

PHYSIOPATHOLOGY EFFECTS IN BOVINES (*Bos taurus* X *Bos indicus*) WITH FASCIOLIASIS IN MÉXICO

EFFECTOS FISIOPATOLOGICOS POR FASCIOLIASIS EN BOVINOS (*Bos taurus* X *Bos indicus*), EN MÉXICO

Fascioliasis in Mexico

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Abstract

Fascioliasis, zoonotic disease is considered endemic in many regions of Latin America (Peru, Bolivia) and the world, however, the development and transmission power is very complex, it produces great economic losses in animal health, the intermediate host varies its species or subspecies (Family Limnaeidae) according to the zoogeographical area in which it presents, as ecopathology can be considered a reemerging disease. Although there are great efforts by many international organizations in determining their distribution and actual prevalence in humans and animals are still unknown, its effect on the physiological and reproductive ruminants in developing countries where prevention is very limited and where degree of infestation depends on the number of flukes present in the liver parenchyma. The aim of this study was to determine the physiopathological changes (metabolic, enzymatic profile, count differential of blood cells) caused by *F. hepatica* in bovines. Blood samples were taken to count white blood cells, and for the measurement of biochemical parameters of metabolic problems (hepatic enzymes, others hepatic components and macro minerals) and hepatic tissue of bovine to know the infestation degree and for the accomplishment of histological cuts to know the cellular damage. It was observed obstruction and increase in the diameter and thickening of the bile ducts, diminutions of the thickness of the left and ventral lobe and the chronic cases displayed cirrhosis; at cellular levels, loss of the cellular morphology, fibrosis, cirrhosis and eggs of *F. hepatica* in the liver parenchyma were observed. The liver damage was directly proportional to the number of the parasites also causing alteration in the metabolic components; the most significant indicators of hepatic damage by *F. hepatica* were Urea/BUN, γ -GT, ALT/GPT, AST/GOT and alkaline phosphatase.

Keywords:

Fasciola hepatica
Liver damage
Metabolic profile
Parenchyma

Palabras clave:

Fasciola hepática
Daño hepático
Perfil metabólico
Parénquima

Resumen

Fascioliasis, enfermedad zoonótica que se considera endémica en muchas regiones de América Latina (Perú, Bolivia) y el mundo, sin embargo, su desarrollo y transmisión es muy complejo, produce grandes pérdidas económicas en la salud animal; el hospedador intermediario varía su especie o subespecie (Familia Limnaeidae) de acuerdo a la zona zoogeográfica en el que se presenta, como ecopatología se puede considerar una enfermedad re-emergente. Aunque hay un gran esfuerzo por muchas organizaciones internacionales en determinar su distribución, la prevalencia real en los seres humanos y los animales, como, su efecto a nivel fisiológico y reproductivo en rumiantes aún se desconocen en países sub-desarrollados, la prevención es muy limitada y donde el grado de infestación dependerá del número de tremátodos presentes en el parénquima hepático. El objetivo de este estudio fue determinar los cambios fisiopatológicos (morfología hepática, perfil metabólico, conteo diferencial de glóbulos blancos) causados por *F. hepatica* en bovinos. Se tomaron muestras de sangre con EDTA, para contar las células blancas de la sangre y para la medición de parámetros bioquímicos (Calcio, fosforo, urea/BUN, bilirrubina, colesterol, proteínas totales, gama glutamil transferasa- γ -GT, alanina

amino transferasa-ALT/GPT, aspartato amino transferasa-AST/GOT, deshidrogenasa láctica y fosfatasa alcalina) en animales con problemas metabólicos (en enzimas hepáticas), se realizó el diagnóstico en el tejido hepático de localizar los dístomas en parénquima y en el líquido biliar, para determinar el grado de infestación y realizar cortes histológicos, para determinar el daño tisular. Se observaron Obstrucción, engrosamiento de los conductos biliares, disminución del espesor del lóbulo izquierdo y ventral y los casos crónicos muestran fibrosis y cirrosis, a nivel celular hay pérdida de la morfología celular, por desarrollar fibrosis y cirrosis, y los huevos de *Fasciola hepatica* fueron localizados en el parénquima hepático, con base a los resultados obtenidos, podemos concluir que el daño hepático es directamente proporcional al número de dístomas presentes, y si comparamos esto con el perfil metabólico los mismos se alteran significativamente y estos fueron UREA/BUN, γ -GT, ALT / GPT, AST / GOT y fosfatasa alcalina.

