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Prevalence and seasonal variation of *Fasciola hepatica* in slaughtered cattle: the role of climate and environmental factors in Mexico

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Abstract

The objective of this study is to determine the prevalence of *Fasciola hepatica* infection in cattle slaughterhouses, as well as its association with climatic/environmental factors (derived from satellite data), seasonality and climate regions in two states in Mexico. Condemned livers from slaughtered animals were obtained from three abattoirs in the states of Puebla and Veracruz. The overall prevalence of the parasite in cattle between January and December of 2017 was 20.6% (1407 out of 6834); the highest rate of condemnation was observed in Veracruz (26.3%; tropical climate), and the lowest rate was found in Puebla (15.5%; temperate climate). The seasonal prevalence of fluke infection was 18.6%, 14.8% and 28.4% during the wet season, and 17.1%, 12.4% and 22.8% during the dry season in the three abattoir sites, located in the districts of Zacatlán, Teziutlán and Ciudad Alemán, respectively. Liver condemnations due to bovine fasciolosis were prevalent in the Zacatlán, Teziutlán and Ciudad Alemán districts during summer, autumn and summer, respectively. Using generalized estimating equations analysis, we determined six variables – rainfall (wet/dry), land surface temperature day, land surface temperature night, normalized difference vegetation index, seasonality and climate regions (temperate/tropical) – to be significantly associated with the prevalence of condemned livers. Climate region was the variable most strongly associated with *F. hepatica* infection (odds ratio (OR) 266.59; 95% confidence interval (CI): 241.90–353.34), followed by wet and dry seasons (OR 25.56; 95% CI: 20.56–55.67).

Introduction

Fasciolosis, a widespread foodborne zoonotic disease that affects a wide range of mammals – particularly grazing animals – is caused by infection with *Fasciola hepatica*. Over 180 million people are at risk of infection globally. Furthermore, approximately 2.4 to 17 million individuals are thought to be infected with liver fluke, and this number is likely increasing (Sabourin *et al.*, 2018). Economic losses in the livestock industry exceed USD 3 billion worldwide, including USD 119 million per year in the Mexican cattle industry (Mehmood *et al.*, 2017; Villa-Mancera & Reynoso-Palomar, 2019b). Infected animals show reduced weight gain, fertility and meat and milk production, as well as liver condemnation in abattoirs, although this condition rarely causes mortality in cattle (Torgerson & Claxton, 1999; Sanchez-Vazquez & Lewis, 2013; Qin *et al.*, 2016). In Mexico, several recent studies using bulk-tank milk enzyme-linked immune sorbent assay (ELISA) in cattle herds have indicated that the *F. hepatica* prevalence in three climate regions ranged from 62.76% to 63.56% (Villa-Mancera & Reynoso-Palomar, 2019a, b). A high prevalence of parasite infection in cattle has been found in north-west Mexico using the indirect ELISA test (24.4%) and sedimentation faecal examination (11.4%) (Munguía-Xóchihua *et al.*, 2007). In addition, dairy cattle herds in the tropical climate had milk production losses of 1.50 kg per day, while dairy cow herds in the temperate climate showed losses of 1.29 kg per day (Villa-Mancera & Reynoso-Palomar, 2019b).

Geographic information systems (GIS) and remote sensing technologies have been useful for extracting data on environmental features to investigate their relationships with disease at known sites, as well as to areas where disease does not exist (Dutra *et al.*, 2010; Charlier *et al.*, 2014). The susceptibility of cattle to the effects of climate change is not restricted to tropical, arid or temperate zones, and recent environmental and climate changes due to global warming may alter the epidemiology, seasonality and geographical distribution of the free-living stages of this parasite, thereby increasing the risk of livestock populations to the disease (Villa-Mancera *et al.*, 2015; Charlier *et al.*, 2016; Villa-Mancera & Reynoso-Palomar, 2019b).

