Morphological classification of biliary disorders of the canine and feline liver

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Update 2021: Ted S.G.A.M. van den Ingh, John M. Cullen, Hille Fieten, Guy C.M. Grinwis

ABSTRACT.

The biliary disorders can be grouped into four major categories: 1) Biliary cystic disease and biliary atresia, 2) Cholestasis and cholate-stasis, 3) Cholangitis, and 4) Disorders of the gallbladder. The biliary cystic diseases comprise solitary usually acquired cysts and congenital cystic diseases of the liver. The latter are considered ductal plate anomalies and the various forms observed in dogs and cats correspond with those seen in humans and can be be subdivided in three different subtypes i.e. juvenile type polycystic liver disease / congenital hepatic fibrosis, congenital dilatation of the hepatic and large intrahepatic bile ducts (Caroli disease) and adult type polycystic liver disease including Von Meyenburg complexes. Extrahepatic biliary atresia is extremely rare and has been described only once in a dog. Cholestasis (bilirubinostasis) is morphologically characterized by the presence of bile in the hepatic parenchyma. Intrahepatic cholestasis is associated with a wide spectrum of liver diseases and microscopic lesions apart from the cholestasis are related to the primary hepatic disease. In extrahepatic cholestasis the lesions are related to the leakage of bile from the bile ducts into the connective tissue of the portal tracts which causes an acute or chronic inflammation in the stromal tissue of these portal tracts; cholestasis is evident in acute cases but may be absent in chronic cases. Cholate-stasis is characterized by swollen pale hepatocytes, preferentially in the periportal region, with centrally located, often copper containing granules. Cholangitis can be differentiated in: 1) Neutrophilic cholangitis, usually resulting from bacterial ascending infection, 2) Lymphocytic cholangitis as observed in cats, 3) Destructive cholangitis, and 4) Chronic cholangitis associated with liver fluke infestation. Disorders of the gallbladder are agenesis and duplication of the gallbladder, mucocele and cystic mucinous hyperplasia, neutrophilic and lymphoplasmacytic cholecystitis, and infarction of the gallbladder. Finally, the role of histopathological evaluation, ultrasonography and examination of bile in the diagnosis of biliary disorders is discussed.

INTRODUCTION

The anatomy of the biliary system and the classification and morphology of the various biliary disorders in dogs and cats is presented. The biliary disorders can be grouped into four major categories: 1) Biliary cystic disease and biliary atresia, 2) Cholestasis and cholate-stasis, 3) Cholangitis, and 4) Disorders of the gallbladder. Histological examination of liver biopsies can substantially aid in the diagnosis of the first three categories. Changes in the portal tracts are the hallmarks of most of these biliary disorders. However, portal inflammation, fibrosis and bile duct proliferation are not restricted to biliary disorders and may also be seen in primary parenchymal disorders such as acute and chronic hepatitis, and primary vascular abnormalities. Differentiation in general is possible through careful histological examination and evaluation of the combination of the parenchymal, vascular and portal tract lesions present in the affected liver. In contrast to the parenchymal and neoplastic liver diseases the diagnosis of diseases of the biliary tract depends not only on histopathological evaluation, but largely on ultrasonography. In neutrophilic cholangitis and cholecystitis culture and cytological examination of bile is required to assess the diagnosis. The role of the different diagnostic methods is summarized by the end of this chapter.

