



## Case Report

# Congenital hepatic fibrosis in Holando Argentino calf: first report in Argentina

Mirentxu Indart<sup>1\*</sup>, María G. de Yaniz<sup>1</sup>, Jorge P. García<sup>2</sup>, Ángel R. Bence<sup>1</sup>, Marcial S. Negrette<sup>3</sup>

<sup>1</sup>Departamento de Fisiopatología. Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Centro de Investigación Veterinaria Tandil (CIVETAN) CONICET, Tandil, Argentina.

<sup>2</sup> Departamento de Clínica, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA).

<sup>3</sup>Patología general y sistemática. Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste (UNNE), Corrientes, Argentina.

\*Corresponding author: E-mail: mindart@vet.unicen.edu.ar

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## Abstract

A case of congenital hepatic fibrosis in a 10-month-old calf is described. The calf had been raised in a feed lot for the past 173 days and exhibited loss of body condition and depression, followed by death. The most remarkable macroscopic lesions observed during necropsy were: jaundice, hepatomegaly with a multilobulated liver surface and diffuse pale brown colour of the parenchyma. Microscopically, the portal tracts were thickened and connected to each other by abundant fibrous tissue, delimiting irregular lobules of hepatocytes and occasional fibrosis of the central veins (perisinusoidal scars) and hyperplasia of embryonal bile ducts. Immunohistochemistry staining of the cytoplasm of the bile ducts was positive to AE1/AE3 and cell proliferation nuclear antigen (PCNA). The findings at necropsy, together with the results of the histopathological and immunohistochemistry studies, confirmed the diagnosis of congenital hepatic fibrosis.

**Key words:** calf, liver, fibrosis, congenital.

## Introduction

Congenital hepatic fibrosis (CHF) is a rare and sporadic cause of mortality in cattle, with few cases described in the literature (3, 9, 16, 24). CHF is characterized by defective remodelling of the ductal plate at the level of interlobular ducts, with an excess of abnormal shaped embryonic bile ducts retained in the primitive ductal plate configuration, abnormal portal veins, and progressive fibrosis of the portal tracts (5).

Ductal plate is defined as a double-layered cylindrical structure of the bile duct epithelium that surrounds the portal ramifications founded during the 8th gestational week. After approximately the 12th gestational week, remodelling of ductal plate begins, and the end of gestation or early postnatal period attains maturity. Biliary ducts are normally formed from remodelling and partial involution of these cylindrical ductal plates. Insufficient remodelling and resorption leads to ductal plate malformation. The timing of defective development

determines the resulting pathologic disorder (7, 17). This condition has also been reported in other veterinary species such as horses (8), cats (13), monkeys (23), dogs (4, 12, 19) and in rats, in this specie, associated with polycystic kidney disease (PKD) (2). Congenital hepatic fibrosis has also been reported in aborted and neonatal calves (3,16). In humans, CHF is a hereditary, autosomal recessive disease, usually associated with autosomal recessive polycystic kidney disease the childhood (14, 21).

The main clinical signs observed in animals are ascites, poor general condition, weakness, decrease in weight gain, diarrhea, dehydration, icterus of the sclera or mucous membranes and neurological clinical signs characterized by delayed reflexes, impaired coordination, convulsions, and opisthotonus associated with secondary hepatic encephalopathy (3, 5, 9).

The macroscopic lesions in the bovine liver, described in the literature are a pale and hard liver (fibrous) with numerous, round, small, approximately 5 mm in diameter or of different size, slightly raised, yellow

foci and extreme irregularity of the hepatic surface. On the cut surface, the fibrosis has a clearly reticulated, hexagonal pattern that delimits the hepatic lobules. The microscopic lesions are characterized by severe portal fibrosis that connected the portal triads to each other, delimiting lobules of different shape and size, and the branches of the portal vein seemed hypoplastic or missing in the portal triads. The bile ducts are small, irregular in outline, some tortuous or branched, mostly solid, but some with a small light, and are formed by a single layer of cuboidal cells. The majority tend to be arranged along the limiting plate in direct contact with the periportal hepatocytes. The centrilobular vein has a fibrous and thick wall in some liver lobules (3, 9, 16, 22, 24).

