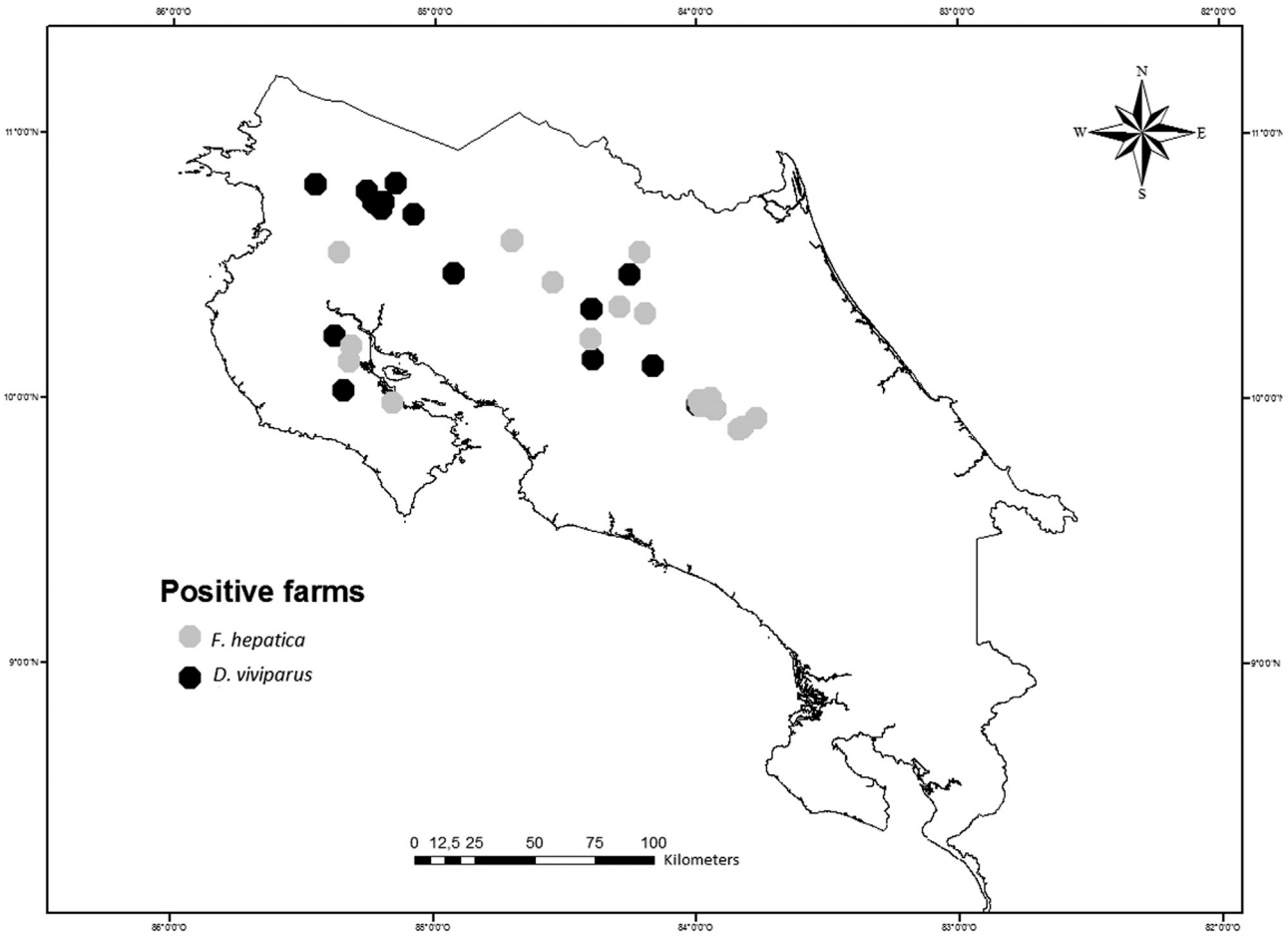

Fig. 1. Distribution of infected dairy herds for *Dictyocaulus viviparus* and *Fasciola hepatica* in Costa Rica.