Although the existing data on liver condemnation in cattle slaughterhouses are not representative of the true infection status, they are useful for enabling herd tracking of regional parasite control programs. The aim of this study is to determine the seasonal prevalence of *F. hepatica* among slaughtered cattle in two climate regions in Mexico, using data collected by the inspection of livers. We also investigate the relationship between climatic and environmental factors and parasite status.

Materials and methods

Study area and geographic information systems

The study was conducted at three abattoir sites in the states of Puebla (Zacatlán and Teziutlán districts, east-central Mexico) and Veracruz (Ciudad Alemán district, eastern Mexico) from January through December of 2017. The states of Puebla and Veracruz cover areas of 33,919 km^2 and 71,826 km^2, respectively. After slaughter, with the presence of veterinary inspectors, the livers of 6834 cattle were inspected by visual examination, palpation and incision. The latitude and longitude of each abattoir were identified using a global positioning system (GPS, Garmin eTrex Vista, Olathe, KS, USA), and the coordinates were georeferenced using ArcGIS 10.1 (ESRI, Redlands, California, USA) and Köppen climate classification maps modified by García (1988) (fig. 1). In addition, we obtained information on elevation data provided by the Shuttle Radar Topography Mission, with a resolution of 1 km (http://srtm.csi.cgiar.org/). This study was approved by the local Animal Care and Ethics Committee of the Meritorious Autonomous University of Puebla, and all procedures complied with the National Legislation Pertaining to Animal Health Research.

Remotely sensed climatic data

Satellite-based precipitation data were averaged to obtain a mean monthly rainfall dataset for January through December, covering a radius of about 50 km around each abattoir site; these data were extracted from the Tropical Rainfall Measuring Mission (TRMM) 3B42 version 7 and TRMM 3B43 (http://disc2.gesdisc.eosdis.nasa.gov). Furthermore, we obtained monthly land surface temperatures (LSTs) and the normalized difference vegetation index (NDVI) from the Moderate Resolution Imaging Spectroradiometer sensor aboard the Terra satellite (https://lpdaac.usgs.gov/) – products MOD11C3 and MOD13C2.005 – with 0.05° spatial resolution. LST data were used as a proxy for day and night temperature, while the NDVI was used as a proxy for soil moisture (Sandholt *et al.*, 2002). Finally, LST and NDVI values were log-transformed to improve normality and to stabilize the variance (Sokal & Rohlf, 1995).

Fasciola hepatica risk index

The calculation of fluke infection risk was carried out using the index developed by Malone *et al.* (1998), based on growing degree-day (GDD) and using climatic factors that impact the *F. hepatica* life cycle:

$$\text{Index} = [\text{GDD} \times \text{days in the month, IF } R\text{–}(\text{PET} \times 0:8) > 0 + (\text{GDD} \times \text{RD})\left(\frac{(R - \text{PET})}{25}\right)\Big), \text{ IF}R\text{–PET} > 0]$$

where GDD is the average monthly mean temperature – 10°C (MOD11C3); R is the total monthly rainfall (mm/month, TRMM 3B43); PET is the potential evapotranspiration as calculated by the Penman method (mm/month); and RD is the number of rain days per month with more than 1 mm of rainfall (GPM_3IMERG-Day 0.1° × 0.1°). Four risk categories were described by Yilma & Malone (1998) to classify the calculated risk index values: (1) no-risk: ≤600; (2) low risk: 601–1500; (3) moderate risk: 1501–3000; and (4) high risk: >3000.

Statistical analysis

Data were analysed using the software IBM SPSS 25 for Windows (SPSS Inc., Chicago, IL, USA). To complete the most comprehensive study of the variability in exposure to *F. hepatica* over time, we compiled datasets for both wet/dry seasons and temperate-climate seasons (spring, summer, autumn and winter). A chi-square test was used for comparison of the prevalence between seasons, and a univariable generalized estimating equation (GEE) analysis was used to identify variables with significant associations between seasonal prevalence of positive cattle for *F. hepatica*. Abattoir data on liver inspection as a repeated measure, a binomial distribution and logit as the link function was specified. Furthermore, a multivariable GEE model was constructed using parasite infection as a dependent variable and monthly rainfall (wet/dry), LST day, LST night, NDVI, season (spring, summer, autumn and winter) and climate region (temperate/tropical) data as independent variables. The results are expressed as odds ratios (ORs), with 95% confidence intervals (95% CIs).

