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Veterinary Parasitology 81 (1999) 119–127

veterinary parasitology

Bovine fasciolosis in Tabasco, Mexico

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Received 7 May 1996; accepted 8 April 1998

Abstract

To establish seasonal trends in infection and maturity of *Fasciola hepatica* in cattle in relation to macroclimatic factors, 2730 condemned livers were examined from March 1989 to February 1992. Livestock came from Jalapa, Tacotalpa and Teapa, all municipalities in Tabasco State. Flukes were collected monthly and separated into three different maturation stages. Mean numbers of flukes collected were determined. The analysis of the maturation stages detected in cattle showed: (a) *F. hepatica* matured throughout the year at all three sites; (b) the greatest mean fluke's burdens were found in Jalapa and the lowest in Teapa; large parasite populations were significantly higher from February to September than in July and/or August; (c) persistence of mature, gravid *F. hepatica* indicated that parasite eggs are shed throughout the year; (d) recruitment of *F. hepatica* occurred throughout the year with two major periods of infection, the first and main period during the dry season (from February to June), and a second minor infection period, during the rainy season (from August to October); (e) a close relationship was observed between the seasonal infection pattern in cattle and the seasonal infection pattern in snails, as well as fluctuations in the snail population according to rainfall and temperature variation. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Trematoda; Fasciolosis; Cattle; Epidemiology; Seasonal trends; *Fossaria*; Mexico

1. Introduction

In the early 1970s, many studies were carried out to establish the transmission periods of fasciolosis in livestock throughout the year. The relationship of transmission to climatic factors has been determined by other studies all around the world including Ross and Morphy (1970) in Ireland; Ollerenshaw (1971) in England; Ross (1977) in Scotland; Mage (1989) in France and Manga-Gonzalez et al. (1990) in Spain. In Asia, studies by

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PII: S0304-4017(98)00152-6

Bhatia et al. (1989) and Pandit et al. (1989) in India, and Morel and Mahato (1987) in Nepal, are relevant. Major contributions have been made in Africa by Mzembe and Chaudhry (1979) in Malawi, and Njau et al. (1990) in Ethiopia and in South America, by Cardozo and Nari (1980) in Uruguay, Amato et al. (1986) in Brazil and Malone et al. (1985) in the USA.

Analysis of seasonal trends of *Fasciola hepatica* infection in its final host is important since, through this, recruitment and highest infection levels can be established. Most studies have been based on fecal examination, post-mortem surveys and immunological studies but were limited mainly to data on prevalence. This is important because consequences are not the same for a host parasitized by one parasite, a hundred or a thousand. In addition, *F. hepatica* can live for months or years in the bile ducts producing eggs constantly. Since *Fasciola* lives for more than 1 year in cattle, this complicates the determination of the major risk periods. Thus, new procedures have been developed or modified in order to obtain the necessary information to determine host infection intensity, its temporal variation and causal factors.

Fasciolosis is present at high prevalence rates in the states of Veracruz and Tabasco along the Gulf of Mexico as well as in Sinaloa, Jalisco and Michoacan on the Pacific Coast. However, it is important to consider that microclimate can vary considerably from one region to another, from one farm to another or between neighbouring open grasslands. Differences in livestock management and zootechniques also have a major influence on the importance of fasciolosis. Studies on *F. hepatica* prevalence in cattle in Mexico are rare.

The aim of this investigation was to determine seasonal trends of *F. hepatica* infection in cattle in three Tabasco sites in relation to climatic factors.

2. Materials and methods

Livers were collected in the main slaughterhouse in Tabasco which is located in Villahermosa city. As it was not feasible to collect healthy and infected livers randomly, only livers condemned due to suspected fasciolosis were processed. As a result, some ecological parameters such as prevalence and abundance could not be obtained. Sample size consisted of 20 livers infected with *F. hepatica* or with evidence of pathology and collected from cattle originating from Teapa, Jalapa and Tacotalpa on a monthly basis. The monthly collections were made from March 1989 through February 1992.