ANATOMY OF THE BILIARY SYSTEM

The liver can be regarded as an exocrine gland and the smallest structures, the bile canaliculi, consist of intercellular spaces formed by the secretory hepatocytes and are sealed off by tight junctions between adjacent hepatocytes. The bile canalicular membrane represents a specialized area of the liver cell surface as evidenced among others by the high concentration of alkaline phosphatase and the presence of a contractile actin filament network in the pericanalicular cytoplasma. In the periportal areas the bile canaliculi drain into cholangioles or terminal ductules (also known as canals of Hering), which are lined by ductal cells and hepatocytes.The ductules extend through the limiting plate and within the smallest portal tracts unite to form the interlobular bile ducts which are lined by cuboidal epithelium. The interlobular ducts anastomose and form the larger intrahepatic (lobar) bile ducts lined by tall columnar epithelium with a basally located nucleus. These finally enter the main hepatic ducts that unite to form the common bile duct (syn: ductus choledochus) which enters the duodenum at the Vater's papilla. The gallbladder, responsible for storage and concentration of bile, communicates with the main hepatic ducts and the common bile duct via the cystic duct.[1-5]

BILIARY CYSTIC DISEASE AND BILIARY ATRESIA

1 Solitary cysts

Solitary cysts are rarely observed in dogs[13] and cats (Fig. 1). They may vary markedly in size and are lined by a single layer of columnar or cuboidal or flat epithelium resting on a basement membrane and a layer of fibrous tissue (Fig. 2). They may be acquired or may be part of congenital cystic disease of the liver.

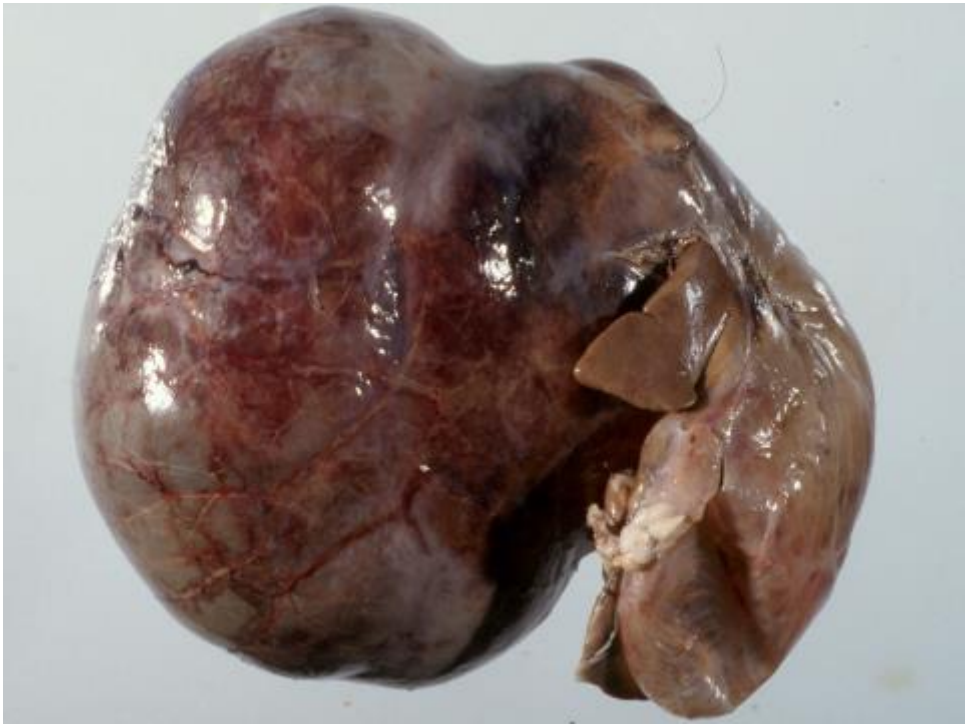

Fig. 1. Cat. Solitary cyst. Formalin fixed specimen.

