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# Ultrasonographic findings in a cow with extra-hepatic cholestasis and cholangitis

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## Summary

This case report describes a 6-year-old Holstein Friesian cow with extrahepatic cholestasis. The most prominent clinical signs were disturbed general condition and behaviour, icterus, yellowish brown urine, and bilirubinuria. An increased concentration of serum bilirubin and an increase in hepatic enzyme activity were the most important laboratory findings. Ultrasonographic examination of the liver and gall bladder revealed severe bile stasis. The intrahepatic bile ducts, the cystic duct, and the gall bladder were severely dilated. Histological examination of a liver biopsy revealed cholestasis, cholangitis, and acute hepatosis. A liver abscess was also found at post mortem examination. It was assumed that cholestasis was caused by the inflammatory products resulting from cholangitis.

**Key words:** ultrasonography – cholestasis – cholangitis – hepatosis – cow

## Introduction

The term cholestasis refers to both intra- and extrahepatic biliary obstruction. In extrahepatic cholestasis, the obstruction usually occurs in the duodenal papilla of the choledochus duct and seldom occurs in the cystic duct or in the hepatic duct. In intrahepatic cholestasis, obstruction to biliary flow occurs in the intrahepatic bile ducts. The most common causes of obstruction in cattle are fascioliasis, fibrinous or purulent inflammatory products, and sediment; other less common causes include gall stones and tissue proliferation (Rosenberger and

## Ultrasonographische Befunde bei einer Kuh mit extrahepatisch bedingter Cholestase und Cholangitis

Es wird eine extrahepatisch bedingte Cholestase bei einer 6jährigen Schweizer Schwarzwälderkuh beschrieben. Die auffallendsten klinischen Befunde waren neben gestörtem Allgemeinbefinden Ikterus, teefarbener Harn und eine Bilirubinurie. Die wichtigsten Laborbefunde waren ein erhöhter Serumbilirubingehalt und erhöhte Leberenzymaktivitäten. Mit Hilfe der Ultraschalluntersuchung der Leber und Gallenblase konnte eine hochgradige Gallenstauung nachgewiesen werden. Die intrahepatischen Gallengänge, der Ductus cysticus und die Gallenblase waren massiv dilatiert. Die histologische Untersuchung einer Leberbiopsie zeigte eine Cholestase, eine Cholangitis und eine akute Hepatose. Bei der postmortalen Untersuchung wurde zusätzlich ein Leberabszess festgestellt. Es wird vermutet, dass die Cholestase durch die im Rahmen der Cholangitis gebildeten Entzündungsprodukte verursacht wurde.

**Schlüsselwörter:** Ultrasonographie – Cholestase – Cholangitis – Hepatose – Kuh

Gründer, 1970). Biliary obstruction can also result from inflammatory products of salmonella, E. coli and proteus infections as well as pathogens causing abscessation and necrosis associated with cholangitis. Such infections ascend via the choledochus duct hematogenously and lymphogenously. In rare cases, obstruction to biliary flow is caused by compression of the major bile duct by tumours, abscesses, or peritoneal changes. Biliary obstruction results in dilatation of the bile channels, hyperbilirubinemia, bilirubinuria, and an increase in the activity of the bile duct enzyme gamma glutamyl transferase. Clinical manifestations of biliary obstruction

include indigestion characterized by constipation. Cases with extensive extrahepatic biliary obstruction may have symptoms of acute to recurring severe colic, yellowish brown urine, and icterus (Rosenberger and Gründer, 1970). Severe hepatogenous dermatitis solaris may occur in affected animals on pasture in the summer.

The following case report describes a 6-year-old Holstein Friesian cow with cholestasis. The cow was 9 months pregnant and according to the owner did not have any abnormal clinical signs until 2 days prior to admission. The cow then became recumbent, anorexic, apathetic and did not pass feces. Increased hepatic enzyme activity and an increased concentration of ruminal chloride were determined by the referring veterinarian. The cow stood and started to eat after an intravenous solution containing calcium was administered. However, her general condition did not improve, and she was referred to our clinic.