Livers were sliced (5 mm thick) to examine the hepatic parenchyma and bile ducts to locate all flukes. In the third sampling year liver slices were shaken in a bucket with water and then sifted through a 0.5 mm screen. Flukes were collected with fine brushes and forceps taking care of collecting only complete specimens. They were transferred to a plastic bowl with saline solution. After collection, the flukes were placed in cold water to have them relaxed and were later fixed in 80% ethanol. In the early sampling months at least one of every 10 flukes was flattened and fixed with Bouin's fixative and permanent mounts were prepared to determine the maturation stage.

Infection level was estimated with the obtained data, according to Margolis et al. (1982); the infection intensity (minimal and maximum number of parasites) and the mean infection intensity (mean number of parasites per parasitized host) were also determined.

2.1. Determination of maturity stages

Parasites were cleared in lactic acid to allow for organ identification and other internal structures in determining maturity stage according to Elkins and Corkum (1976); these authors considered the development of structures such as genital system, presence or absence of eggs, vitelline glands and size. The first stage corresponded to immature parasites which had just entered the host, the second was an intermediate stage, where several organs were still in development and the third included parasites with full reproductive capacity. Great variability was observed in shape and size of flukes as well as in the development of internal organs mentioned above. All three characteristics were considered collectively to determine the maturity stage, since there was no consistency between shape, size and maturity of worms.

Stage 1. (1) absence or little development of genital organs, mainly testis and ovary, (2) absence or little development of vitelline follicles, (3) vitelline reservoir absent or poorly defined, (4) no eggs in uterus, (5) very simple digestive system (6) small body size.

L. max.	6.40–18.00 (10.55)	W. max.	2.70–5.10 (3.85)
L. o.s.	0.30–0.55 (0.41)	W. o.s.	0.35–0.70 (0.50)
L. v.s.	0.59–0.90 (0.73)	W. v.s.	0.45–0.85 (0.64)
L. p.	0.20–0.60 (0.46)	W. p.	0.20–0.55 (0.28)

L=length, W=width, max=maximum, o.s.=oral sucker, v.s.=ventral sucker, p=pharynx. All measurements in mm.

Stage 2. (1) very simple testis and ovary, (2) poorly defined vitelline follicles, (3) more developed vitelline reservoir and better defined than in stage 1, (4) absence or few transparent eggs in the uterus, (5) more developed digestive system than in stage one, (6) medium body size.

L. max.	8.70–17.3 (14.04)	W. max.	3.20–9.60 (6.53)
L. o.s.	0.40–0.80 (0.55)	W. o.s.	0.45–0.80 (0.82)
L. v.s.	0.63–1.35 (0.89)	W. v.s.	0.45–0.99 (0.84)
L. p.	0.40–0.85 (0.63)	W. p.	0.23–0.80 (0.34)
D. ov.	0.27–0.63 (0.45)	D. oo.	0.07–0.30 (0.17)

D=diameter, ov=ovary, oo=ootype; all measurements in mm.

Stage 3. (1) well-defined contour of testis and ovary, (2) well-defined vitelline follicles occupying the whole area of the lateral fields (3) well-defined vitelline reservoirs and full of vitellum (brown colour) (4) uterus full of eggs, most of them yellow, (5) well-developed digestive system, (6) large body size.

L. max.	16.50–25.40 (20.78)	W. max.	7.10–12.30 (9.75)
L. o.s.	0.50–1.35 (0.73)	W. o.s.	0.45–0.97 (0.82)
L. v.s.	0.75–1.35 (1.01)	W. v.s.	0.80–1.35 (0.99)
L. p.	0.66–0.97 (0.79)	W. p.	0.23–0.75 (0.44)
D. ov.	0.50–0.90 (0.75)	D. oo.	0.15–0.37 (0.26)

All measurements in mm.

With the exception of seasonal trends obtained from all 3 years of data, all statistics were analysed as individual year data. In the third year, the change in the fluke sampling (washing and sifting), increased in the efficiency of collection, mainly for stages 1 and 2. In order to contrast differences among the monthly samples, means for each site were subjected to a one-way analysis of variance using ranks of the Kruskal–Wallis test. The seasonal rate for mean intensity on the total stages or for the sum of the immature stages 1 and 2 was used to determine the presence of a cyclical and seasonal trend in infection. The monthly average and totals of the means of the 3 years were obtained to establish the seasonal rate for mean intensity. Then, the rate was obtained by the method of mean percentage. Using this method, monthly data were presented including percentages of the annual average. The 12 resulting percentages determined seasonal trends.

