sciendo

Acta Veterinaria-Beograd 2024, 74 (2), 313-321
UDK: 636.2.09:616.36-003.6
DOI:10.2478/acve-2024-0022

Case report

CONGENITAL HEPATIC FIBROSIS IN AN ABORTED HOLSTEIN-FRIESIAN FETUS

Božidar SAVIĆ[1,2*], Bojan MILOVANOVIĆ[1], Slobodan STANOJEVIĆ[1], Branislav KURELJUŠIĆ[1], Nemanja JEZDIMIROVIĆ[1], Aleksandra Fejsa LEVAKOV[3]

[1]Scientific Institute of Veterinary Medicine of Serbia, J. Janulisa 14, 11000, Belgrade, Serbia; [2]University of Novi Sad, Agriculture Faculty, Department of Veterinary Medicine, D. Obradovic Square 8, 21000, Novi Sad, Serbia; [3]University of Novi Sad, Clinical Centre of Vojvodina, Faculty of Medicine, Centre for Pathology and Histology, Department of Histology and Embryology, Hajduk Veljkova 3, 21000 Novi Sad, Serbia.

(Received 24 October 2023, Accepted 05 April 2024)

Congenital hepatic fibrosis (CHF) is a rare condition characterized by abnormal accumulation of fibrous tissue in the liver, leading to liver dysfunction. While CHF has been documented in various animal species, it has rarely been reported in cattle. This report presents a case of CHF in an aborted Holstein-Friesian fetus. During the necropsy, the most notable macroscopic findings included an enlarged liver with an irregular "cobblestone appearance" on the surface and firm parenchyma, as well as moderate ascites. Histopathological examination revealed extensive hepatic fibrosis in the portal areas, accompanied by bridging fibrosis extending between portal tracts and bile duct proliferation within the fibrous tissue. Screening for infectious agents as the cause of abortion or liver lesions was unremarkable. The necropsy and histopathological findings confirmed CHF, constituting the first case described in Holstein-Friesian calves in Serbia. Further research is required to determine whether this condition has a genetic basis or is influenced by yet to be identified factors in Holstein-Friesian cattle.

Keywords: congenital hepatic fibrosis, Holstein-Friesian calf.

INTRODUCTION

Congenital hepatic fibrosis (CHF), first described by Kerr et al. [1] is a condition characterized by the aberrant accumulation of fibrous tissue within the liver, leading to hepatocellular dysfunction, portal hypertension, and compromised liver function. This disease is relatively rare in humans, and has been reported in some veterinary species such as horses [2], rat [3], cat [4], an equine fetus [5] and dogs [4]. CHF is a rare and sporadic cause of perinatal mortality in cattle, however in literature there are few reports of occurrence of congenital hepatic fibrosis in calves [6-10].

*Corresponding author: e-mail: bozidar.savic@nivs.rs

In this report, we describe the macroscopic and histopathological aspects of hepatic fibrosis in an aborted Holstein-Friesian fetus. Additionally, we tested the serum from the aborting dam, parts of the placenta, and organs of the aborted fetus to identify infectious agents commonly associated with cattle abortions. We also examined the fetal liver tissue for the potential presence of hepatitis E virus (HEV). Our goal was to identify infectious agents that could have been the cause of the cow's abortion and liver lesions in the aborted fetus.

Case presentation

An abortion in a third parity Holstein-Friesian cow, in a village in Mladenovac municipality, Belgrade, Serbia, was reported to the local veterinarian. An aborted female fetus (at the 8th month of gestation) was sent for necropsy to the Department of Pathology of the Veterinary Institute, with no history of disease in the cow during pregnancy, as part of the active program of veterinary authorities for the detection of infectious abortive agents in domestic animals. Accordingly, a serum sample from the aborting cow, as well as parts of the placenta were also sent to the institute for analysis. According to the submission form, there were two family milk cows in the backyard, raised in an extensive system, both cows were subjected to artificial insemination at different times, and only this cow had an abortion. The cow had no previous history of disease, she aborted late at night, and in the morning the owner did not notice any clinical signs in the cow.