On the other hand, significant difference was found for *F. hepatica* in the latitude of farms ($p < 0.05$), but not in longitude ($p > 0.05$) (Fig. 3). Significant differences were found soil orders of Alfisol and Vertisols, as well as in precipitation in the category of < 2000 mm; finally in the elevation variable in the category of 1201 to 1800 m.a.s.l. a significant difference was found (Table 2).

4. Discussion

The ELISA tests carried out in bulk milk tank samples have been used for many years for monitoring infectious diseases (Forschner et al., 1986; Niskanen, 1993; Hoorfar et al., 1995), and parasitic diseases (Björkman et al., 1997; Bennema et al., 2009; Fiedor et al., 2009); however, this is the first report of *D. viviparus* and *F. hepatica* prevalence at the herd level, determined with milk tank samples in Costa Rica.

Most reports on *D. viviparus* and *F. hepatica* prevalence determined in bulk milk tank samples were from temperate countries, where prevalences for the first parasite presented ranges between 2.6% and 31.2% (Bennema et al., 2009; Fiedor et al., 2009; Schunn et al., 2013; Ploeger et al., 2014; Dank et al., 2015), while prevalences for the second agent were determined ranging between 6.5% and 75.4% (Bennema et al., 2009; Höglund et al., 2010; Bloemhoff et al., 2015). In Costa Rica, the prevalence of *D. viviparus* obtained in this study (3.8%) was low with respect to that reported by Jiménez et al. (2008) ranging between 15% and 44% in different farms, whereas the prevalence of *F. hepatica* (3.6%) was slightly higher than the 1.9% reported by Rojas (2015). For *D. viviparus*, the greatest percentage of positive farms was found in the Chorotega region (13%), which is statistically significant and may be related to the variables of precipitation, altitude, life zone and biotemperature, that showed a significant difference between *D. viviparus* positive and negative farms; or eventually to management variables in farms, which were however, not analyzed in this study, but reported previously (Jiménez et al., 2010). The presence of this parasite in dairy farms located in the counties of Liberia, Hojancha and Nicoya of the Chorotega region is reported for the first time in Costa Rica.

For *F. hepatica*, the highest percentage of positive farms was found in the Central region (5.63%), with no statistically significant difference between the regions. However, positive farms were found in the county of Turrialba in this region, where several cases have been reported in humans and in bovines (Mora et al., 1980; Chang and Cartín, 1983; Alpízar et al., 2013; Rojas, 2015). In addition, a study of *F. hepatica* prevalence in the Central and Caribbean regions found the highest number of positive animals, which came from places with a high probability of infection (Rojas, 2015).

The use of GIS in this study made it possible to analyze environmental variables as possible explanations for *D. viviparus* and *F. hepatica* spatial distribution. The analysis revealed differences in spatial distribution of positive farms when comparing both parasites, as well as when comparing farms that were positive and negative for each agent. This finding may be related to variations in latitude and longitude, which are related to environmental conditions such as temperature, precipitation, and life zone, or may be related to differences in the management practices in the regions, which are important characteristics in determining presence or absence of the parasites (Bennema et al., 2009; Khan et al., 2009). A risk-predictive factor for both agents is precipitation, which has been used in different studies for the construction of risk models (McCann et al., 2010; Selemetas et al., 2015).

In this study, presence of *D. viviparus* was related to forests with average annual precipitations ranging between 2000 and 4000 mm, and a dry season between 0 and 5 months, in contrast to dry forest

