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Technical note

Factors associated with the seizure of livers positive to *Fasciola* sp. in an endemic area of southeastern Mexico

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Abstract:

This study aimed to determine the frequency for the seizure of livers with damages attributed to the presence of *Fasciola* sp. and associated risk factors. It was conducted a prospective observational study with daily visits, for 12 months, to a municipal slaughterhouse in the Sierra area in the state of Tabasco. Of the seized livers, 25.8 % tested positive for the parasite presence; seizure was the same in male and female animals (X^2= 0.011, gl= 1, P<0.05). The highest proportion of seized livers was observed during the rainy season (9.36 %). This study concludes that the prevalence of fascioliasis in the Jalapa area has not decreased in recent years and is related to animal origin. Fascioliasis is a disease that must be monitored to detect the factors that allow it to remain in a geographic region, in order to establish and propose strategic control and preventive measures adapted to the particular conditions of endemic areas.

Keywords: Seizure, *Fasciola* sp., Liver, Tabasco

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Fascioliasis is a zoonotic parasitic disease caused by the presence of *Fasciola* sp. trematodes in the hepatic ducts of ruminants[1,2]. It is an economically important disease, since it affects productive species such as cattle, sheep, horses, and hogs, in addition to wild animals[3]. The economic costs associated with its presence are estimated to amount to 3 million dollars worldwide[4]. In Mexico, losses ascend to 130 thousand dollars[5], in addition to being a parasitic disease included in the priority research list of neglected tropical diseases[1]. In recent years, fascioliasis prevalence in domestic animals and humans has increased due to climate change, animal movement from one area to another, and the traffic of travelers and migrants[6,7].

In Tabasco, one of the main agricultural activities is cattle farming, based on intensive or extensive grazing systems, which favor the presence and transmission of the disease in the ruminants of the region. The disease occurs when animals ingest infective metacercariae, which are cysts attached to the grass, hence the importance of this disease in the production system.

Fascioliasis has been reported as common parasitism in animal populations, with state prevalence of 19.7 %[8], with variations according to the area and the characteristics of each region. Rangel and Martínez[8], identified areas of high, medium, and low prevalence, which classifies the state as an endemic area of the disease, along with Chiapas and Veracruz.

In the absence of a sensitive and specific diagnostic technique for the detection of positive animals, one way to determine the true prevalence is by monitoring slaughterhouses; this can provide relevant epidemiological information at a relatively low cost[9]. The analysis of this information can be used to determine the behavior and significance of the disease in a specific region[9]. Sanitary inspection is a routine procedure during the slaughter of beef cattle for human consumption and is crucial for epidemiological studies that allow seeing the disease scenario. The presence of livers positive to *Fasciola* is a cause for immediate seizure; however, more remains to be known about the factors related to the presence of the disease. This study aimed to determine the prevalence in slaughterhouses and the factors associated with the seizure of livers in an endemic area of southern Mexico.

The study was carried out in the State of Tabasco, on the municipal slaughterhouse of Jalapa, which is located in the Rio Grijalva region, Sierra subregion of the state. The area is characterized by having a tropical rainforest climate (Af) with year-round rainfall, according to the Koppen climate classification modified by García[10]. Mean temperature of 24.9 °C and annual mean precipitation of 3,711 mm are recorded[11]. The bodies of water of the Jalapa municipality are made up of two large rivers, the Rio de la Sierra and Puente Grande, as well as streams and lagoons. This complex orographic system overflows during the rainy season, causing flooding in the area.

It was conducted a prospective observational study for 12 mo (January to December of 2014), with daily visits to a municipal slaughterhouse. Inspections were carried out following the slaughtering procedures of the slaughterhouse, which adhere to the slaughter guidelines in NOM 033-ZOO-1995. After the slaughter and once the visceral package was removed from the carcass, organs were inspected following the zoosanitary standard NOM-194-SSA-2004, which specifies that the revision of the viscera intends to look for the presence of parasites, and these must be absent.

Livers with injuries suggestive of the presence of the parasite were separated for further inspection. Seized livers were inspected for injuries and for adult parasites or migratory forms, for which incisions were made in the parenchymal tissue and classified as positive and negative in the presence of *Fasciola*. Positive livers met any of the following criteria: macroscopic injuries like abscesses and thickening of the walls of bile ducts, living or dead adult or developing

parasites in hepatic ducts or the parenchyma. A liver was considered negative when no injuries or parasites were found.