In animals, including bovine, equine, canine and feline, the definitive diagnosis is made through ultrasonographical, histopathological and immunohistochemical studies (IHC) for cytokeratin 7 and cell proliferation nuclear antigen (PCNA) (4). In Feline IHC is performed for cytokeratin 19 and typing polymorphism of the length of the PCR fragment for the mutation of feline PKD to confirm the diagnosis (13). In humans, the definitive diagnosis of CHF is established mainly through the histopathological study of samples obtained by liver biopsy and genetic markers. The presence of cytokeratin 7 is important for the diagnosis, indicating persistence of embryonic bile ducts (10, 21).

The objective of this study is to report the first case in Argentina of CHF in a 10-month old Holando Argentino calf.

### Case report

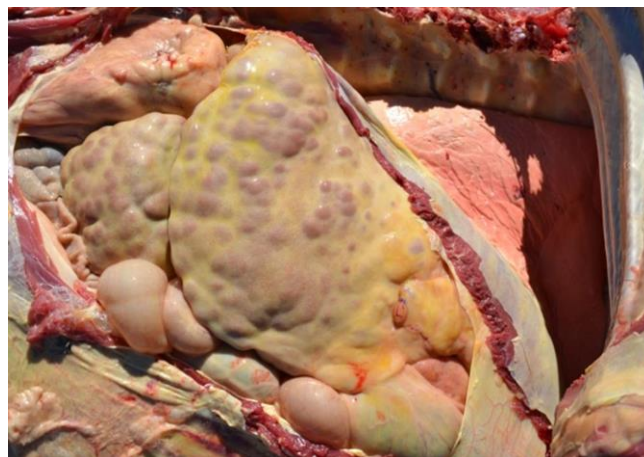
The case occurred in April 2017, when a 10-month-old male Holando Argentino calf died. The calf was for 173 days in a feedlot located at Tandil city, in Buenos Aires province. At the time of the animal's death, the facility was visited, collecting epidemiological information; record of the clinical history and the necropsy of the dead calf was performed.

The clinical signs reported by the personnel from the feedlot, prior to the death of the animal were: loss of body condition, with incoordination and depression, followed by death. Before death, the calf had received treatment with antihistamine, dexamethasone and oxytetracycline with no response.

At necropsy, the subcutaneous tissue, abdominal serous, tracheal and esophageal mucosa, liver and heart presented a yellow colouring, interpreted as jaundice. The liver shows hepatomegaly with firm consistency of the parenchyma and round edges with an irregular surface due to multiple nodular formations of variable size (Fig. 1), at cut surface the nodules were surrounded by fibrous tissue of yellowish colour (Fig. 2).

Tissue samples of liver, lung, brain, spleen, kidney, heart, hepatic and mesenteric lymph nodes and small intestine were fixed in 10% neutral buffered formalin

(pH 7,2) for 48 hr. All tissues were processed by standard histological techniques. 4- $\mu$ m-thick paraffin sections were cut and stained with hematoxylin and eosin (HE). Selected sections of the liver were stained with Masson's trichromic stain.



**Figure 1.** Liver, irregular surface with multiple nodular formations of variable size.



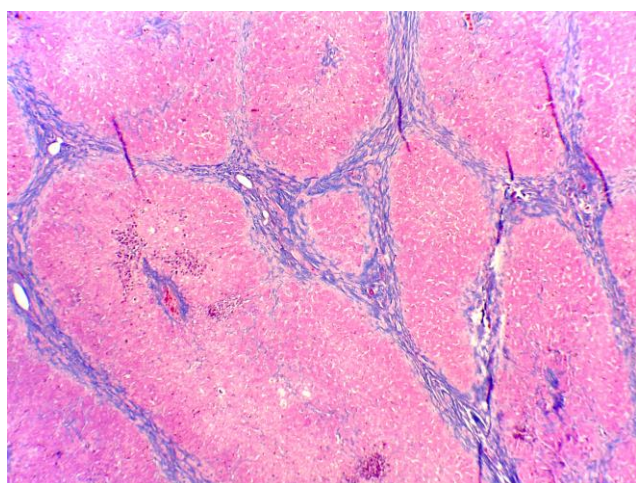
**Figure 2.** Liver, at cut surface, the nodules were surrounded by fibrous tissue of yellowish colour.