At the necropsy of the calf fetus, significant changes were observed in the liver, which appeared enlarged with rounded edges, measuring approximately 20 x 15 x 7 cm. The liver's capsule surface exhibited irregularities and increased hardness. On the cut surface, extensive irregular white-gray firm structures were observed extending into the hepatic parenchyma (Fig. 1A). Additionally, a larger amount of red-tinged ascites fluid had accumulated in the peritoneum, notably in the omentum and mesentery. The thoracic and pericardial sacs were also filled with a moderate amount of free reddish fluid (Fig. 1A).

Furthermore, the face of the fetus appeared slightly shortened, and the mucous membranes of the eyes, mouth, vagina, and anus exhibited cyanosis. In the subcutaneous tissue, noticeable edema composed of a reddish liquid was observed. No gross lesions were noted in other organs.

Tissue samples from the liver were fixed in 10% neutral buffered formalin and processed routinely for histological examination, and embedded into paraffin blocks. Sections of the liver tissue were cut to 5 μm and stained with haematoxylin and eosin (HE), Masson's trichrome (MT) and Van Gieson (VG), and examined histologically.

Serum from the aborting dam was tested by rapid slide agglutination test (Rose Bengal test – RBT) for identifying *Brucella* spp. antibody, as well as by serological tests for detection of antibodies against BVDV, BoHV-1, *C. burnetii*, *C. abortus*, *N. caninum* and *Leptospira* spp. (ELISA for anti-BVDV, BoHV-1, *C. burnetii*, *C. abortus* and *N. caninum*

antibodies and microscopic agglutination test for anti-*Leptospira* spp. antibodies) [11-17]. Placenta and fetal tissues were tested by real-time PCR, targeting *EGFP* gene of BVDV, *gB* genes of BoHV-1, *IS1111* of *C. burnetii*, *lipL32* gene of *Leptospira* spp., *pNC-5* gene of *N. caninum*, *B1* gene of *T. gondii* and 16s rDNA of *Chlamydia*-related bacteria, and subjected to bacterial isolation of *Listeria* spp. [18-25]. Since several pigs were kept in the same backyard with cows, the cow serum was tested for the presence of anti-HEV antibodies by a species-independent competitive enzyme-linked immunosorbent assay (ELISA) [25] and fetus liver tissue for presence of HEV RNA by RT-PCR [26].

Microscopically, extensive hepatic fibrosis was observed in the portal areas (Fig. 1B), along with bridging fibrosis extending from one portal tract to another, as demonstrated by specialized MT staining (Fig. 1C). The hallmark of portal fibrosis was an increase in extracellular matrix (ECM) components, including collagen type I deposits, as revealed by the specialized VG staining (Fig. 1D). This resulted in the expansion of the portal tract, accompanied by the proliferation of bile ducts. Bridging fibrosis was characterized by hyperplastic proliferation of fine fibers, forming fibrotic bands that connected the portal spaces (porto-portal bridging fibrosis).

Short cords or islands of vacuolated hepatocytes could be observed in the liver parenchyma and besides, we did not observe any notable inflammatory reaction in the liver parenchyma. Additionally, we observed large cells with a dark, displaced nucleus at the periphery and cytoplasm containing droplets, which were localized in proximity to the collagen fibers within fibrotic areas. These cells were assumed to be stellate cells (Fig. 1B).

The serum of the aborting cow was negative for antibodies against BVDV, BoHV-1, *Brucella* spp., *C. burnetii*, *C. abortus*, *N. caninum*, *Leptospira* spp., and HEV, while placenta and fetal tissues were negative by real-time PCR for all seven agents, including fetus liver tissue for HEV-RNA, and cultivation of *Listeria* spp.

The diagnosis of congenital hepatic fibrosis in this aborted calf fetus was established through macroscopic observations, consistent with previously described findings of this condition in calves [6-10]. Confirmation was obtained through histopathological evaluation, as well as collagen fiber staining using MT and VG techniques. These staining methods provided evidence of portal and bridging fibrosis, along with bile duct proliferation and the presence of cells resembling stellate cells in the liver parenchyma, as observed through HE staining. Furthermore, the absence of positive results for the most common infectious abortive agents in both the cow and fetal tissues, along with the absence of HEV RNA in the fetal liver tissue, supports the diagnosis of CHF in the aborted calf.