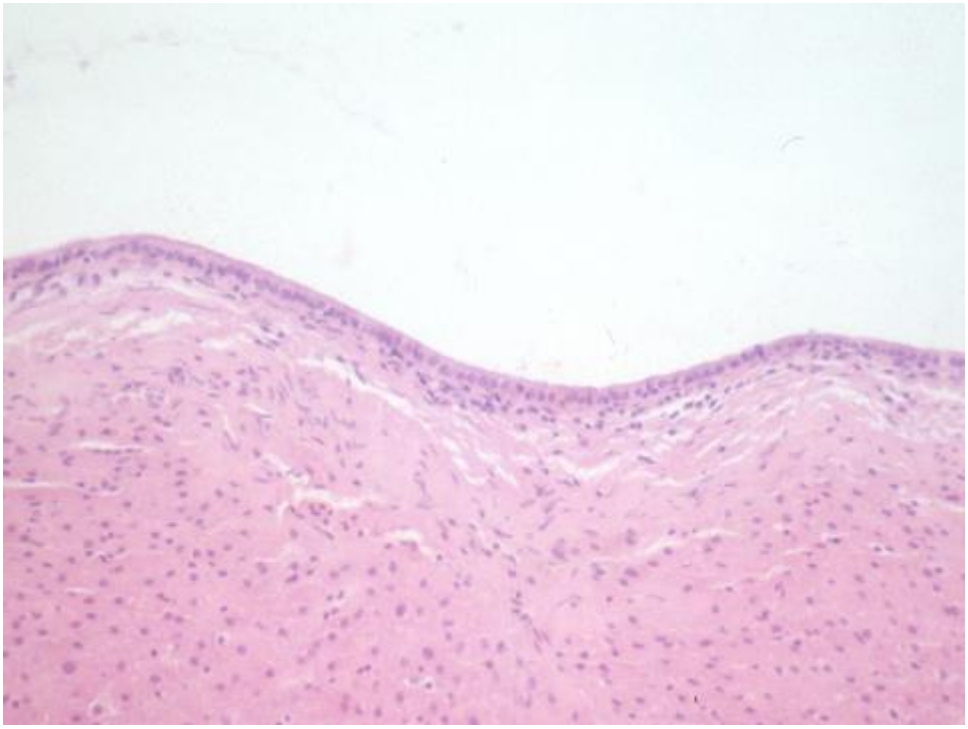

Fig. 2. Cat. Solitary cyst lined by a single layer of epithelium resting on a fibrous layer. HE.

2 Congenital cystic disease of the liver

Congenital cystic disease of the liver is a complex and difficult group of diseases, which has caused much confusion in veterinary literature with respect to nomenclature and classification of the various disease entities. The proposed nomenclature and classification of congenital cystic diseases in dogs and cats therefore is only understandable with knowledge of the embryological development of the biliary tree and the most recent human classification.

The various congenital cystic diseases of the liver, although quite different in appearance, are thought to represent anomalous development of the intrahepatic bile ducts, i.e. ductal plate anomalies, at different levels of the biliary tree.[6, 7] In early gestation the liver develops from the hepatic diverticulum of the foregut, which comprises two parts: a cranial or hepatic part and a caudal or cystic part. The hepatic part gives rise to primitive liver cells growing into the vascularized mesenchyme of the septum transversum and thus forming plates of liver cells alternating with hepatic sinusoids. The cystic part gives rise to the gallbladder and the common bile duct. In the initial stages the developing liver has no intrahepatic bile duct system ornd hepatic artery, but only primitive portal areas consisting of a portal vein surrounded by a myofibroblast-rich stroma.The intrahepatic bile ducts develop from a ring of primitive liver cells surrounding the primitive stroma around the portal veins, called ductal plate. By regional duplication with subsequent focal dilatation and tubular formation and finally incorporation in the stromal tissue of the primitive portal area they form individual bile ducts.[1, 7-9] The development of the hepatic artery, likely by vasogenesis from myofibroblasts in the primitive portal areas, precedes and is essential for the incorporation of the tubular part of the ductal plate in the primitive portal area.[10] As the formation of the intrahepatic bile ducts follows the outgrowth and development of the portal vein branches, the process results in a continuous development of bile ducts throughout fetal life from the liver hilus to the periphery.[1, 7-9,34-36]

Congenital cystic diseases of the liver are characterized by dilatation of segments of the intrahepatic bile ducts and variable degrees of fibrosis and are frequently associated with polycystic kidney disease. Although the hepatic lesions may show overlapping features, in man they can be classified according to their morphology and inheritance in three basic categories: 1) autosomal recessive polycystic kidney disease (ARPKD) or *childhood type of polycystic liver disease,* 2) autosomal dominant polycystic kidney disease (ADPKD) or *adult type of polycystic liver disease, and 3) Caroli disease* with an autosomal recessive inheritance.