## Clinical examination

The general attitude and behaviour of the cow were moderately disturbed. The cow was in good bodily condition, occasionally had bruxism, and had a very poor appetite. The conjunctival, oral, and vestibular mucous membranes were icteric. The cow had a rectal temperature of 39.4 °C, a heart rate of 76 beats/minute, and a respiratory rate of 42 breaths/minute. Other than increased vesicular sounds, there were no abnormal findings auscultated over the lungs. Ruminal activity, filling, and stratification of ingesta were slightly reduced. Intestinal activity could be auscultated. The pole test and the back grip were positive. Pain percussion of the reticulum, liver, and abomasum were negative. Swinging auscultation and percussion auscultation on both sides of the abdomen revealed no abnormalities. The abdominal wall was slightly tense. Rectal examination verified an advanced pregnancy, and a live calf was palpated. The feces were olive coloured, were reduced in quantity, and had a soupy consistency. Spontaneous urination produced yellowish brown urine; a chemical reagent test strip revealed a pH of 7.0, ++ bilirubin, and + protein. The glutaraldehyde test reading was 9 minutes.

Based on the clinical findings, a diagnosis of icterus was made. In order to determine whether the icterus was pre-, intra- or posthepatic, hematological and biochemical examination of blood and ruminal fluid, histologic examination of a liver biopsy, ultrasonography of the liver, and parasitological examination of the feces were performed.

## Clinical pathology

Hematological examination of the blood revealed an increased hematocrit and a mild leukocytosis (Table 1). The concentrations of plasma protein and fibrinogen were increased. The concentrations of bilirubin and bile

*Table 1: Hematological and biochemical analyses of blood from a cow with cholestasis, cholangitis, and hepatosis.*

Measurement	Value for the diseased cow	Reference values
Hematocrit (%)	44	28-38
Leukocyte count (/µl)	10600	4000-10 000
Total protein (g/litre)	88	60-80
Fibrinogen (g/litre)	7	4-6
Total bilirubin (µmol/litre)	56.1	0.8-8.6
Direct bilirubin (µmol/litre)	20.0	-
AST (U/litre)	449	40-80
GLDH (U/litre)	341	2.0-12.0
Gamma-GT (U/litre)	113	6.0-17.0
SDH (U/litre)	123	0-12.0
Bile acids (µmol/litre)	281	<60
Urea (mmol/l)	6.2	2.5-7.5
Sodium (mmol/litre)	144.0	140-150
Chloride (mmol/litre)	111.0	95-105
Potassium (mmol/litre)	3.8	4.0-5.0
Calcium (mmol/litre)	2.47	2.0-2.6
Phosphorus (mmol/litre)	1.30	1.30-2.25
Magnesium (mmol/litre)	0.88	0.70-1.10