The relationship between monthly variation of infection rates and climatic parameters (temperature and rainfall) was determined by Spearman's correlation analysis. Parasitological rates were analysed: mean intensity (INT X), intensity of stages 1–3 (S1, S2 and S3) with climatic parameters such as temperature (TEMP), rainfall (RAIN) and rainfall frequency (RFRE).

Samples were collected in the first 15 days of every month and the monthly data were considered results of the previous month because infection values corresponded to the recruitment carried out the previous month. The first year was considered from March 1989 to February 1990, the second year from March 1990 to February 1991 and the third year from March 1991 to February 1992.

3. Results

Between March 1989 and February 1992, 2730 cattle livers from Teapa, Tacotalpa and Jalapa were examined. From the 2049 parasitized livers observed, a total of 52 756 *F. Hepatica* were recovered; this resulted in a mean of 25.75 flukes per liver.

3.1. Analysis of infection intensity

A annual values for mean fluke numbers were highest in the cattle from Jalapa, with a 3-year average of 29.10. 3-year average values were lower for Tacotalpa (24.96) and Teapa (22.81). Annual mean values for fluke collections in each site are shown in Table 1. The general trend of fluke infection from all three sites was similar. As no significant differences were observed between sites, results were integrated in a set of data showing the regional trend of *F. hepatica* infection. The highest mean intensity values occurred during the dry season, that is, from March to June, with values from

Table 1
Annual mean data on *Fasciola hepatica* infections in cattle from three sites of the Sierra region in Tabasco State

Municipality	Year	Livers examined	Livers infected	Number of flukes	Range/liver[a]	Mean/liver[b]
Jalapa	1	312	240	6873	1-338	28.64
	2	267	212	5400	1-283	25.47
	3	266	237	7997	1-277	33.74
Tacotalpa	1	349	241	5596	1-232	23.22
	2	287	230	3940	1-90	17.13
	3	279	219	7687	1-319	35.10
Teapa	1	405	239	4922	1-324	20.59
	2	311	226	3894	1-216	17.23
	3	254	205	6467	1-514	31.55

[a]Infection intensity.
[b]Mean of infection intensity.

47.95 to 118.85 worms per infected liver. Lowest values were observed during the rainy winter season, that is, from November to February with values ranging from 37.80 to 26.85 worms (Fig. 1).

3.2. Analysis of maturity stages

The mean intensity of stages 1 and 2 showed a similar pattern. For stage 1, during the dry season, values ranged from 7.3 to 13.1 and for the rainy winter season, from 1.9 to 8.9. For stage 2 during the dry season, variability was from 8.5 to 12.9 and in the rainy winter season from 3.2 to 3.8 (Fig. 2)

Throughout the 3 years of study, the monthly mean temperatures were at maximum values during May (28.8°C) and June (28.6°C) and at minimum values in December (21.7°C) and January (23.2°C). The highest rainfall was in September and October with 639.9 and 462.4 mm, respectively, and the lowest in March, April and May with 80.3, 30.6 and 85.5 mm, respectively, (Fig. 3).

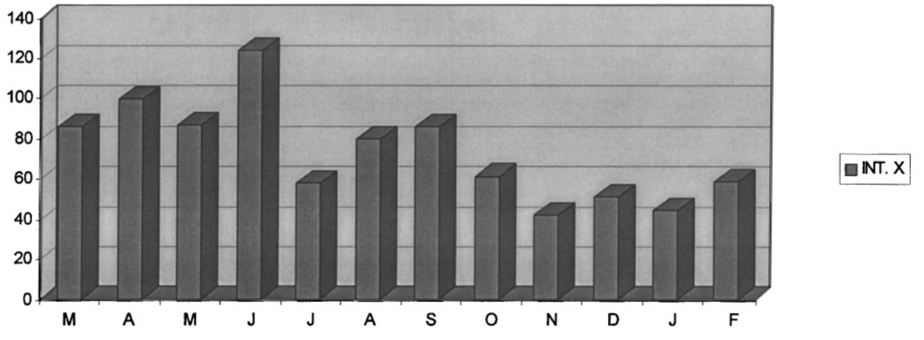

Fig. 1. *Fasciola hepatica* mean monthly intensity in Tabasco State, Mexico. INT. X=mean intensity.