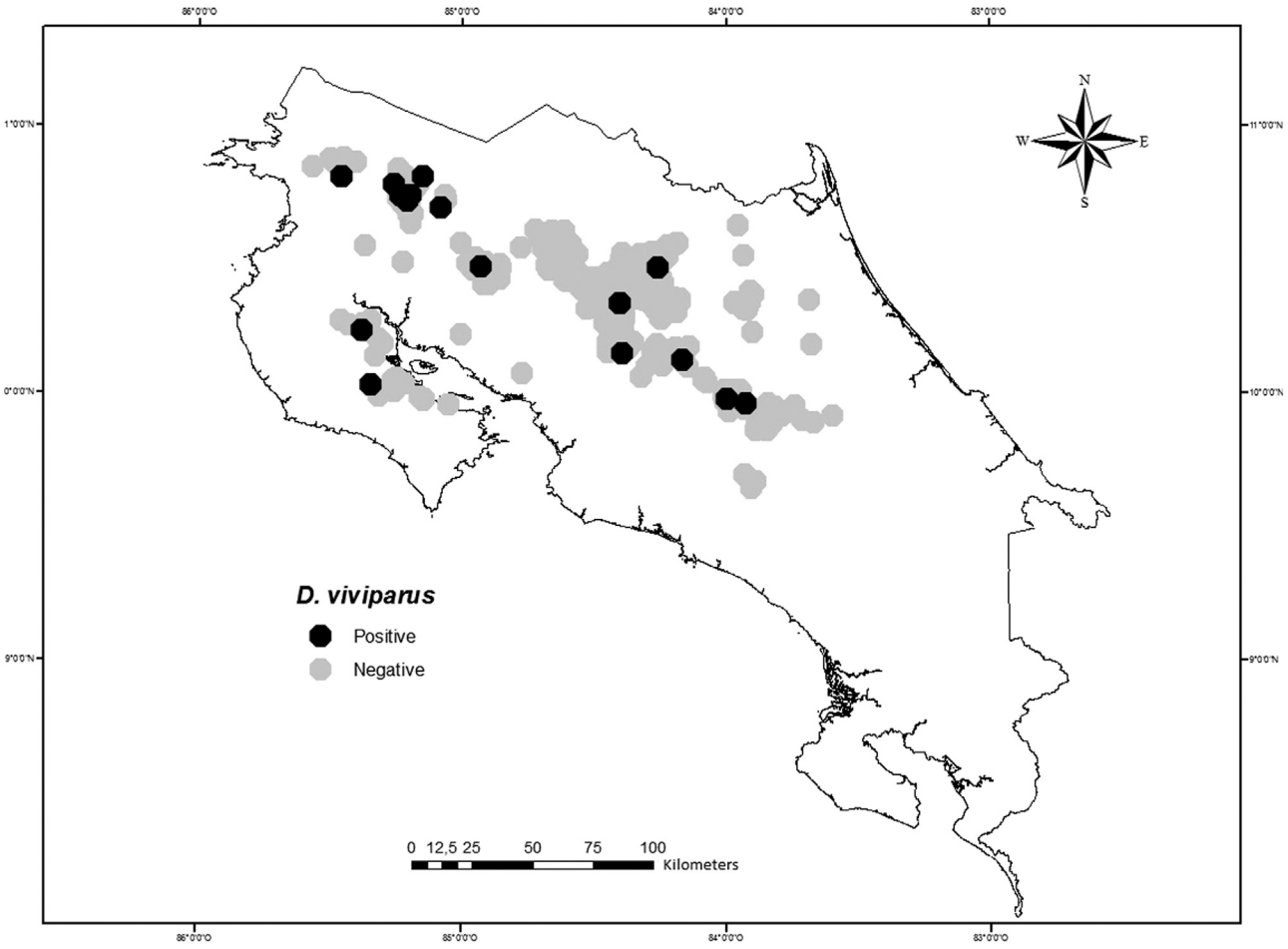

Fig. 2. Distribution of infected dairy herds for *Dictyocaulus viviparus* in Costa Rica.

characteristics (1000 to 2000 mm average annual precipitation, and seven months of dry season), and other types of forests in Costa Rica, where there is practically no dry season. Studies carried out by Jiménez et al. (2007) reported that precipitation had a significant effect on *D. viviparus* prevalence where average annual precipitation was 3333 mm, a value which is within the ranges found in this study. Precipitation conditions may favor survival of larvae, since they are found in excrement, which is susceptible to desiccation. Excess precipitation may also be an unfavorable condition for larvae, causing them to come into hypobiosis.

Fasciola hepatica has as the primary intermediate host the snail of the genus *Lymnaea*, whose distribution is determined by its requirements for specific humidity and vegetation (Pritchard et al., 2005; Bennema et al., 2009; Freitas et al., 2014). The environmental variables

Table 1
Environmental variables with significant differences between positive and negative farms for *D. viviparus*.