Results

Prevalence of condemned livers

In total, we inspected the livers of 6834 cattle – 1861 from Zacatlán district, 1735 from Teziutlán district and 3238 from Ciudad Alemán district (table 1). The overall prevalence of *F. hepatica* infection was 20.6% (n = 1407); the highest rate of condemnations was found in animals from Ciudad Alemán (26.0%; CI: 24.09–27.86), followed by Zacatlán (17.5%; CI: 14.78–20.29) and Teziutlán (13.2%; CI: 12.27–14.07).

Climate and environmental data

The elevation above sea level, Köppen climate classification and the risk index of the abattoir for each site were as follows: Zacatlán district: 2028 m, temperate climate and 632; Teziutlán district: 1890 m, temperate climate and 440; and Ciudad Alemán district: 24 m, tropical climate and 1082. A graphical representation of the prevalence of *F. hepatica* in slaughtered cattle compared to climate and environmental factors is shown in supplementary fig. S1.

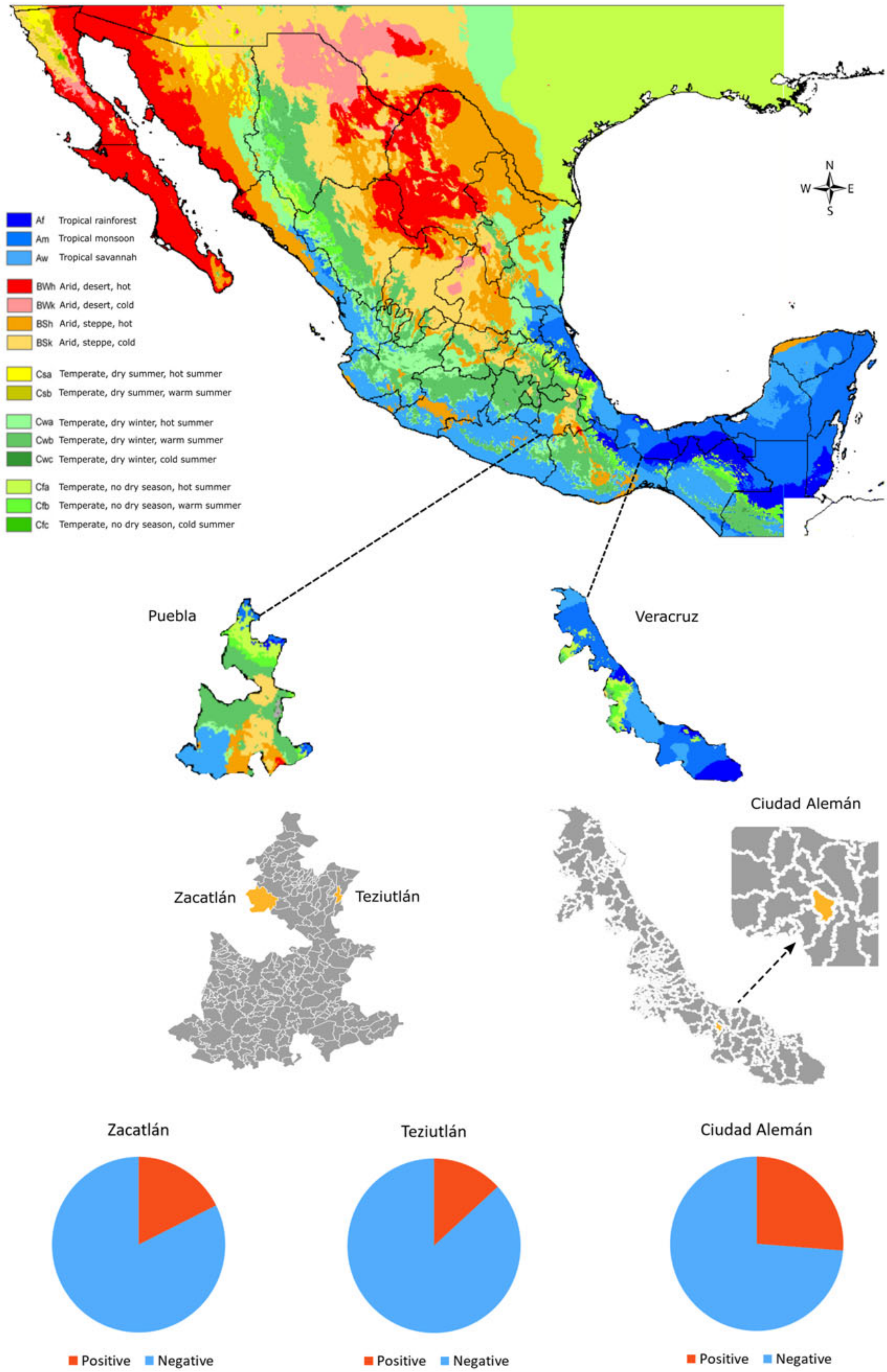

Fig. 1. Map of Mexico in the Köppen climate classification system showing the slaughterhouse locations in the districts of Zacatlán, Teziutlán and Ciudad Alemán.

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Table 1. Monthly prevalence of *Fasciola* in the livers of slaughtered cattle during the year 2017 in three abattoirs in the states of Puebla and Veracruz.

	Puebla state						Veracruz state		
	Zacatlán district			Teziutlán district			Ciudad Alemán district		
Month	No. of animals slaughtered	No. of livers condemned	Prevalence (%)	No. of animals slaughtered	No. of livers condemned	Prevalence (%)	No. of animals slaughtered	No. of livers condemned	Prevalence (%)