In ***childhood type polycystic liver disease*** the liver involvement is primarily microscopic and the changes range from persistent ductal plates or a striking increase of abnormally structured sometimes dilated biliary channels which arise in portal areas and extent irregularly into the parenchyma, are lined by a single layer of low columnar or cuboidal cells and show limited supporting stroma *(infantile type)* to moderate or marked portal fibrosis with porto-portal bridging and presence of often abnormally structured or slightly dilated bile ducts *(juvenile type/ congenital hepatic fibrosis);* as the development of the biliary sytem mirrors the development of the portal veins, portal vein hypoplasia and concomitant arteriolar proliferation is a common histological finding in congenital hepatic fibrosis. In ***adult type polycystic liver disease*** the liver is grossly characterized by multiple unilocular or multilocular, non-communicating cysts ranging from less than one millimetre to more than 12 cm in diameter, which contain a clear colorless fluid and microscopically are lined by columnar or cuboidal epithelium; *Von Meyenburg complexes* i.e. discrete fibrotic areas with round or irregularly formed bile ducts are considered as part of the spectrum of ADPKD. In

Caroli disease the lesion is characterized by macroscopically recognisable moniliform or saccular dilatations of the larger bile ducts, i.e. the hepatic ducts and the segmental ducts. Microscopically the cystic ducts are lined by cuboidal to tall columnar epithelium and the lumen may contain inspissated mucin, bile and/or calcareous material.

The dilatation of the larger bile ducts as seen in Caroli disease, is thought to represent an early defect in the formation of the bile ducts. The childhood type of polycystic disease including congenital hepatic fibrosis with abnormally structured cystic bile ducts then represents a ductal plate anomaly at an intermediate level. The unilocular and multilocular hepatic cysts of the adult type polycystic disease and the Von Meyenburg complexes then represent a ductal plate anomaly at a late peripheral stage, whereby the large cysts may result from progressive dilatation of the Von Meyenburg complexes [6, 9,34,36].

In ADPKD the kidneys are characterized by multiple rounded tubular cysts of varying size (millimetres to several centimetres), which may form in any segment of the nephron but involve only a small percentage of the nephron population. In ARPKD and Caroli disease identical renal lesions, also known as polycystic kidney type I, are seen characterized by diffuse involvement of the kidneys with dilatation of collecting ducts, thus producing a radial cystic pattern from papilla to cortical surface. [6, 11, 12]

Although often not clearly defined with regard to inheritance similar morphological entities can be discriminated in cats and dogs.

In **cats** until now only congenital hepatic fibrosis and adult type polycystic disease of the liver have been reported with a predisposition for Persian cats and Persian crossbreds[15,37]. In **congenital hepatic fibrosis** grossly the liver is enlarged and firm (Fig. 3). The disease is histologically characterized by moderate to marked porto-portal bridging fibrosis with irregularly formed often slightly dilated bile ducts which may extend in the surrounding parenchyma, as well as hypoplasia of portal veins and concomitant arteriolar proliferation (Fig. 4). Sometimes congenital hepatic fibrosis is associated with macroscopically recognisable cysts[15] which may contain mucinous material or inspissated bile. **Adult type polycystic disease of the liver** (Figs. 5, 6) is characterized by multiple, non-communicating, unilocular or multilocular cysts containing a clear watery fluid and lined by a single layer of epithelium. Both congenital hepatic fibrosis as well as adult type polycystic liver disease are usually accompanied by polycystic kidney disease [15] characterized by multiple rounded, unilocular or multilocular cysts (Fig. 7). More than one mutation of the feline PKD1 gene may be involved depending on the phenotype of the liver lesions[37-40].