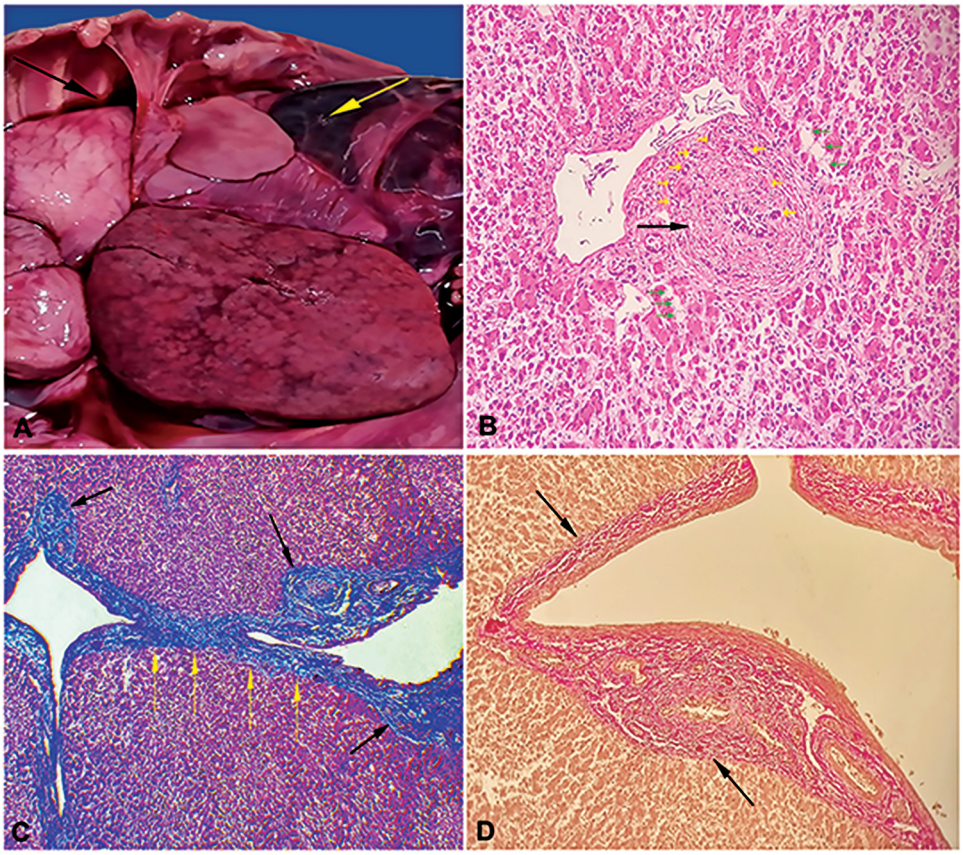


Figure 1. Liver; calf. Congenital hepatic fibrosis in a Holstein-Friesian calf. **(A)** Irregularities on the surface of the calf's liver, showing a "cobblestone appearance". A larger amount of red-tinged ascites fluid accumulated in the peritoneum and omentum (yellow arrow). The thoracic sac filled with free reddish fluid (black arrow). Histopathological evaluation of CHF in a Holstein-Friesian calf. **(B)** Excessive deposition of connective tissue in the portal and periportal space (black arrow) with proliferation of bile ducts (yellow arrows). Large cells with a nucleus at the periphery and cytoplasm filled with droplets near the collagen fibers within the fibrous area, which we suspect are stellate cells (green arrows), hematoxylin and eosin staining × 40 objective. **(C)** Increased fibrous tissue expanding the portal tract (portal fibrosis) (black arrows) and fibrotic bands connecting portal tract to portal tract (porto-portal bridging fibrosis) (yellow arrows), Masson Trichrome staining × 10 objective. **(D)** Type I collagen deposits in the portal area (black arrows), Van-Gieson staining × 40 objective.

DISCUSSION

Congenital hepatic fibrosis is a rare developmental disorder pathologically based on ductal plate malformation, resulting from abnormal embryogenesis of the biliary ductal system [6]. In humans, CHF is a hereditary, autosomal recessive disease, usually associated with autosomal recessive polycystic kidney disease childhood [28,29]. In cattle, CHF has been diagnosed in fetuses, newborns [6-8], and in a ten-month-old

calf [9]. However, unlike CHF cases in horses, cats, dogs, and rats, this fibrocystic hepatic disease in calves is not associated with malformations in other organs, which is also a feature of this case. The sporadic occurrence and the absence of other etiologies indicate that congenital hepatic fibrosis in prenatal and postnatal calves is similar to the disease in humans and may result from a heritable or spontaneous genetic mutation, possibly related to biliary dysgenesis [6].