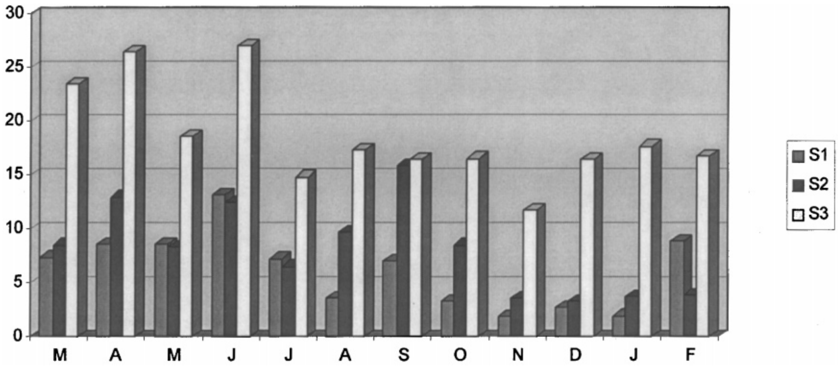

Fig. 2. Mean monthly intensity of *Fasciola hepatica* maturity stages in Tabasco State, Mexico.

3.3. Seasonal trends

The seasonal rate of monthly mean intensity was as follows: increases occurred during March–June and August–September (Table 2). The maximal values occurred in April and June.

The results obtained from correlation analysis using the Spearman rank test showed a negative correlation between parasitological values and rainfall amount and frequency and a positive correlation of parasitological values with temperature.

4. Discussion

The highest *F. hepatica* fluke numbers were found in Jalapa with smaller numbers in Tacotalpa and the lowest in Teapa. This could be attributed to topography of each site in that Jalapa has the largest lowland area with many flood plains that favour the

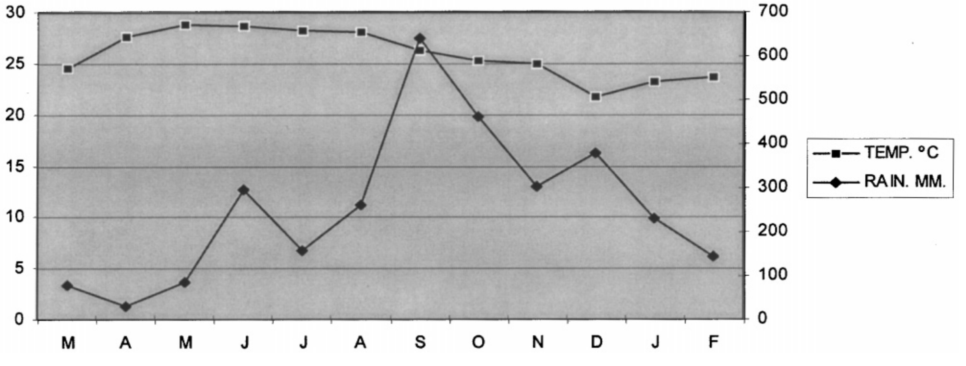

Fig. 3. Climatic parameters in Tabasco State, Mexico, 1989–1992.

Table 2
Monthly average and yearly means for the 3-year seasonal trends calculated by the mean percentage method of *Fasciola hepatica* in the Tabasco State

Year			1		2			3			
Total			859.0		667.0			1093.0			
Monthly average			72.0		56.0			91.0			
Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
66.6	154.8	130.4	165.1	87.1	122.7	109.4	111.5	37.3	95.4	52.5	60.8
68.0	208.7	93.2	129.3	70.0	109.4	91.6	72.8	92.5	70.4	94.7	107.4
189.2	77.7	124.7	198.4	79.7	98.3	139.6	69.2	51.5	48.9	45.3	78.8
Total											
323.8	441.2	348.3	492.8	236.8	330.4	340.6	253.5	181.3	214.7	192.5	247.0
Seasonal trends											
Maximum value					Maximum value			Minimum value			
108	147	116	164	79	110	114	84	60	72	64	82

establishment of suitable micro-environmental growth of snail intermediate hosts and transmission of larval stages of free-living *F. hepatica* (miracidia and cercariae).