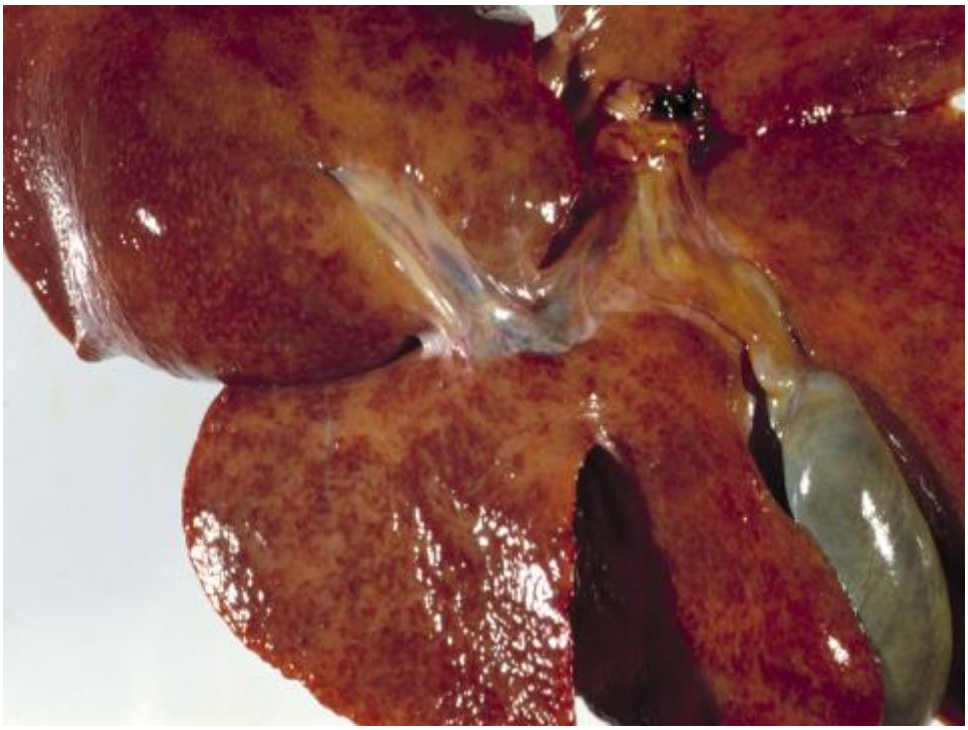

Fig. 3. Cat. Congenital hepatic fibrosis. Enlarged and firm liver with a pale ramifying pattern.