Jan	148	17	11.5	135	14	10.4	212	48	22.6
Feb	149	18	12.1	137	15	10.9	239	55	23.0
Mar	162	24	14.8	139	17	12.2	243	55	22.6
Apr	152	18	11.8	140	17	12.1	195	42	21.5
May	154	22	14.3	141	18	12.8	241	63	26.1
Jun	155	26	16.8	142	21	14.8	256	73	28.5
Jul	148	31	20.9	135	20	14.8	274	76	27.7
Aug	154	28	18.2	132	19	14.4	320	97	30.3
Sep	156	29	18.6	144	22	15.3	315	99	31.4
Oct	160	37	23.1	160	21	13.1	248	68	27.4
Nov	165	40	24.2	162	22	13.6	340	90	26.5
Dec	158	38	24.1	168	23	13.7	355	84	23.7
Total	1861	328	17.5	1735	229	13.2	3238	850	26.0

Seasonal prevalence of F. hepatica

To investigate the seasonality of *F. hepatica* during the wet and dry seasons for each slaughterhouse, we tabulated the percentage of condemned livers (table 2). The highest average prevalence for all abattoirs was detected in the wet season (24.1%), whereas the lowest prevalence was observed in the dry season (17.6%). Overall, there was a significant difference between the wet and dry seasons ($P = 0.019$). The wet season in Zacatlán and Teziutlán is the four-month period from June through September, while the dry season lasts from October through May. For the tropical climate in the Ciudad Alemán district, the wet season extends from May to November and the dry season stretches from December to April. The highest percentages of prevalence during the study period were found in the wet (28.4%) and dry (22.8%, $P = 0.022$) seasons from Ciudad Alemán district, with a tropical climate, low altitude and low risk category. The lowest estimated prevalence of condemned livers was observed in Teziutlán district, which has a temperate climate, high altitude and a no-risk classification.