Immunohistochemistry of liver sections were carried out using the streptavidin-biotin immunoperoxidase system. Mouse monoclonal anti-PNA antibody and mouse monoclonal anti-cytokeratins antibody (AE1/AE3) were used as primary antibodies.

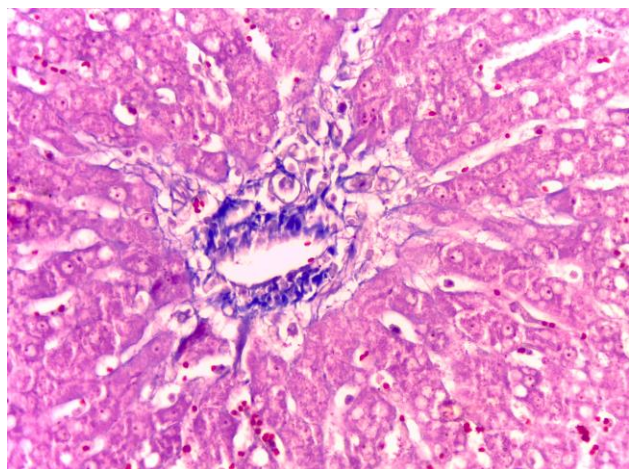
Microscopically, the portal tracts were thickened and connected to each other by abundant fibrous tissue, delimiting irregular lobules of hepatocytes (Fig. 3). In the portal spaces no branches of the portal vein were observed. Occasional hepatic lobules showed fibrosis of the central vein with extension to the Disse spaces in the centrilobular regions, forming perisinusoidal scars between the hepatocyte cords, that showed strong basophilic stain when was dyed with the Masson's trichrome stain (Fig. 4). This type of fibrosis is known in human medicine as "chicken wire" characteristic of alcoholic and non-alcoholic steatofibrosis. Hyperplastic bile ducts were observed at the



periphery of the ductal plate and in contact with the hepatocytes (Fig. 5). The bile ducts were numerous, small, irregularly contoured and mostly solid, without lumen. They were formed by a simple cubic epithelium with basal flat nuclei with stipple fine chromatin without obvious nucleoli. In the peripheral areas to the fibrotic parenchyma, the centrilobular lobules showed moderate necrosis with a severe inflammatory infiltration mainly formed by viable and apoptotic neutrophils. Most of the lobules presented hepatocytes with macrovacuolar fatty degeneration with midzonal distribution. Numerous nuclei of hepatocytes and fibroblasts were positive for PCNA immunohistochemistry (Fig. 6). The cytoplasm in most of the epithelial cells of the bile ducts was positive for AE1/AE3 (Fig. 7) and negative for PCNA.



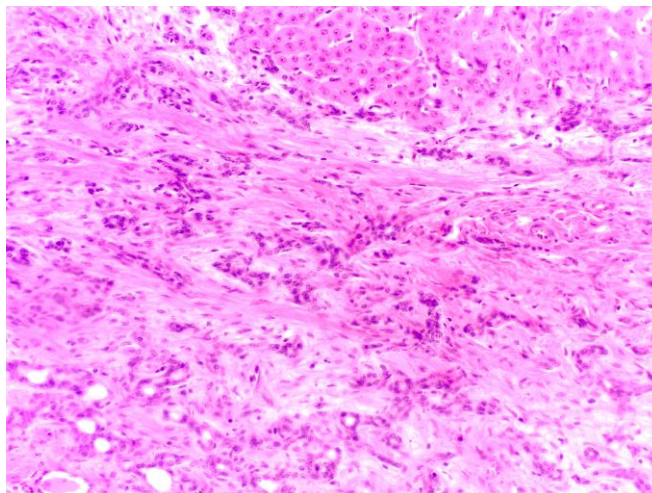
**Figure 3.** Liver, portal tracts were connected to each other by abundant fibrous tissue, delimiting irregular lobules of hepatocytes. (40X, Masson's trichrome).