From each animal, regardless if they tested positive or not, it was recorded the slaughter date (day and month), sex (male or female), locality of origin of the introducer (locality), and liver seizure (positive or negative). The number and frequency of seized livers during the study period were calculated by month and by the season of the year. The frequency of seized livers was calculated using the equation to determine prevalence described by Thrusfield[12] and was expressed as the percentage of the total number of cattle slaughtered compared to the number of animals that entered the slaughterhouse. Data were grouped by sex, month of seizure, season of the year (rainy, dry, and norther), and by population origin to determine its association with the seizure of livers positive to *Fasciola* sp. The month of seizure data were grouped into three seasons: dry (March-June), rainy (July-October) and norther (November-February).

The data grouped by sex were analyzed using a Chi-squared test in a 2 x 2 contingency table. It was used a Kruskal-Wallis test to analyze the data and detect differences between months. Association analyses were performed, through a correspondence test, for the season of the year and locality. 3 x 2 and 27 x 2 contingency tables were used, respectively. The analyses were performed using the SPSS statistical program, version 8.

The number of animals that entered the municipal slaughterhouse during the year varied every month, between 42 to 108, for January and May, respectively. Still, and within this context, the number of seized positive livers was 278, which represent 25.8 % of the total seized (Table 1), which is within the range of prevalence reported for the State of Tabasco[8,13,14], a percentage that remains without apparent change from the first studies carried out on Tabasco slaughterhouses[8].

Table 1: Number of slaughtered cattle and seized livers during a year in a municipal slaughterhouse of the southeastern area, Jalapa, State of Tabasco, Mexico

		Seized livers			
Cattle	**n**	**Positive**	**Negative**	**Positive %**	**P value**
Females	1025	264	761	25.7	
Males	53	14	39	26.4	0.005
Total	1078	278	800	25.8	

In cattle, the risk factors associated with disease prevalence are those related with the environment, like temperature and humidity, and water resources such as rivers; the presence of the intermediate host and the factors associated to the definitive host such as age, breed, animal species, and the exploitation system and feeding management. Likewise, the farm location and

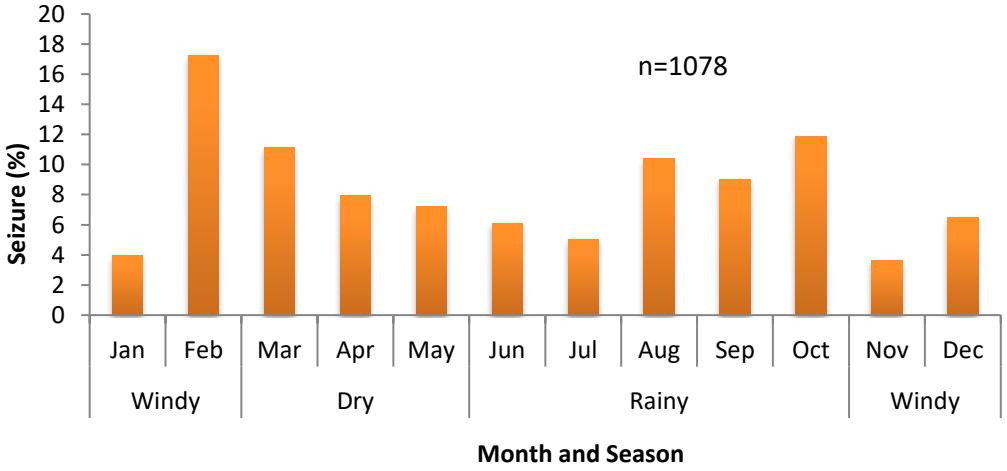
the prevailing microclimatic conditions in the grazing area are of great importance in the epidemiology of the disease[15].

In this study, regardless of the number of animals that entered the slaughterhouse, the probability of males being positive was the same for females, as there was no relation between the seizure of positive livers and sex (X^2=0.011, gl=1, P<0.05). These results are similar to those of Ticona[16], who mentions that sex is not a risk factor for the disease in Peru, age was the risk factor more associated with the presence of the disease[17,18].

A factor that can be confused with sex and age is the breeding production system. Usually, females are bred fundamentally under grazing conditions[19] and remain for a more extended period in the farms, while males are usually sold to be fattened under intensive systems. However, males are also susceptible to parasitism when managed under a grazing system.

In the present study, the monthly variation in the seizure of positives ranged between 3.6 and 12.3 %. We could detect *Fasciola* sp. throughout the year with monthly variations (X^2=51.918, gl=11, P=0.000). However, February and November were the months with the highest and lowest percentage of positive livers, 17.3 % and 3.6 %, respectively (Figure 1). The rainy season showed the highest percentage of positive livers with 9.36 % (June-October), followed by the dry season with 8.34 % (March-May), and the norther season with 8.07 % (November-February). These results show a relationship between the sampling period and seizure (X^2=6.511, gl 2, P=0.039). The latter is similar to the reported by Feunmayor and Ojeda-Robertos[14,19], who determined a relationship between the time of the year and the presence of animals positive to *Fasciola*, both authors used the sedimentation technique to determine the prevalence in animals under grazing production systems.