The lowest percentage of livers condemned for liver flukes occurred in winter (16.8%), while the highest percentage was found in summer (23.7%, table 3). A significant difference between seasons and districts was also observed ($P < 0.05$). The greatest numbers of condemned livers were reported from Ciudad Alemán district, which showed the highest prevalence (29.9%) in the study. Statistically significant differences were found in Zacatlán and Ciudad Alemán districts between seasons ($P < 0.05$).

GEE*s*

The univariable GEE analysis of condemned livers at slaughter over time is outlined in supplementary table S1. Supplementary table S2 presents the significant differences between seasons (spring, summer, autumn and winter) and between the wet and dry seasons. The results of the multivariable GEE analyses are presented in table 4. Among confirmed fasciolosis cases in the abattoirs, temperate vs. tropical climate regions (OR 266.59; 95% CI: 241.90–353.34) and wet vs. dry season (OR 25.56; 95% CI: 20.56–55.67) were all strongly associated with increased odds of condemned livers.

Discussion

The present study is the first to determine the seasonal prevalence of bovine fasciolosis with different climate regions, elevations and risk factors in Mexico through the inspection of livers, as well as the first study to link the presence of this disease to climate and environmental factors. The percentages of prevalence of condemned livers in Zacatlán and Teziutlán districts with temperate climate ranged from 13.2% to 17.5%, similar to the range in prevalence reported in Brazil (10.14–18.66%) (Dutra *et al.*, 2010) but higher than those observed in Portugal (2.2%) (Barbosa *et al.*, 2019) and lower than those reported in Brazil (37.6%), Algeria (26.7%), Portugal and Spain (28%), Uruguay (33.9%) and Peru (55.72%) (Arias *et al.*, 2011; Ouchene-Khelifi *et al.*, 2018; Quevedo *et al.*, 2018; da Costa *et al.*, 2019; Arias-Pacheco *et al.*, 2020). This study shows that the prevalence of *F. hepatica* in the tropical climate was 26.0%, which is consistent with the prevalence (25.8%) reported in cross-sectional surveys in tropical climates in Mexico (Ojeda-Robertos *et al.*, 2020), although it is higher than the prevalence found in Costa

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Table 2. Significant differences between the wet and dry seasons in the number and prevalence of condemned livers due to *F. hepatica* infections at three Mexican abattoirs.

State/district	Wet season			Dry season			*P*
	No. of animals slaughtered	No. of livers condemned	Prevalence (%)	No. of animals slaughtered	No. of livers condemned	Prevalence (%)	
Puebla							
Zacatlán	613	114	18.6[a]	1248	214	17.1[c]	0.739
Teziutlán	553	82	14.8[a]	1182	147	12.4[c]	0.250
Veracruz							
Ciudad Alemán	1994	566	28.4[b]	1244	284	22.8[d]	0.022
Total	3160	762	24.1	3674	645	17.6	0.019

Rainy season: [a]June–September, [b]May–November; dry season: [c]October–May, [d]December–April.

Rica (1.83%) (Rojas & Cartín, 2016). Therefore, climate and environmental variables, the livestock system and general management factors – such as the length of the grazing season and the proportion of grazed grass in the diet – are important considerations in the prevalence of condemned livers and for comparing results across studies and countries. For instance, in Switzerland, Rapsch *et al.* (2006) estimate the true prevalence of *F. hepatica* in slaughtered cattle at 18.0%; the high diagnostic sensitivity was found using coproscopy, bile examination and antibody ELISA rather than condemned livers, resulting in a lower prevalence. One of the limitations of the present study was the failure to obtain faeces and serum samples due to financial, technical and political reasons.