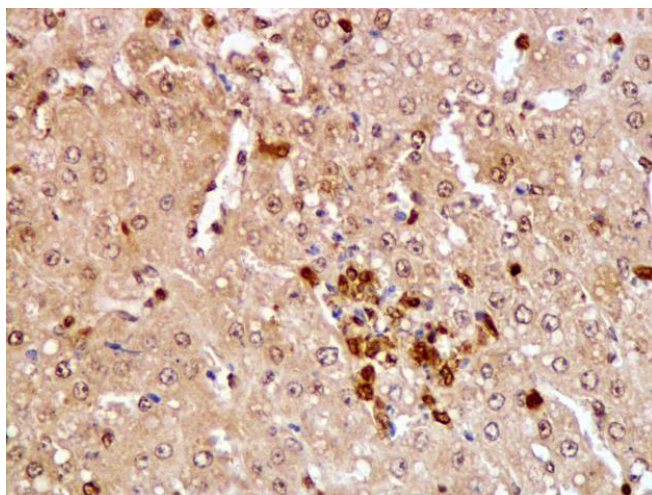
**Figure 4.** Liver, fibrosis of the central vein with extension to the spaces of Disse in the centrilobular regions. (400X, Masson's trichrome).

Both kidneys showed diffuse membranous proliferative glomerulonephritis, characterized by an increase in the number of mesangial

cells and endothelial cells admixed with neutrophils that fill the glomerular space. Also thickening of the basement membrane and accumulation of hyaline material in the glomerular space were observed. The tubules in the cortical zone showed vacuolar degeneration and intratubular hyaline cylinders and were dilated in the subcapsular area (Fig. 8).



**Figure 5.** Liver, hyperplastic bile ducts were observed at the periphery of the ductal plate. (200X, H&E).



**Figure 6.** Liver, numerous nuclei of hepatocytes and fibroblasts were positive for PCNA (400X).

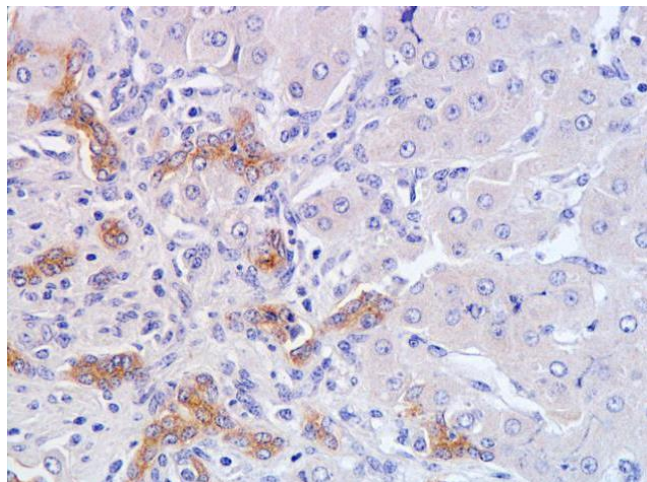
## Discussion

CHF has been described in different species, horse, cat, dog and rat, generally associated with malformations in other organs, mainly with polycystic kidneys (2, 8, 12, 13). In bovines, the few cases studied of CHF were not associated with malformations in other organs (3, 9, 16, 24), a feature shared by the present case.

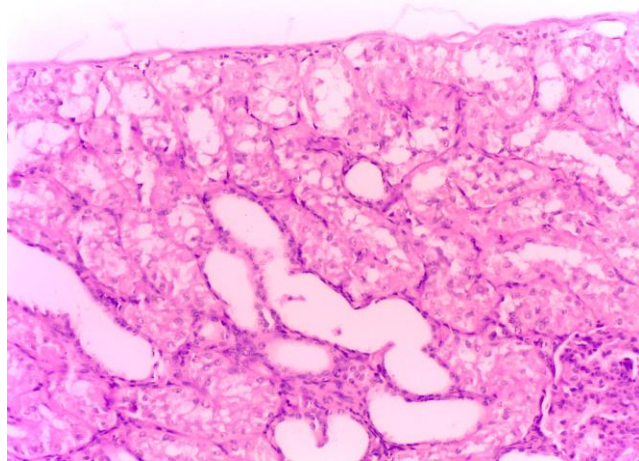
In cattle, the CHF was diagnosed in fetuses and newborns (3, 16, 24), as well as in a five-month-old calf (9). However, the present case shows CHF in an older



animal. Regarding the breed, most of the cases were reported in dairy breed cattle (3, 9, 16, 24). This case have similar clinical presentation with the case reported by Dutra Quintela (9) in a Red Angus calf that from birth showed poor body condition, weakness, low or no weight gain, anaemia, dehydration and intermittent diarrhea.