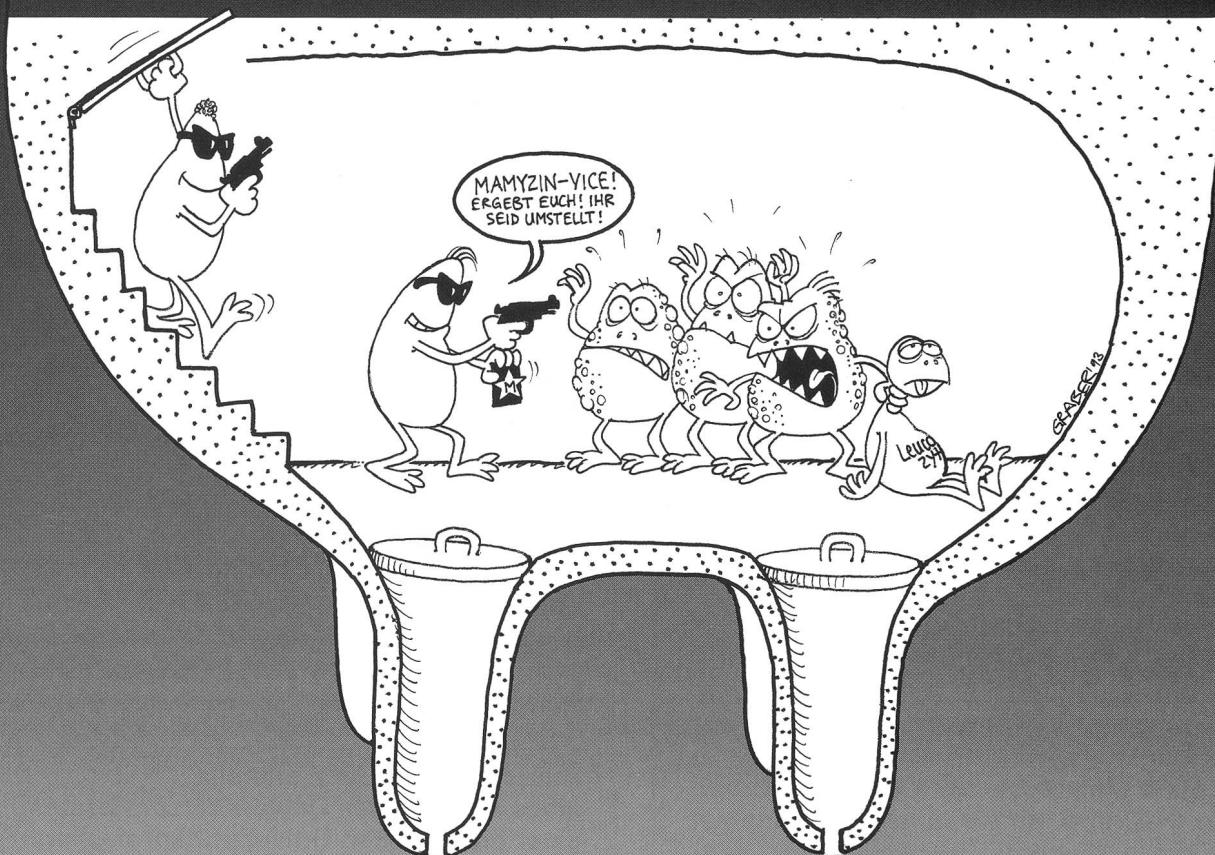
acids and the activity of the hepatic enzymes aspartate amino transferase (AST), glutamate dehydrogenase (GLDH), sorbit dehydrogenase (SDH), and gamma glutamyl transferase ( $\gamma$ -GT) were markedly increased. Venous blood gas analysis and serum electrolyte concentrations were normal except for a mild hypokalemia. Examination of ruminal fluid revealed inactivity of the ruminal flora and fauna. The concentration of ruminal chloride (43 mmol/l) was moderately increased. Results of parasitological examination of the feces were normal.

## Ultrasonographic examination of the liver and gallbladder

The area over the last 7 intercostal spaces on the right side of the cow was clipped, and the remainder of the hair was removed with depilatory cream. Contact gel was applied, and the cow was examined ultrasonographically using a 3.5-MHz linear and a 2.25-MHz convex scanner (LSC 7000, Picker International GmbH, Leu AG, CH-6004 Luzern). The liver was examined ultrasonographically from caudal to cranial beginning high dorsally in the last intercostal space and proceeding ventrally with the scanner as previously described (Braun, 1990). The most striking findings were a markedly dilated gall bladder and a severely dilated cystic duct (Fig. 1). The liver parenchyma appeared ultrasonographically normal; however, there were longitudinal, band-shaped, anechoic structures, up to 4 cm in width, running almost parallel to each other. These structures joined similar ones with a wider lumen (Fig. 2, 3). Based on the dilated gall bladder and cystic duct, it was presumed that these anechoic structures were dilated bile ducts. Ultrasound-guided centesis of these structures using a 0.09 × 90 mm cannula yielded bile and confirmed the diagnosis of