This study shows that the prevalence of *F. hepatica* at altitudes higher than 1800 m above sea level in Puebla (which has a temperate climate) ranges from 13.2% to 17.5%, which is lower than the prevalence in Peru (55.4% and 55.72% at 3300 m and 3350 m above sea level, respectively) (Arias-Pacheco *et al.*, 2020; Caravedo *et al.*, 2021). Our survey also shows that the prevalence of this parasite in the tropical climate (24 m above sea level) was 26.0% – higher than that reported in Cuba (18.27%) – with an altitude of 122 m above sea level (Palacio Collado *et al.*, 2017). In addition, Malone *et al.* (1998) report that the optimum thermal conditions in tropical sites for the development of *F. hepatica* are found in lower-elevation areas, providing an example of previous studies that have reported a significant relationship between elevation and parasite infection risk (Dutra *et al.*, 2010; Martins *et al.*, 2012; Villa-Mancera & Reynoso-Palomar, 2019a, b).

In this study, the wet and dry seasons had a significant influence on the bovine fasciolosis occurrence in the tropical climate (in Veracruz); the prevalence rate was greater during the wet season (28.4%) than in the dry season (22.8%). These results align with those of a study by Bernardo *et al.* (2011), who report a higher rate of fasciolosis cases in cattle slaughtered during the dry season (25.79%) and a lower rate in the wet season (23.87%) in Brazil. Again, this is consistent with previous reports from Tabasco, Mexico indicating a prevalence of between 9.36% and 8.34% during the wet and dry seasons, respectively (Ojeda-Robertos *et al.*, 2020). A high condemnation rate in the wet season is likely due to increased populations of infected snails in grazing pastures and humid microhabitats that enhance the survival of the infective metacercariae, as well as the lack of metacercariae exposure during the dry season. Villa-Mancera *et al.* (2019b) propose seven specific factors that serve as significant predictors of parasite infection, based on the detection of *F. hepatica*-specific antibody levels in bulk-tank milk samples: rainfall, elevation, the proportion of grazed grass in the diet, contact with other herds, herd size, parasite control use and education levels.

No significant difference in the prevalence of condemned livers was found between the wet and dry seasons in a temperate climate at altitudes of 1890 m and 2028 m. Consequently, the findings in this study are similar to those of another study conducted in Peru, in which prevalence rates of 56.3% and 53.9% in the wet and dry seasons were found, at 3300 m above sea level (Arias-Pacheco *et al.*, 2020). Persistent infection throughout the year may result from continued exposure of the cattle to encysted metacercariae in grazing pastures.

In our study, the highest seasonal prevalence of *Fasciola* infection in the tropical climate was observed in summer (29.9%), followed by autumn (25.7%), spring (25.7%) and winter (22.8%). These results are similar to those reported in Brazil (Bernardo *et al.*, 2011) and show significant seasonal pattern for fasciolosis infection in livers in Zacatlán district, which have temperate climates. Furthermore, these results are similar to those reported in Algeria, where the prevalence was significantly different for all seasons (Ouchene-Khelifi *et al.*, 2018). However, this finding contrasts with that of a study in Greece reporting that infection prevalence was not significantly different for all seasons (Theodoropoulos *et al.*, 2002).

We used a GEE approach to construct a multivariable model, showing that six variables were significantly associated with parasite infection: rainfall (wet/dry), LST day, LST night, NDVI, season and climate region (temperate/tropical) (table 4). Climate region was clearly one of the most significant factors in *F. hepatica* infection in cattle; tropical climates present a 266.59-times higher risk of infection in slaughtered animals than a temperate climate (95% CI: 241.90–353.34). Similar odds were observed with LST day (OR 11.18; 95% CI: 10.98–42.09) and LST night (OR 11.20; 95% CI: 11.00–42.11). Our results are reinforced by the fact that several researchers have found that temperature is a positive predictor of infection in Ireland, England and Wales, and Mexico (McCann *et al.*, 2010; Selemetas & de Waal, 2015; Villa-Mancera & Reynoso-Palomar, 2019b). However, this finding contrasts with previous studies in Brazil that have reported no significant differences in prevalence based on temperature (Dutra *et al.*, 2010). Cattle slaughtered in the wet season had 25.56-times higher liver fluke infection rates than those

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Table 3. Seasonal prevalence of bovine fasciolosis in three slaughterhouses in the states of Puebla and Veracruz.

