Biliary hyperplasia in alpacas (*Lama pacos*) experimentally infected with *Fasciola hepatica* metacercariae

Hiperplasia biliar en alpacas (*Lama pacos*) infectadas experimentalmente con metacercarias de *Fasciola hepatica*

Vicente Maco, Vicente P. Maco, Olga Timoteo, Jose R. Espinoza

Comunicación Corta / Short Communication

Abstract

*Fasciola hepatica* infection is one of the major causes of economic loss in livestock, causing a significant morbidity among South American camelids such as alpacas (*Lama pacos*). In order to better understand the host-parasite interaction and histopathological insult in the liver, 6 *Huacaya* alpacas were infected with a single dose of 200 viable *F. hepatica* metacercariae. Adult worms and eggs were found in tissue preparations of livers of infected animals after 6 month of experimental infection. Hepatic lesions as wall thickening of bile ducts, disorganization of the liver parenchyma, stellate scar with fibrosis and destruction of bile ducts and liver parenchyma were observed in all infected animals. The livers of infected animals showed regions with chronic inflammation, granuloma containing parasite eggs, necrosis and cirrhosis. Biliary hyperplasia with multiple foci of proliferative bile ducts formed by several layers of transformed epithelial cells was observed adjacent to a region with extensive liver damage. Chronic *F. hepatica* infection in alpacas causes an extensive liver damage and leads to bridging fibrosis and, characteristically, adenomatous biliary hyperplasia, suggesting that these camelids are highly susceptible to this trematode.

Key words: *Fasciola hepatica* / anatomy & histology | *Fasciola hepatica* / metabolism | Camelids, New World | Immunohistochemistry | Models, Biological | (Source: DECS BIREME)

Resumen

La infección por *Fasciola hepatica* representa una de las mayores causas de pérdidas económicas en el ganado a nivel mundial, causando morbilidad importante entre algunos camélidos sudamericanos como las alpacas (*Lama pacos*). Con el objetivo de entender mejor la interacción hospedero-parásito y el daño histopatológico que causa el parásito en el hígado, 6 alpacas tipo Huacaya fueron infectadas con una dosis única de 200 metacercarias de *F. hepatica*. Se visualizó parásitos adultos y huevos en los tejidos del hígado de los animales infectados luego de 6 meses de la infección experimental. En todos los animales, se observó engrosamiento de las paredes de los conductos biliares, parénquima hepático desorganizado, cicatrizaciones estelares con fibrosis y destrucción de los conductos biliares y del parénquima hepático. Los hígados de los animales infectados también mostraron inflamación crónica, granulomas conteniendo huevos parasitarios, necrosis y cirrosis. Se observó hiperplasia biliar con múltiples focos de conductos biliares proliferativos formados por múltiples capas de células epiteliales transformadas, adyacentes a la región con daño hepático extenso. La infección crónica por *F. hepatica* en alpacas causa un daño hepático extensor y lleva a fibrosis portal extensa y, característicamente, a la formación de adenomas de hiperplasia biliar, lo cual sugiere que estos camélidos son altamente susceptibles a la infección por este distoma.

Palabras clave: *Fasciola hepatica* / anatomía & histopatología | *Fasciola hepatica* / metabolismo | Camélidos Americanos | Inmunohistoquímica | Modelos Biológicos | (Fuente: DECS BIREME)

Citation: Maco V, Maco VP, Timoteo O, Espinoza JR Biliary hyperplasia in alpacas (*Lama pacos*) experimentally infected with *Fasciola hepatica* metacercariae. Peru j parasitol. 2012;20(1): e25-e29
Asciola hepatica (F. hepatica) infection is one of the major causes of economic loss in livestock worldwide, with a great impact in the economy of endemic areas in Peru. A wide range of domestic species is susceptible to the liver fluke infection, as alpacas (Lama pacos), in which the infection causes anemia, eosinophilia, weight loss, emaciation and mortality. Despite the importance of this infection by its negative impact in fiber productivity and quality, little is known of the liver pathology caused by F. hepatica in South American cameldids, and of the parasite factors that produce the pathogenesis of the disease.

A differential susceptibility of the host to the fluke is a feature of the disease, being cattle able to mount a strong immunological response that immunoprotects to subsequent infections, and sheep develop little or absent immunoprotective response to F. hepatica infection. It is not known whether alpacas become resistant to F. hepatica after a primary infection. However, field studies suggest that animals acquire multiple infections in endemic areas and they are re-infected after treatment with flukicide. Alpacas produce antibody response to liver fluke infection, which peaks 8 weeks after primary infection, but this response seems unable to confer immunoprotection to re-infection and does not prevent the liver damage observed in infected animals.

Until now, no description of the histopathological insults caused by the fluke infection was reported in alpacas, as it is well described in sheep, cows and goats. In alpacas, the liver penetration appears to start 4 weeks after infection suggested by the elevation of hepatic enzymes observed in infected animals and bile ducts resident mature flukes shed eggs 8 weeks after the infection.