The pathological lesions described in our case are similar to those reported in cases of CHF in calves of the Holstein-Friesian [6,7], Nellore [10], and Hollando-Argentino [9] cattle breeds, as well as liver fibrosis described in cases of 'Paunch Calf Syndrome' (PCS) in Romagnola cattle [30]. Subsequent investigations of PCS cases identified a missense mutation in the *KDM2B* gene (c. 2503G>A) on BTA17, which is associated with this condition [31]. In our case, in addition to the defined liver fibrosis, we also observed moderate ascites and mild subcutaneous edema, likely resulting from postmortem changes or alterations in hydrostatic and osmotic blood pressure caused by liver disease. No pathological lesions were observed in other organs or systems. Therefore, even though we did not perform genetic testing for PCS genotyping, we ruled out PCS as the cause of fetal death in this case, which, along with hepatic fibrosis, is characterized by multiorgan developmental dysplasia. To the authors' knowledge, there have been five reported cases of this condition in Holstein-Friesian calves, with one case described in a 1-day-old calf [7], two in aborted fetuses at 8 month of gestation [6,8] and two in aborted fetuses at 7 month of gestation [6]. This suggests that CHF is a very rare condition in this breed of cattle. Therefore, additional research will be necessary to confirm a genetic etiology for this condition with convincing evidence that the defect might be regulated by a single autosomal locus acting in a recessive manner, or to detect the other factors responsible for the occurrence of this liver disease in Holstein-Friesian calves.

Since an infectious etiology was absent in our case, and a toxic etiology, metabolic abnormalities, nutritional deficiencies, or trauma were unlikely due to the lack of clinical signs in the dam, the characteristic macroscopic "cobblestone appearance" of the lesions on the surface of the fetal liver, associated with marked fibrosis in the liver parenchyma as observed in the histopathological evaluation, favored the diagnosis of CHF in the aborted fetus.

In conclusion, this is the first report of liver disease with a description of fibrosis and proliferation of bile ducts in fibrous tissue in a Holstein-Friesian calf in Serbia, and the described lesions are characterized as congenital hepatic fibrosis. This disease may have a genetic basis in Holstein-Friesian cattle, or it may be caused by factors not identified in our research. In both cases, it is necessary to continue the research on aborted and stillborn fetuses in this breed of cattle to detect the causes of this fatal liver disease. Given its potential genetic basis as well as unrecognized factors that cause its appearance, it is crucial to recognize and monitor this disease. This recognition will serve to make decisions regarding the use of breeding animals that carry genes for this condition and their exclusion from further breeding, or if other causes are responsible for its occurrence, they need to be addressed.

Acknowledgements

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract number 451-03-66/2024-03/200030.

We would especially like to thank the veterinarians from the "Tikavet" veterinary services, for all the obtained anamnestic and clinical data and collected samples.

Authors' contributions

BS interpreted the results, wrote the manuscript, assisted with the calf's necropsy, captured microscopic images, and oversaw the anatomo-pathological and histological interpretations. BM, SS, and BK conducted the calf's necropsy, interpreted the macroscopic lesions, and assisted in obtaining microscopic images. NJ and AFL carried out the laboratory diagnostics.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ORCID iDs

Božidar Savić https://orcid.org/0000-0001-7705-6457
Bojan Milovanović https://orcid.org/0000-0001-8573-0771
Slobodan Stanojević https://orcid.org/0000-0001-6913-7986
Branislav Kureljušić https://orcid.org/0000-0003-4281-1672
Nemanja Jezdimirović https://orcid.org/0000-0003-1958-9487
Aleksandra Fejsa Levakov https://orcid.org/0000-0002-9510-3090

REFERENCES

1. Kerr DNS, Harrison CV, Sherlock S, Milnes WR: Congenital hepatic fibrosis. Q J Med 1961, 30:91-117.
2. Haechler S, Van Den Ingh TS, Rougivue C, Ehrensperger F and Welle M: Congenital hepatic fibrosis and cystic bile duct formation in Swiss Freiberger horses. Vet Pathol 2000, 37:669-671.
3. Bettini G, Mandrioloi L and Morini M: Bile duct dysplasia and congenital hepatic fibrosis associated with polycystic kidney (Caroli Syndrome) in a Rat. Vet Pathol 2003, 40:693-694.
4. Brown DL, Van Winkle, T, Cecere T, Rushton S, Brachlente C and Cullen JM. Congenital hepatic fibrosis in 5 dogs. Vet Pathol 2010, 47:102-107.
5. Brown DL, Anderson, M and Cullen JM: Mesenchymal hamartoma of the liver in a late-term equine fetus. Vet Pathol 2007, 44:100-102.
6. Bourque AC, Fuentealba C, Bildfell R, Daoust P-Y and Hanna P: Congenital hepatic fibrosis in calves. Can Vet J 2001, 42(2):145-146.