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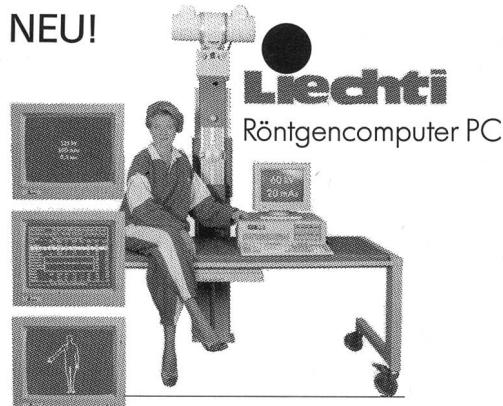
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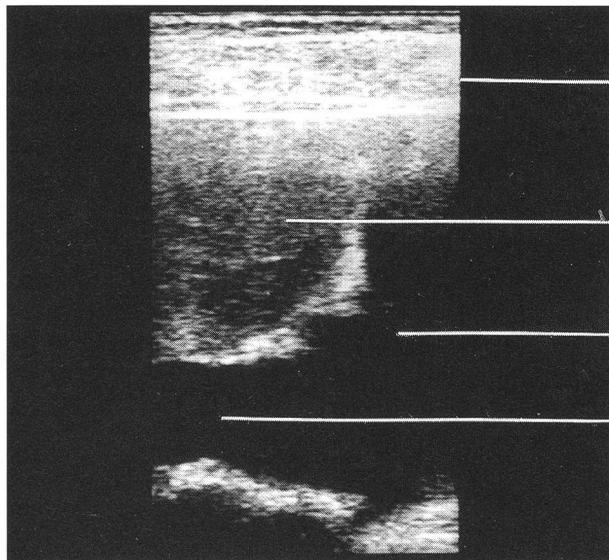
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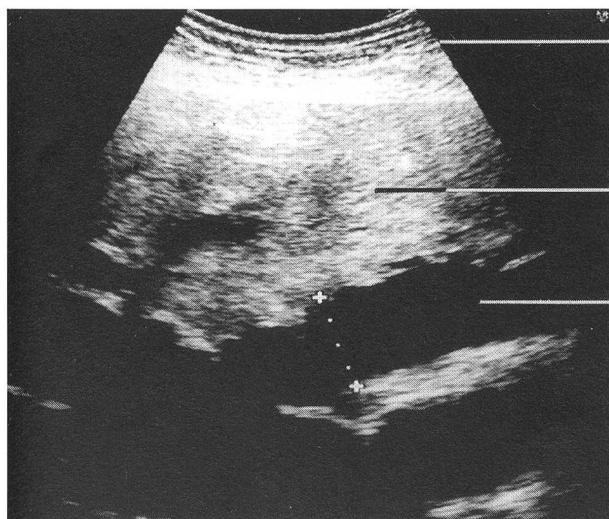
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cholestasis. The caudal vena cava could not be imaged, and the portal vein was unremarkable. Ultrasound-guided centesis of the gall bladder was performed according to the method of Braun and Gerber (1992). No liver fluke eggs were observed in the aspirated bile, and the concentration of bile acids was 12.5 mmol/l.



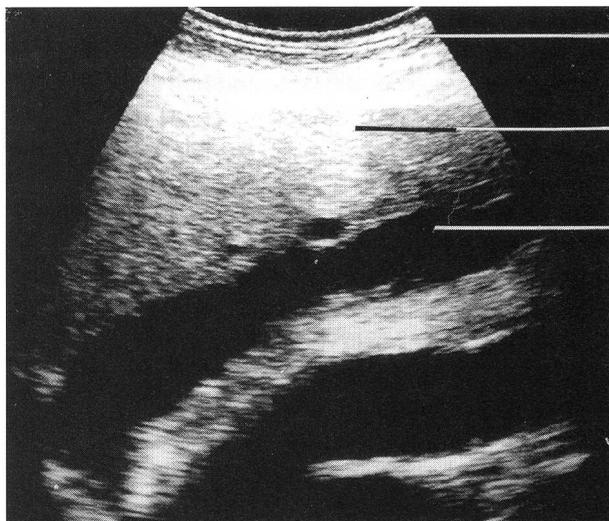
*Fig. 1: Ultrasonogram of the liver and the gallbladder imaged from the 11th intercostal space of a cow with cholestasis using a 3.5-MHz linear scanner. The cystic duct, which is normally difficult to see, is markedly dilated because of cholestasis.*

*Top line = abdominal wall, second line = liver texture, third line = gallbladder, bottom line = dilated cystic duct.*



*Fig. 2: Ultrasonogram of the liver imaged from the 11th intercostal space of a cow with cholestasis using a 2.25-MHz curved convex scanner. The intrabhepati bile ducts, which are not visible under normal circumstances, are markedly dilated because of cholestasis and are anechoic. The bile ducts run almost parallel to each other.*

*Top line = abdominal wall, second line = liver texture, third line = dilated bile ducts.*



*Fig. 3: Ultrasonogram of the liver imaged from the 11th intercostal space of a cow with cholestasis using a 2.25 MHz-curved scanner. The intrabhepati bile ducts, which are not visible under normal circumstances, are markedly dilated because of cholestasis, and are anechoic. The bile ducts run almost parallel to each other and then join with a larger bile duct. Top line = abdominal wall, second line = liver texture, third line = dilated bile ducts.*

## Diagnosis, treatment and course of the disease

A diagnosis of icterus attributable to extrahepatic cholestasis was made based on the icteric mucous membranes, hyperbilirubinemia, bilirubinuria, increased hepatic enzyme activity, and the ultrasonographic findings in the liver and gall bladder. In addition, based on the high hepatic enzyme activity, there was probably severe hepatic parenchymal disease. An indwelling catheter was placed in the jugular vein of the cow, and 20 litres of sodium chloride and glucose solution were administered over a period of 24 hours. Within 24 hours of admission, the cow had symptoms of colic (increasing restlessness and treading). Intravenous administration of novaminsulfonic acid did not improve the cow's condition, and she became recumbent. Emergency slaughter of the cow in extremis was performed. The calf was removed alive but died shortly thereafter.