Variable	Category	Positives	Total	Percentage	χ2	P
Soils	Alfisols + Vertisols	2	23	8.70	2.73	> 0.05
	Inceptisol	18	461	3.90		
	Ultisol	0	32	0.00		
Precipitation (mm)	< 2000	1	63	1.59	5.75	> 0.05
	2000–4000	17	306	5.56		
	> 4000	2	148	1.35		
Altitudinal belt	Basal	11	142	7.75	8.19	< 0.05
	Montane	0	5	0.00		
	Lower Montane	4	132	3.03		
	Premontane	5	238	2.10		
Life zone	Moist Forest	9	103	8.74	8.72	< 0.05
	Wet Forest	11	383	2.87		
	Rain Forest	0	29	0.00		
Biotemperature (°C)	< 18	4	137	2.92	8.07	< 0.05
	18–24	5	238	2.10		
	> 24	11	142	7.75		
Elevation (m.a.s.l)	≤ 600	7	254	2.76	11.79	> 0.05
	600–1200	9	96	9.38		
	1201–1800	2	87	2.30		
	> 1800	2	82	2.44		

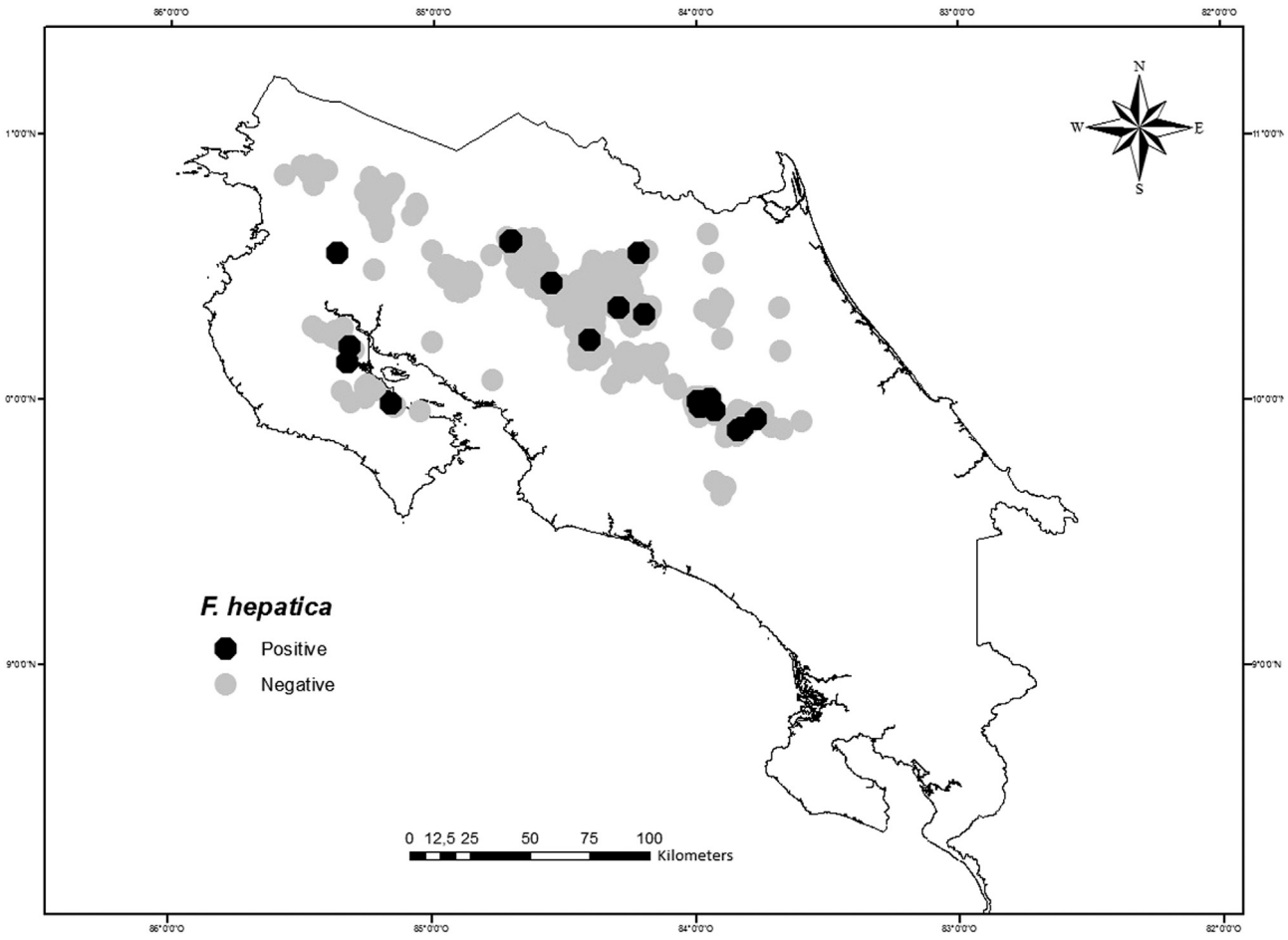

Fig. 3. Distribution of infected dairy herds for *Fasciola hepatica* in Costa Rica.