Introduction

The Health status of animals depends on the ability to prevent and eliminate disease and restore health status, on the other hand, the capacity to prevent and avoid the onset of disease, when animal health system is functional, this latter seems to be very complex, but there are elements that are crucial to diagnose the health of animals and that they influence, these elements are: a) the environment, b) the way of life that animals develop, c) animal biology (animal breeding) and d) health system are implement, based on disease characteristic of each zoogeographic region (Ortega, 1994 and Caicedo *et al.*, 2010). Alterations in only one of these factors and their interactions are what we call ecopathology. One of the most common ecopathology well as brucellosis and tuberculosis in México is a fascioliasis, it occurs mainly in the rainy season (July to December). The prevalence of this disease is 23-29% in tropical and subtropical regions, even in areas adjacent to the Gulf of México; the prevalence is a 80-95 %, the migration of animals from one region to another make the disease is spreading throughout the country to reach areas where none existed. The fascioliasis is a parasitic disease that affects herbivores, preferably to cattle and sheep's, and less frequently the man, which is caused by the parasite *Fasciola hepatica* (Dalton, 1999). This disease is emerging or re-emerging in many countries, and its prevalence, intensity and geographic distribution are increasing (Mas-Coma, 2004). Nowadays, fascioliasis is the vector-borne disease presenting the widest latitudinal, longitudinal and altitudinal distribution known (Mas-Coma *et al.*, 2003). The purpose of this study was to evaluate the physiopathological effects caused by *F. hepatica* in cattle

Materials y methods

Blood samples and livers were collected of cattle clinically healthy and animals with fascioliasis slaughtered in the municipal slaughterhouse of Atlixco, Puebla, Mexico, during two years. The number of flukes in the hepatic parenchyma was determined by examination of the affected livers. To observe the parenchymal damage, samples of liver tissue were taken and were subjected to a histological process and stained with H & E.

Blood samples were taken to count white blood cells. The metabolic profile analysis was performed on blood serum samples, and various enzymes and metabolites were measured with different diagnostic kits (Bio-System/Randox). An analysis of variance (ANOVA) with the statistical program Stat2 was used (Olivares, 1994) and to determinate the significance between averages was used Duncan's new multiple range test.

Results and discussion

Fascioliasis is a zoonotic disease that causes economic losses in México. On the other hand, numerous studies on the incidence of this infection in several regions of southeastern and Central Mexican highlands. Parameters have been determined ranging from 27 to 75 % prevalence of fascioliasis diagnosis through infected livers, but these values depend on the location and time of year that sampling be carried. To some extent the evaluation of economic losses is difficult to determine because of the multiplicity of factors, not only analyzing the losses of livers and milk, but also mortality and morbidity resulting consequences of wasting the delay in growth and development of recurrent disease. The decrease in the growth and yield has been determined by several investigators (Dalton, 1999), found that the loss in milk production was ranging from 4.5 % to 34.3 % or weight loss ranging from 15 kg to 40 kg (per month) per head between infected animals and compared with healthy animals (Caicedo *et al.*, 2009). Economic losses can be estimated in this study based on traces livers seized in