7. Yoshikawa H, Fujida, T, Oyamada, T and Yoshikawa T: Congenital hepatic fibrosis in a newborn calf. Vet Pathol 2002, 39:143-145.
8. Movassaghi AR, Naseri Z and Rezaee Oghazi M: Congenital hepatic fibrosis in an aborted calf. Iran J Vet Res 2011, 12(3):262-264.
9. Indart M, María GY, Jorge PG Ángel RB, Marcial SN: Congenital hepatic fibrosis in Holando Argentino calf: first report in Argentina. Braz J Vet Pathol 2019, 12(2):58-62.
10. Dantas DG, Sousa IV, Barbosa FMS, Neto JFS, Sampaio RAG, Simões SVD, Dantas IM, Lucena RB: Congenital hepatic fibrosis associated with ascites in a nellore calf. Ciênc Rural 2020, 50(5):e20190511.
11. Morgan WJB, Mackinnon DJ, Lawson JR and Cullen GA: The rose bengal plate agglutination test in the diagnosis of brucellosis. Vet Rec 1969, 85:636-641.
12. Edwards S: The diagnosis of bovine virus diarrhea-mucosal disease in cattle. Rev Sci Tech 1990, 9:115-30.
13. Kramps JA, Banks M, Beer M, Kerkhofs P, Perrin M, Wellenberg GJ and van Oirschot JT: Evaluation of tests for antibodies against bovine herpesvirus 1 performed in national reference laboratories in Europe Vet Microbiol 2004, 102:169-181.
14. Tissot-Dupont H, Thirion X and Raoult D: Q fever serology: cutoff determination for microimmunofluorescence. Clin Diagn Lab Immunol 1994, 1:189-196.
15. Sachse K, Vretou E, Livingstone M, Borel N, Pospischil A and Longbottom D: Recent developments in the laboratory diagnosis of chlamydial infections (Review). Vet Microbiol 2009, 135:2-21.
16. Bartels CJM, Arnaiz-Seco JI, Ruiz-Santa-Quitera A, Björkman C, Frössling J, von Blumröder D, Conraths FJ, Schares G van Maanen C, Wouda W and Ortega-Mora LM**:** Supranational comparison of *Neospora caninum* seroprevalences in cattle in Germany, the Netherlands, Spain and Sweden. Vet Parasitol 2006, 137**:**17-27.
17. Goris M and Hartskeerl R: Leptospirosis serodiagnosis by the microscopic agglutination test. Curr Protoc Microbiol 2014, 32, Unit 12E.5.
18. Hoffmann B, Depner K, Schirrmeier H and Beer M: A universal heterologous internal control system for duplex real-time RT-PCR assays used in a detection system for pestiviruses J Virol Methods 2006, 136:200-209.
19. Wang J, O'keefe J, Orr D, Loth L, Banks M, Wakeley P, West D, Card R, Ibata G, van Maanen K, Thoren P, Isaksson M and Kerkhofs P: An international inter-laboratory ring trial to evaluate a real-time PCR assay for the detection of bovine herpesvirus 1 in extended bovine semen. Vet Microbiol 2008, 126:11-19.
20. Klee SR, Tyczka J, Ellerbrok H, Franz T, Linke S, Baljer G and Appel B: Highly sensitive real-time PCR for specific detection and quantification of Coxiella burnetii. BMC Microbiol 2006, 6:2.
21. Nayeri T, Moosazadeh M, Sarvi S, Daryani A: Neosporacaninum infection in aborting bovines and lost fetuses: A systematic review and meta-analysis. PLoS ONE 2022, 17(5): e0268903.
22. Thaipadungpanit J, Thaipadunpanit J, Chierakul W, Wuthiekanun V, Limmathurotsakul D, Amornchai P, Boonslip S, Smythe L, Limpaiboon R, Hoffmaster A, Day N, Peacock S: Diagnostic accuracy of real-time PCR assays targeting 16S rRNA and lipL32 genes for human leptospirosis in Thailand: a case-control study. PloS One 2011, 6(1).