State/district	Spring		Summer		Autumn		Winter	
	Slaughtered/ condemned	Prevalence (%)	Slaughtered/ condemned	Prevalence (%)	Slaughtered/ condemned	Prevalence (%)	Slaughtered/ condemned	Prevalence (%)
Puebla								
Zacatlán[a]	461/66	14.3	458/88	19.2	483/115	23.8	459/59	12.9
Teziutlán	423/56	13.2	411/61	14.8	490/66	13.5	411/46	11.2
Veracruz								
Ciudad Alemán[a]	692/178	25.7	909/272	29.9	943/242	25.7	694/158	22.8
Total[a]	1576/300	19.0	1778/421	23.7	1916/423	22.1	1564/263	16.8

[a]Prevalence of *F. hepatica* is significantly different ($P < 0.05$, chi-square test).

Table 4. Significant associations between prevalence of condemned livers at three Mexican slaughter and seasonal, climate and environmental variables, using multivariable GEE analysis.

Variable	Odds ratio	Confidence interval (95%)	*P*-value
Intercept			<0.001
Annual rainfall			
Wet vs. dry	25.56	20.56, 55.67	<0.001
LST day	11.18	10.98, 42.09	<0.001
LST night	11.20	11.00, 42.11	<0.001
NDVI	11.54	11.40, 42.50	<0.001
Seasonal	5.86	3.06, 57.43	0.015
Climate regions			
Temperate vs. tropical	266.59	241.90, 353.34	<0.001

slaughtered in the dry season (95% CI: 20.56–55.67). Furthermore, rainfall has been found to have a consistent relationship with prevalence based on data on fasciolosis in Belgium, England and Wales, and Mexico (McCann *et al.*, 2010; Bennema *et al.*, 2011; Villa-Mancera & Reynoso-Palomar, 2019a). In China, temperature, rainfall and elevation have been associated with *F. hepatica* infestation (Qin *et al.*, 2016). Temperatures greater than 10°C are required for the development of the free-living larval stages and intra-snail stages of the trematode, as well as the development of the parasite's intermediate molluscan (Andrews *et al.*, 1999; Torgerson & Claxton, 1999). In Mexico, for the life cycle to be complete, *Lymnaea humilis* and *Lymnaea bulimoides* must be frequently present, appearing during or immediately after rainfall peaks (Cruz-Mendoza *et al.*, 2011). The NDVI includes several factors (land cover, temperature, rainfall and vapour pressure) that have been identified as positive predictors of disease in Australia (Durr *et al.*, 2005). However, there are also negative aspects to our approach. For example, our study is limited to using data from one or two slaughterhouses for each climatic region. While our study reports ranges in prevalence similar to those in other studies, more accurate and robust outputs may be obtained by using existing data on liver condemnation in slaughterhouses throughout all of Mexico.

In conclusion, this is the first study to provide epidemiological data on the seasonal prevalence of fasciolosis in cattle slaughterhouses from different climate regions in two states of Mexico, demonstrating that the overall prevalence of *F. hepatica* was 20.6%. Moreover, our study examines and compares seasonal data with regards to climate and environmental factors, and it highlights the importance of continuous monitoring programmes for liver fluke infection. Lastly, we identify some of the factors associated with the prevalence of condemned livers, including rainfall, LST, NDVI, season and climate region. Further studies are necessary to generate more knowledge on shifts in levels of exposure between years and climatic regions.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X21000444

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Conflicts of interest. None.

Ethical standards. This study was approved by the local Animal Care and Ethics Committee of the Meritorious Autonomous University of Puebla, and all procedures complied with the National Legislation Pertaining to Animal Health Research.

Author contributions. Conceptualization, methodology, funding acquisition, project administration, supervision, writing – review and editing: K.H. and P.M. Investigation, supervision, formal analysis: J.O. Conceptualization, methodology, writing – review and editing: Y.A. Supervision, data analysis, writing – original draft: A.O. and A.C. Analysis tools, writing – review and editing: A.V. All authors read and approved the final manuscript.

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