In order to gain insight into the histopathological changes of the fluke in the liver and biliary track of this South American cameldid, 6 Huacaya alpacas (4 males, 2 females, aged 18 to 24 months) from the department of Junin (Central Highlands of Peru) were infected with a single dose of 200 metacercariae of F. hepatica administered orally, after a 30-days adaption period. The metacercariae were obtained from lymnaeid snails infected with laboratory-raised F. hepatica miracidia. Prior to inoculation, fluke eggs were not detected in stool samples by the Rapid Sedimentation Technique (RST) by Lumbreras, and enzyme-linked immunosorbent assays (ELISA) using Fas1, Fas2 and excretory/secretory products were negative. All of the infected alpacas and 1 uninfected control underwent necropsy 6 months after the first single dose. The study protocol was approved by the Institutional Ethics Committee for Animals. Tissue samples from livers were fixed in formaldehyde, included in paraffin and processed by haematoxylin-eosin (H&E) and Masson's trichrome (MT) stains, the latter used to differentiate between collagen fibers and smooth muscle from tumors. Von Kossa, a stain intended for use in the evaluation of calcium deposits, was not performed since standard field studies suggest that animals are re-infected after treatment with flukicide. Alpacas produce antibody response to liver fluke infection, which peaks 8 weeks after primary infection, but this response seems unable to confer immunoprotection to re-infection and does not prevent the liver damage observed in infected animals. Alpacas produce antibody response to liver fluke infection, which peaks 8 weeks after primary infection, but this response seems unable to confer immunoprotection to re-infection and does not prevent the liver damage observed in infected animals. Until now, no description of the histopathological insults caused by the fluke infection was reported in alpacas, as it is well described in sheep, cows and goats. In alpacas, the liver penetration appears to start 4 weeks after infection suggested by the elevation of hepatic enzymes observed in infected animals and bile ducts resident mature flukes shed eggs 8 weeks after the infection.

In order to gain insight into the histopathological changes of the fluke in the liver and biliary track of this South American cameldid, 6 Huacaya alpacas (4 males, 2 females, aged 18 to 24 months) from the department of Junin (Central Highlands of Peru) were infected with a single dose of 200 metacercariae of F. hepatica administered orally, after a 30-days adaption period. The metacercariae were obtained from lymnaeid snails infected with laboratory-raised F. hepatica miracidia. Prior to inoculation, fluke eggs were not detected in stool samples by the Rapid Sedimentation Technique (RST) by Lumbreras, and enzyme-linked immunosorbent assays (ELISA) using Fas1, Fas2 and excretory/secretory products were negative. All of the infected alpacas and 1 uninfected control underwent necropsy 6 months after the first single dose. The study protocol was approved by the Institutional Ethics Committee for Animals. Tissue samples from livers were fixed in formaldehyde, included in paraffin and processed by haematoxylin-eosin (H&E) and Masson’s trichrome (MT) stains, the latter used to differentiate between collagen fibers and smooth muscle from tumors. Von Kossa, a stain intended for use in the evaluation of calcium deposits, was not performed since standard field studies suggest that animals are re-infected after treatment with flukicide. Alpacas produce antibody response to liver fluke infection, which peaks 8 weeks after primary infection, but this response seems unable to confer immunoprotection to re-infection and does not prevent the liver damage observed in infected animals. Until now, no description of the histopathological insults caused by the fluke infection was reported in alpacas, as it is well described in sheep, cows and goats. In alpacas, the liver penetration appears to start 4 weeks after infection suggested by the elevation of hepatic enzymes observed in infected animals and bile ducts resident mature flukes shed eggs 8 weeks after the infection.

In order to gain insight into the histopathological changes of the fluke in the liver and biliary track of this South American cameldid, 6 Huacaya alpacas (4 males, 2 females,
H & E sections of infected liver tissue showed extensive tracks of fibrosis (Fig. 2b) as a response to the liver damage caused by the fluke. The infection produced an extensive liver damage that ranges from inflammatory response to cirrhosis. The liver of infected animals showed patches of acute and chronic inflammation to the liver fluke and to the eggs. Granuloma containing parasite ova was a common finding in H&E sections of the liver with chronic infection (Fig. 3a). Inflammatory response recruited lymphocytes, macrophages and plasma cells. We did not detect eosinophils in the inflammatory foci, in spite that peripheral blood eosinophilia in blood was observed in infected alpacas. A peripheral ring of fibroblasts with multinucleated giant cells, lymphocytes and plasma cells surrounded granuloma containing *F. hepatica* ova (Fig. 3b). Multinucleated cell and lymphocytes were abundant in mineralized granuloma and in areas with cirrhosis. Mild to severe grades of bile duct hyperplasia with biliary adenoma formation (Fig. 4).
The presence of multiple non-encapsulated biliary adenomas in infected animals is a feature of this infection not described in other species. The neoplastic tissue consists of biliary epithelial proliferation with no evidence of nuclear or cellular atypia or the presence of mitotic figures. Multiple foci of proliferative bile ducts formed by several layers of transformed epithelial cells were observed adjacent to a region with extensive liver damage (Figs. 4a and 4b). A severe form of neoplasia that produced the atrophy of the liver right lobe was caused by the hyperplasia of biliary epithelia was previously described in *F. hepatica* infected alpaca. A similar finding, hyperplasia of the gall bladder was described in a man with a chronic infection. The development of biliary hyperplasia associated to *F. hepatica* infection is probably not an uncommon event and it is suggestive of the high susceptibility of the alpacas and other hosts to the liver fluke infection.

In conclusion, alpacas are a highly susceptible species to *F. hepatica* as suggested by the extensive liver damage with a distortion of the liver architecture that even leads to tissue transformation with characteristics of biliary adenomas in experimentally infected animals.
Acknowledgments. This work was partially funded by a grant B/2856-1 from the International Foundation for Science an INCAGRO CONTRACT 007-2003 to JRE. OT received support from the Consortium of Francophones Universities of Belgium (CIUF) and RED SAREC. E. Chavarry for providing the Fas2 antiserum.

Author’s contribution: VM, OT, and JRE designed the study. VM and VPM performed all histopathological stains and analyzed the paraffin-embedded liver tissues.

Conflict of Interest: The authors declare none.

References