the same city and are estimated from 1988-1989, in the State of Tabasco of USD \$ 281.8 million, in the State of Sinaloa losses ranged in USD \$ 124.3 to 138.0 million in the year 1997, in the town of Tampico (State of Tamaulipas), losses were by more than USD \$ 13.7 million, in the State of Mexico (1996), were detected losses at USD \$ 14.5 million and recent studies in the State of Puebla were lost from 2006 to 2010 by more than USD \$ 53.7 million (Rangel y Martinez, 1994 y Caicedo *et al.*, 2010), however, these losses only in bovine, ovine are not contemplated or goats. In Mexico, only two zoogeographic areas there are no reported cases of fascioliasis these are the Peninsula of Baja California and Yucatán, this is due in part to the Peninsula of Baja California is geographically located in a semidesert-desert areas and Yucatan Peninsula has a typical relief above the sea (from coral origin) and soil salinity contributes to disease is not present throughout the intermediate host (mollusks of the genus *Lymnaea*), nevertheless, the most prevalent regions are areas that are irrigated by rivers coming from mountainous regions and that these rivers bathe the lower floors and the same are for grazing or pasture production for livestock, also anthropomorphic factors contribute to the spread, transhumance and migration of animals from endemic regions to areas where no disease exists, but if the intermediate host, on the other hand, remains a high prevalence because there is no control programs, prevention and diagnosis of the disease nationwide. This study considers the fascioliasis due to *Fasciola hepatica*, a zoonotic disease that causes more damage to productive and reproductive level in bovine (*Bos taurus* X *Bos indicus*) in Mexico. This study shows the degree of infestation through municipal slaughterhouses in the liver ducts of the confiscated livers, flukes were found in a variable number, with a minimum of 1 individual and a maximum of 353 individuals for liver, the largest number of flukes found in the liver of females was of 157, whereas for males was 273. This variation is due to the number of metacercariae ingested by each animal, which was impossible to detect in this study because the analyzed animals were naturally infected by *Fasciola hepatica*. According to Dalton (1999), the majority of the confiscated livers showed an increase of about 3 cm in diameter of the liver ducts; besides their color was whitish-yellowish, due to the obstruction of these ducts by the parasite, as well decrease in the thickness of the affected lobes (left and ventral) as the disease progresses and loss of liver consistency, caused by fibrosis and cirrhosis in chronic cases. As a result, the liver loses its metabolic faculties and allows the accumulation of substances rich in salt, which crystallizes causing obstruction of ducts bringing favorable conditions for the reproduction of bacteria as *Clostridium* spp (Robles, 1998), *Escherichia coli*, *Enterococcus faecalis*, y *Klebsiella pneumoniae* (Valero *et al.*, 2006). All the above characteristics and based on literature (Mas-Coma, 2005; Guy *et al.*, 2001) indicates that most animals with *F. hepatica* that were sampled where in the chronic phase of the disease. At cellular level, comparing with clinically healthy liver tissue, we could observe loss of the morphology, chronic hepatitis, inflammation forming micro-abscesses, proliferation of bile ducts, intracytoplasmic cholestasis, chronic inflammatory infiltrate based of lymphocytes, dilation of the sinusoids, thickening of the capsule of Glisson by a chronic process, presence of fibroblasts, fibrosis, cirrhosis, and eggs of *F. hepatica* in the ducts and in the hepatic parenchyma. About the counting of white blood cells, we found significant differences ($P < 0.01$) in eosinophils values in the analyzed groups, also in monocytes in males. According to the number of flukes, there were significant differences in males who presented a decrease of neutrophils, an increase of lymphocytes; and in both groups (males and females) there was a decrease of monocytes and an increase of eosinophils, all of this according to the number of flukes increase. The increase in the number of eosinophils in animals with *F. hepatica* agree with the diagnostic characteristics of the disease (Mas-Coma, 2005; Guy *et al.*, 2001); however, this increase is more evident during parenchymal fase and this cells increase when the parasite enter into the bile ducts (Poitou *et al.*, 1992, 1993; Jemli *et al.*, 1993). In relation to metabolic profile, there were significant differences ($P < 0.05$ y 0.01) in the values of Urea/BUN and phosphorus between the two groups of males, whereas females only in Urea/BUN. Based on metabolic profile of animals with fasciolosis grouped according to the number of flukes, we observed in males that the values of urea/BUN decrease when the number of flukes in the liver parenchyma increases. The values of alkaline phosphatase (AP), alanine aminotransferase (GPT), aspartate aminotransferase (GOT) and Cholesterol in males and GOT in females showed a declining trend line when the number of flukes in the liver increases. As a result of damage, this parasitic disease, caused heavy economic losses in livestock, by causing a negative effect on production, causing a decrease of weight gain (Loyacano *et al.*, 2002), reproductive efficiency and milk production (Chirinos y De Chirinos, 1993) and the confiscation of liver (Ortega *et al.*, 2007, Torres, 2010, Caicedo *et al.*, 2010).

Conclusions

We conclude that fascioliasis in Mexico is a zoonotic-ecopathology is broken up throughout country (although in tropical and subtropical endemic, there is the intermediate host), global environmental changes are contributing to the spreading of this infestation. On the other hand, the physiopathological damage depends on the number of metacercariae ingested the animal and then re-infested. A federal health standards fascioliasis animal disease in not priority, however, produces large physiological changes in the animal, decreasing productivity and reproductivity of animals that have it; many areas with high animal density are located in areas endemic for fascioliasis and greater dedication is the mobilization of animals to areas where the disease didn't exists and finally there is the high prevalence should be considered an important zoonotic disease ecopathological in animal health.

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