## Post mortem findings

The liver was markedly enlarged and yellow. There was an abscess, approximately 10 cm in diameter, on the diaphragmatic surface of the liver which contained creamy, yellow, purulent material. The intrahepatic bile ducts, the gall bladder and the cystic duct were severely dilated and contained foul smelling bile. There was no calcification of the bile ducts, and they did not contain

any liver fluke eggs. The gall bladder contained mucoid material and an approximately 3 cm × 3 cm × 3 cm structure that was soft and friable and had a cauliflower-like surface which was easily compressed with the fingers. The choledochus duct was also dilated due to obstruction and had a diameter of 1.5 cm. The liver and the gall bladder were separated from the intestines at slaughter; thus, the junction of the choledochus duct and the duodenal papilla could not be examined. Other than severe reddening of the abomasal mucosa, no other abnormal findings were observed in the remaining organs. Histological examination of the liver biopsy revealed acute hepatosis and chronic active cholangitis with portal fibrosis. Diffuse hyaline areas were observed in the cytoplasm of the hepatocytes. The hepatic nuclei varied in size and a few were pyknotic. The amount of nuclear chromatin in hepatocytes varied. More mitoses than normal and a few bile thrombi were also observed. The portal triads were dilated, and there was prominent bile duct proliferation with bile stasis, periductal edema, a mixed cell infiltration including eosinophils and neutrophils, and marked proliferation and activation of connective tissue. Sudan-stained sections revealed a mild increase in hepatocellular adipose accumulation.

## Discussion

In this case report, the diagnosis of icterus could be made based on the icteric mucous membranes, the yellowish brown urine and the bilirubinuria. Basically, the icterus was either prehepatic, hepatocellular or post-

hepatic. Prehepatic icterus attributable to hemolytic anemia and causing hyperbilirubinemia could be quickly ruled-out because the hematocrit was not decreased. The clinical findings and the increase in bilirubin are usually less marked in cows with hepatocellular icterus. The marked increase in the activity of the bile duct enzyme gamma glutamyl transferase was an indication that the bile duct was involved. Severe damage to the liver parenchyma was also indicated by the increase in direct and indirect serum bilirubin and in the liver enzyme activity.

It was possible to verify the cholestasis via ultrasonography. This technique has been successfully used for years in human beings to differentiate between hepatocellular and obstructive icterus; dilatation of the bile ducts can be seen immediately after an obstruction via ultrasonography even when there is no cholestasis as determined by measuring the serum bilirubin concentration and the activity of the bile duct enzyme gamma glutamyl transferase (Banholzer and Weigold, 1988). In human beings, other techniques such as computer tomography (Partanen et al., 1990), endosonography (Amouyal et al., 1989), endoscopic retrograde cholangiography (Borsch et al., 1988) and cholescintigraphy (Borsch et al., 1988, Klingensmith and Ashdown, 1991) are used to diagnose cholestasis. Based on the pattern of dilatation of the intra- and extrahepatic bile ducts and of the gall bladder, ultrasonography can locate the site of obstruction and provide information about the adjacent structures e.g. presence of liver abscesses (Wurbs, 1987). Bile duct stones can be ultrasonographically identified in human beings using a more subtle examination technique. In

### **Observations ultrasonographiques chez une vache avec une cholestase et cholangite d'origine extrahépatique**

Une cholestase d'origine extrahépatique d'une vache tachetée noire suisse agée de six ans est décrite. Les observations cliniques les plus frappantes étaient, mis à part un état général perturbé, un ictere, une urine couleur thé et une bilirubinurie. Les analyses de laboratoire ont révélé une concentration élevée de la bilirubine sérique et des concentrations élevées des enzymes hépatiques. A l'aide d'un examen ultrasonographique du foie et de la vésicule biliaire, une stase biliaire prononcée au pu être mise en évidence. Les canaux biliaires intra-hépatiques, le ductus cysticus et la vésicule biliaire étaient fortement dilatés. L'examen histologique d'une biopsie du foie a révélé une cholestase, une cholangite et une hépatose aigüe. Durant l'autopsie, un abcès du foie a été décelé. Il est supposé que la cholestase a été causée par les produits de l'inflammation de la cholangite.