**Figure 7.** Liver, the bile ducts were positive to cytokeratin (AE1-AE3) (400X).



**Figure 8.** Kidney, tubules with dilated in the subcapsular area. (200X).

The distinctive histological characteristic in CHF is the presence of abundant fibrous tissue in the portal spaces in the form of portal-portal bands, in response to the persistence and hyperplasia of embryonic bile ducts and hypoplasia or absence of portal veins. Coinciding these anatomohistopathological aspects with our description.

In bovines, the anatomo-physiopathological aspects of CHF are characterized by affecting only the liver, without lesions in other organs, coinciding with the present case. The characteristic lesions of the CHF correspond to malformation of the ductal plate with permanence and hyperplasia of bile ducts and fibrosis of portal tracts reported by Desmet (6), was also observed in

our case. The lack of remodelling of the ductal plate results in persistence of embryonic bile ducts, which has been called "ductal plate malformation". This failure in the remodelling is associated with abnormalities of the portal veins with obliteration and hypoplasia and the absence of portal veins (10). Portal vein hypoplasia is a common concurrent histologic finding in humans with congenital hepatic fibrosis. During organogenesis, the development of the biliary system mirrors that of the portal venous system, and ductal plate malformations are often accompanied by portal vein malformations (15, 18) that have a characteristic pattern of growth, in which large venous branches abruptly give rise to numerous small branches, without intervening intermediate-sized portal veins (6).

In the present case, necrosis and apoptosis of hepatocytes in centrolobular location and polymorphonuclear infiltrate were observed. This microscopic lesion was not described in other presentations of FHC in cattle, in which they indicate morphological preservation without lesions of the hepatocytes. Probably, in our case, changes in blood circulation caused hepatocyte necrosis, as well as fatty degeneration.

The membranous proliferative glomerulopathy and dilation of tubules in the subcapsular regions observed in the kidney, was not previously described in other species, although other renal abnormalities were also associated with this disease. However, we could not associate the renal lesions observed in this case with FHC.

The immunohistochemical study allowed the detection of hepatocyte immunostaining positive to the PCNA and cytokeratin (AE1/AE3) in the cytoplasm of bile ducts as was observed in other reports. However, in those report the immunohistochemical studies were carried out in other species, such as canines (4) and felines (13) in which the CHF was also associated with injuries in other organs.

Studies of ductal plate malformations in humans have documented expression of cytokeratin 7, 8, 18, 19 and laminin in the cells that form the primitive biliary structures from week eight of gestation and persistence of its positivity in residual cells of the ductal plate in congenital fibrosis (1, 10, 11, 20).

AE1/AE3 immunohistochemistry detects certain high and low molecular weight keratins. AE1 detects the high molecular weight cytokeratin 10, 14, 15, and 16, and also the low molecular weight cytokeratin 19. Clone AE3 detects the high molecular weight cytokeratin 1, 2, 3, 4, 5, and 6, and the low molecular weight cytokeratin 7 and 8. In this way, the immunohistochemistry for AE1/AE3 involves the cytokeratin 7 and 19, that are reference to demonstrate the presence of embryonic ducts, as was observed in the present case. In the calf of this report, immunohistochemistry positive cytokeratin expression was observed in the cytoplasm of bile ducts, identifying them as immature cells of bile ducts. These results are consistent with a congenital ductal plate anomaly. PCNA-

positive hepatocytes are probably related to regeneration of hepatocytes in areas of necrosis.

The necropsy, histopathological and imunohistochemistry findings allowed to confirm the diagnosis of the disease, constituting the first case described in a bovine in Argentina.

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