Annual mean fluke collections in the three sites were over 20 parasites per parasitized liver except for the second year in Tacotalpa and Teapa. In general, decrease in annual mean fluke collections occurred from all sites during the second year but all increased during the third year. Increase during the third year was mainly due to greater efficiency in collections of stages 1 and 2 as a result of improved recovery in the techniques (washing and sieving of sliced livers).

With the exception of the monthly variation of fluke collections for stages 1 and 2, total fluke numbers were similar in the three sites. The general pattern of maturity stage numbers did not show any correlation between stage 1 peaks and the increment of stage 2 a month after. However, during the 3 years, the highest and lowest recruitment periods occurred at the same times of the year.

Monthly mean fluke collections were higher from February to September except for July and/or August where a significant decrease was observed. The fewest flukes were recovered in November–January, the period in which the lowest temperatures of the year occur. For mean numbers of stages 1 and 2, monthly variation was similar to that of all three stages combined. The lowest figures of intensity in stages 1 and 2 were observed when they were considered independently. Mature flukes of stage 3 were observed during the entire study period in all three sites due to reinfection and parasite longevity, and some egg shedding by *F. hepatica* occurred throughout the year.

Infection occurred throughout the year as indicated by the presence of stages 1 and 2 which were the two major periods of infection. The time periods for stages 1 and 2 began in February or March and ended in September or October including a month of lower values for stages 1 and 2 in July or August. Increase in infection rates was associated with a period of low rainfall amount and frequency and an increase in temperature which was for typical of the area (West et al., 1987).

Of the two infection periods the first was considered most important; it occurred during the dry season, the second occurred in the rainy season. This pattern was similar to the one reported by Hernandez et al. (1990), who studied Fasciolosis in sheep in 34 'Tzotziles' communities in Chiapas; they found that *F. hepatica* infection was higher during the dry season (26%) than in the rainy season (9.5%). In Uruguay, Cardozo and Nari (1980) also found the highest infection rates in the dry season.

It was difficult to establish seasonal infection patterns in the area studied because seasonality is not as clear-cut and precise as in temperate regions. Major infection occurred during spring, summer and autumn with a significant decrease in mid-summer (August) and during winter. Cardozo and Nari (1980) in Uruguay reported similar results using ovine tracers during a major infection period in spring and autumn and greatly reduced infection rates in winter. Mage (1989) in Cerdagne, France, reported major infection rates during the summer months with 44.7% of 163 bullocks being positive in September. Variable climatic patterns in different regions have been reported by a number of authors to determine major periods of *Fasciola* transmission in divergent world agroecologic zones (Malone et al., 1985; Amato et al., 1986; Morel and Mahato, 1987; Bhatia et al., 1989; Pandit et al., 1989; Manga-Gonzalez et al., 1990).

The results obtained from the analysis by the Spearman rank correlation coefficient showed a negative or opposite relationship between parasitological values and rainfall amount and frequency; a positive or direct relationship was observed in the case of temperature. A negative correlation between the mean numbers of stage 1 and rainfall was observed following the main snail infection period and maximum abundance of *Fossaria viatrix* which is intermediate host of *F. hepatica* in this region (Rangel-Ruiz, 1995a, b).

5. Future directions

Significant progress has been made in recent years in determining infection patterns of *Fasciola hepatica* in its two hosts; *Fossaria viatrix* and cattle. The understanding of fasciolosis in Tabasco State will be improved by determining the efficiency of molluscicidal substances extracted from local plants to control the intermediate host of fasciolosis and by application as an integral part of control on farms. Based on seasonal variation in mean intensity of flukes recovered in this and other studies carried out in Tabasco, strategic control programs will have to be improved to reduce the huge economic losses due to *F. hepatica* infection in this region (Rangel-Ruiz and Martínez-Duran, 1994).

Acknowledgements

This work was supported by funds from the Ministry of Public Education (SEP) and the National Council of Science and Technology (CONACYT).

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