23. Wastling JM, Nicoll S and Buxton D: Comparison of two gene amplification methods for the detection of Toxoplasma gondii in experimentally infected sheep. J Med Microbiol 1993, 38:360-365.
24. Ehricht R, Slickers P, Goellner S, Hotzel H and Sachse K: Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mol Cell Probes 2006, 20:60-63.
25. Eld K, Danielsson-Tham ML, Gunnarsson A and Tham W: Comparison of a cold enrichment method and the IDF method for isolation of Listeria monocytogenes from animal autopsy material. Vet Microbiol 1993, 36:185-189.
26. Tialla D, Cissé A, Ouédraogo GA, Hübschen JM, Tarnagda Z, Snoeck CJ: Prevalence of hepatitis E virus antibodies in cattle in Burkina Faso associated with swine mixed farming J Vet Sci 2022, May;23(3):e33.
27. Tritz SE, Khounvisith V, Pommasichan S, Ninnasopha K, Keosengthong A, Phoutana V, et al.: Evidence of increased hepatitis E virus exposure in Lao villagers with contact to ruminants. Zoonoses Public Health 2018, 65(6):690-701.
28. Janowski K, Goliszek M, Cielecka-Kuszyk J, Jankowska I, Pawłowska J: Congenital hepatic fibrosis in a 9-year-old female patient – a case report. Clin Exp Hepatol 2017, 3(3):176-9.
29. Strazzabosco M and Fabris L: Development of the bile ducts: Essentials for the clinical hepatologist. J Hepatol 2012, 56(5):1159-70.
30. Testoni S, Militerno G, Rossi M, Gentile A: Congenital facial deformities, ascites and hepatic fibrosis in Romagnola calves. Vet Rec 2009, 164:693-694.
31. Testoni S, Bartolone E, Rossi M, Patrignani A, Bruggmann R, Lichtner P, Tetens J, Gentile A, Drögemüller C. KDM2B is implicated in bovine lethal multi-organic developmental dysplasia. PLoS One. 2012, 7(9):e45634.

KONGENITALNA FIBROZA JETRE KOD ABORTIRANOG FETUSA HOLŠTAJN-FRIZIJSKE RASE GOVEDA

Božidar SAVIĆ, Bojan MILOVANOVIĆ, Slobodan STANOJEVIĆ, Branislav KURELJUŠIĆ, Nemanja JEZDIMIROVIĆ, Aleksandra Fejsa LEVAKOV

Kongenitalna fibroza jetre (KFJ) je poremećaj koji se karakteriše nakupljanjem vezivnog tkiva u parenhimu jetre koje uzrokuje poremećaj funkcije jetre. Oboljenje je opisano kod različitih životinjskih vrsta, međutim kod goveda se ovo stanje retko javlja. U ovom radu, opisujemo slučaj KFJ kod abortiranog fetusa goveda Holštajn-Frizijske rase. Najznačajniji makroskopski nalazi kod obdukovanog fetusa bili su, uvećana jetra, tvrde konzistencije parenhima sa nepravilnostima na površini, karakterističnog „kockastog izgleda" i umeren ascit. Histopatološki, u parenhimu jetre uočena je fibroza portalnih prostora kao i fibroza premoštavanjem koja se širila od jednog portnog prostora do drugog, sa proliferacijom žučnih kanala. Dijagnostička ispitivanja na najznačajnije infektivne abortogene agenese, kao uzrok pobačaja ili ustanovljenih promena na jetri fetusa, su bila negativna. Patoanatomski nalaz i histopatološka ispitivanja su potvrdila dijagnozu KFJ kod abortiranog fetusa, što predstavlja prvi slučaj ovog oboljenja jetre

kod fetusa Holštajn-Frizijske rase goveda u našoj zemlji. Neophodna su dodatna istraživanja kako bi se utvrdilo da li ovo oboljenje ima genetsku osnovu ili je uzrokovano sa do sada, još uvek nepoznatim faktorima kod Holštajn-Frizijskih goveda.