Table 2
Environmental variables with significant differences between positive and negative farms for *F. hepatica*.

Variable	Category	Positives	Total	Porcentage	χ2	P
Soils	Alfisols + Vertisols	5	23	21.74	26.11	< 0.05
	Inceptisol	11	459	2.40		
	Ultisol	3	32	9.38		
Precipitation (mm)	< 2000	6	63	9.52	6.89	< 0.05
	2000–4000	9	306	2.94		
	> 4000	4	146	2.74		
Altitudinal belt	Basal	4	140	2.86	5.02	> 0.05
	Montane	0	5	0.00		
	Lower Montane	9	132	6.82		
	Premontane	6	238	2.52		
Life zone	Moist Forest	7	103	6.80	3.48	> 0.05
	Wet Forest	11	381	2.89		
	Rain Forest	1	29	3.45		
Biotemperature (°C)	< 18	9	137	6.57	4.39	> 0.05
	18–24	6	238	2.52		
	> 24	4	140	2.86		
Elevation (m.a.s.l)	≤ 600	10	251	3.98	8.71	< 0.05
	600–1200	0	95	0.00		
	1201–1800	7	87	8.05		
	> 1800	2	82	2.44		

which showed significantly different values between *F. hepatica* positive and negative farms were soil type (Alfisols and Vertisols), annual precipitation < 2000 mm and elevation from 1201 to 1800 m.a.s.l. The types of soil that showed significant differences with respect to the presence or absence of *F. hepatica*, presented some characteristics that could contribute to the creation of optimal environments for the survival of snails. Snails require clay soils that accumulate a great amount of moisture, and that contain minerals such as calcium (Vertisols) needed to form shells (Morales and Pino, 2004; Schnieder, 2006; Deplazes et al., 2013). Some studies have found that soil characteristics may be predictive factors for fasciolosis (McCann et al., 2010; Selemetas et al., 2014; Selemetas et al., 2015). Selemetas et al. (2015) found that regions that were positive for the disease had deep soils with poor drainage, which is consistent with the characteristics of soils of

positive regions in Costa Rica, which have poor drainage and may flood during the rainy season. In relation to the significant variables of altitude and precipitation found in this study, the presence of *F. hepatica* has been associated elsewhere with environmental factors (precipitation, temperature, moist, presence of ponds or flows of water, soil type, vegetation type, type of snail habitat, presence of snails, presence of bodies of water, and pasture drainage) (Howell et al., 2015).

5. Conclusion

Spatial distribution of positive farms for *D. viviparus* showed significant clustering in contrast with the distribution of positive farm for *F. hepatica*. This distribution may be associated with environmental variables that were statistically related to both parasites. Precipitation, altitudinal belt, life zone and biotemperature were environmental variables associated to *D. viviparus,* whereas soil, precipitation and elevation were for *F. hepatica*. The results of the analysis of the environmental variables can be used in the future as an input in the construction of risk maps. It is recommended investigate the possible relationships of the presence of parasites with other environmental characteristics not considered in this study, such as humidity, soil pH, type of vegetation, and presence of water bodies. Likewise, variables related to management, such as grazing duration, deworming program, which have been reported in other latitudes, and have shown to be related to significant risks for *D. viviparus* and *F. hepatica,* should also be studied in detail.

Acknowledgements

We are very thankful to the Vicerrectoría de Investigación, Universidad Nacional, for financial support. We also thank the German Service of Academic Exchange (DAAD) for the research scholarship given to Victor Montenegro, M.Sc. Manrique Oviedo for the sample collection, and Sandra Buschbaum for the technical assistance. The collaboration of farm owners is also gratefully acknowledged.

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