Figure 1: Percentage of livers positive to *Fasciola* sp. by month and time of the year



In this study, the detection of positive livers and postmortem hepatic damage show that the animals, at some point in their lives, were in contact with the infective stage of *Fasciola* sp. These animals acquired the disease when the external environmental conditions in the farm were optimal for the development of the exogenous infective stage of the parasite, which is confirmed by the presence of different developmental stages in the hepatic ducts. The number of parasitic developmental stages found in positive livers was not determined.

The season of the year is directly related to the development and survival of the intermediate host and the parasitic infective stages[20]. *Fasciola hepatica* possesses a complex life cycle that includes the presence of obligate hosts, the definitive (ruminants and humans), and the intermediate (*Fossaria* and *Pseudosuccinea* mollusks of the Lymnaeidae family).

In both hosts, the elimination of the parasitic developmental stages allows the parasite to fulfill its life cycle. Eggs enter the environment in the feces of the definitive host, while the snail releases the cercariae, which form cysts in the aquatic vegetation, grass, or at the side of water bodies, transforming into metacercariae (infective stage)[21,22].

The definitive host becomes infected by ingesting metacercariae-contaminated grass or water. The metacercariae mature until its juvenile stage, in which they can migrate through the intestinal wall, subsequently staying in the abdominal cavity, peritoneum, Glisson's capsule, and hepatic parenchyma; they later establish in the bile ducts, where metacercariae reach their adult stage and sexual maturity, which enables them to produce fertile eggs, which are eliminated in the feces to the environment[15].

During the postmortem examination, the liver shows hypertrophy, bleedings, with different degrees of fibrosis, calcification and hyperplasia of bile ducts, as well as the presence of parasitic forms in the hepatic tissue, which are reason for seizure, regardless of the degree of liver damage[23].

A study in northeastern Nigeria, in the African continent, determined the degree of damage of livers seized due to the presence of *Fasciola*, the results show that severe liver damage was the most frequent (55.3 %), followed by moderate damage, these damages include fibrosis to total liver damage and atrophy[24]. In this study, is describe the degree of damage in positive livers, which could be a future research subject.

Herein, the time of year with the largest number of seized livers was possibly related to the largest number of animals that came from a locality where the prevalence and probability of sending a positive animal to the slaughterhouse are higher since the local conditions favor the presence of infective stages (floodable soils, proximity to the river and water blankets).

The origin of the total seizures (n= 1,078), regardless of the cause, were distributed in 27 towns belonging to the municipality of Jalapa. Of the total number of registered localities, seven contributed more than half of the total number of positives (89.2 %, 248/278), ten localities contributed the remaining 10.8 % (30/278), and in the remaining ten, no seizures were detected. The contribution of the seven localities varied from 16 to 45 positive livers, the second group of localities contributed 1 to 7 positive livers, and the remaining ten, zero. From each town, the number of animals received for slaughter varied and depended on the needs of the producers to send animals for slaughter.

The population-adjusted prevalence was 16.19 % (Huapacal) for the highest and 5.40 % for the lowest (Figure 2); a relationship between the localities of origin and the presence of positive livers was determined (X^2=59.621, gl 26 p=0.000). The origin of the animals is an important factor related to seizure, since the regional environmental conditions of the locality, as well as the breeding production system, are reflected in the number of positive animals, and it is highly likely that the animals from these regions have had greater contact with the infective stages of the parasite due to the particular conditions of the breeding region[24].

Figure 2: Frequency of seized positive livers by locality in the Jalapa municipal slaughterhouse, Tabasco, Mexico

It is concluded that the prevalence of Fascioliasis in the Jalapa area, Tabasco area, has not decreased in recent years, despite traditional control measures, including the use of fasciolicides for the control of immature and adult stages, as well as the restriction to graze areas near rivers, to avoid ingestion of metacercariae. The prevalence of the disease is related to the location or origin of the animal. However, other factors influence the presence of the disease and must be studied, including the type and system of livestock production, the use of fasciolicides, and the microclimatic conditions of each breeding location.

Fascioliasis, a disease that affects animals and humans, should be monitored in order to increase knowledge about its epidemiology and propose control and preventive measures, depending on the microclimatic conditions. Amid climate change, state and nationwide monitoring are recommended; this could provide information to better understand the ecology of fascioliasis and its presence in ruminant animals.

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