### **Rilevamenti ultrasonografici in una vacca con colestasi extraepatica e colangite**

Si descrive il caso di una vacca della razza pezzata nera svizzera di sei anni che soffriva di una colestasi extraepatica. I reperti clinici principali furono uno stato generale alterato, ittero, coloramento marrone dell'urina e bilirubinuria. I reperti di laboratorio principali furono un contenuto elevato di bilirubina nel siero ed attività elevate degli enzimi del fegato. Per mezzo degli esami ultrasonografici del fegato e della cistifellea si potè rilevare una marcata stasi della bile. I dotti biliferi intraepatici, il dotto cistico e la cistifellea erano estremamente dilatati. L'esame istologico di una biopsia del fegato evidenziò una colestasi, una colangite ed una epatosi acuta. All'esame macroscopico postmortale venne inoltre scoperto un ascesso nel fegato. Si presuppone che la colestasi sia stata provocata da prodotti infiammatori risultanti dalla colangite.

cattle with reduced food intake, the gall bladder is often dilated, and therefore it is not necessarily an indication of bile stasis. A markedly dilated cystic duct is a clear indication that there is an obstruction because even in cows with very large gall bladders, this duct is not normally dilated.

Severe cholangitis was diagnosed in this case report based on the mucoid, foul smelling nature of the bile and on the results of histological examination of the liver. The resulting inflammatory products, some of which were found in the gall bladder, caused intra- and extrahepatic obstruction of the bile ducts and bile stasis. They were also responsible for the colic symptoms which progressively worsened and which did not respond favourably to therapy. Cholestasis in cattle is usually attributable to sediment from liver fluke infection and seldom to inflammatory products from other causes or to bile stones. Parasitological examination of the feces and bile for liver fluke eggs was negative, and post mortem examination of the liver did not reveal adult liver flukes. This did not rule out fascioliasis if the liver flukes had died before the cow became ill. Inflammatory processes of the bile ducts can be caused by various bacteria such as, salmonella, *E. coli* and *proteus* as well as by pathogens resulting in abscessation and necrosis. It is very likely that the acute hepatosis resulted from bile stasis, and that the liver abscess was a complication of the cholangitis (Rosenberger and Gründer, 1970). In human beings, liver abscesses are the most common complication of cholangitis besides other septic changes such as, perihepatic abscesses, pylephlebitis, and general sepsis (Wurbs, 1987). In this report, the liver abscess could not be ultrasonographically imaged because it was located on the diaphragmatic surface of that part of the liver located under the lung. Liver abscesses are seldom located in this area. Usually they are situated high dorsally in the last intercostal space near the caudal vena cava or near the cranoventral part of the reticulum where they can easily be seen via ultrasonography. Liver abscesses, identified using ultrasonography, have been reported to occur in cattle inoculated with *Fusobacterium necrophorum* via the portal vein (Itabisashi et al., 1987) and in intensively fed beef cattle (Jönsson et al., 1988).

The results of the determination of bile acid concentrations in the serum and in the bile were informative; the concentration was markedly increased in the serum and clearly decreased in the bile. The concentration of bile acids in the serum of fasted healthy cattle is on average 22.3 µmol/l (Gül and Gründer, 1988). Serum concentrations of over 60 µmol/l indicate liver disease (Pearson, 1990). The concentration of bile acids in the bile is normally much higher than that in the serum. The average concentration of bile acids in the bile of 20 cows was

$45.3 \pm 3.05$  mmol/l (Braun and Gerber, 1992). There is a high correlation between the concentration of bile acids in the serum and that in the bile. In this report, there was a marked increase in the concentration of bile acids in the serum (281 µmol/l) and a very low concentration in the bile (12.5 mmol/l) in comparison to healthy cows. This was probably because the bile acids could no longer be removed from the portal blood and secreted into the bile due to hepatocellular dysfunction. Thus, the bile acids entered the peripheral blood.

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