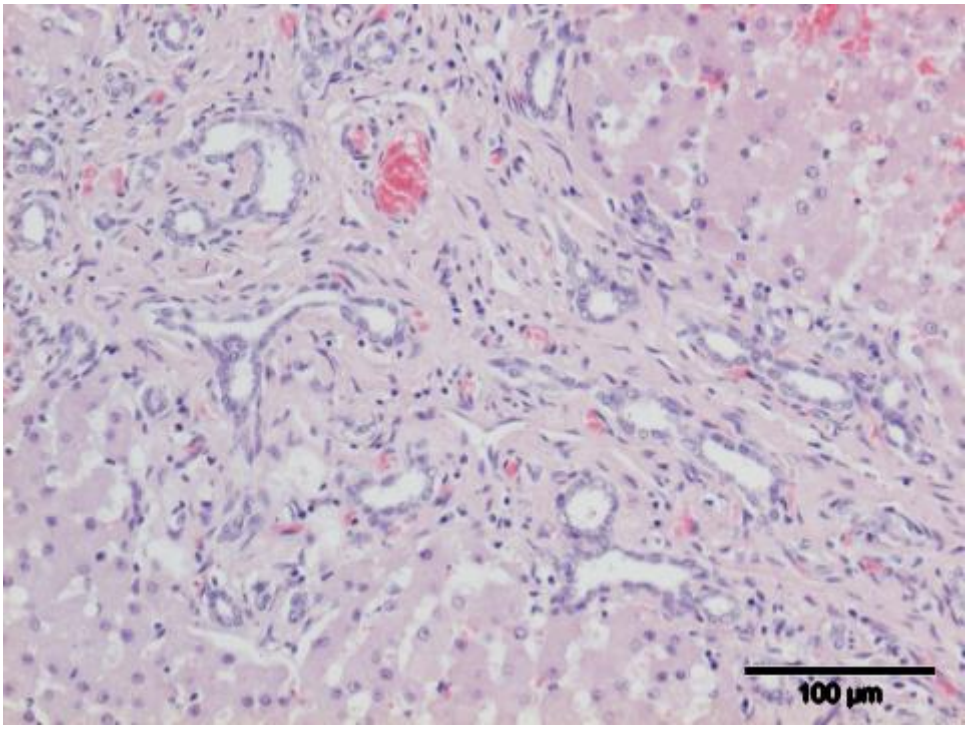

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Fig. 4. Cat. Congenital hepatic fibrosis. Fibrotic portal area with irregularly formed slightly dilated bile ducts, hypoplasia of the portal vein and arteriolar proliferation. HE.

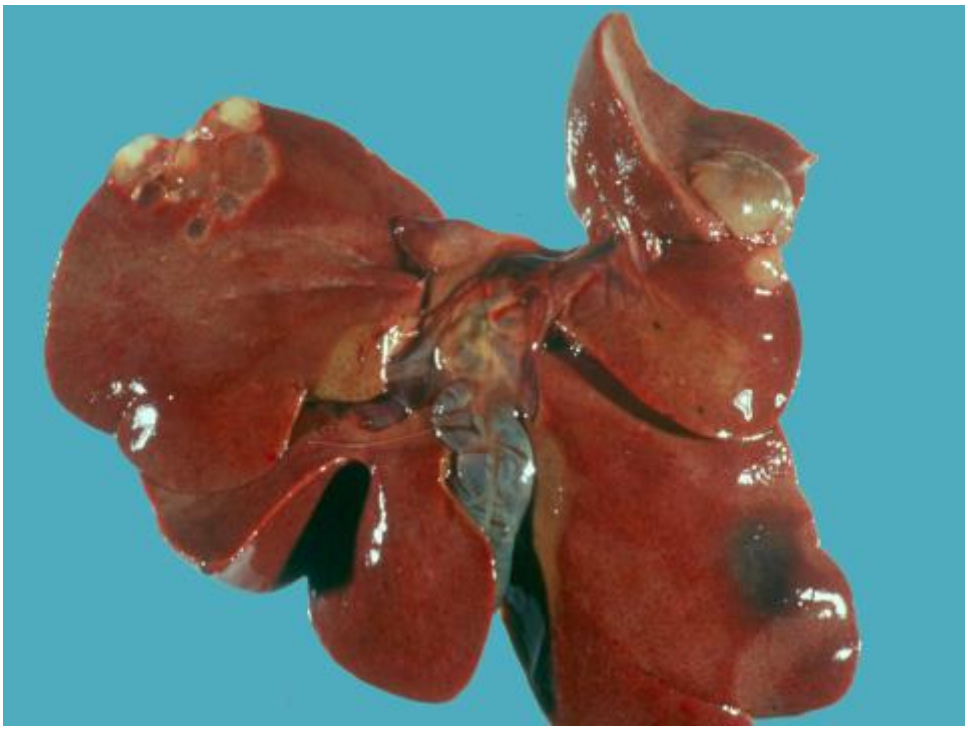

Fig. 5. Cat (Persian). Adult type polycystic liver disease. Multipe rounded cysts in the liver. (Bosje et al[15])

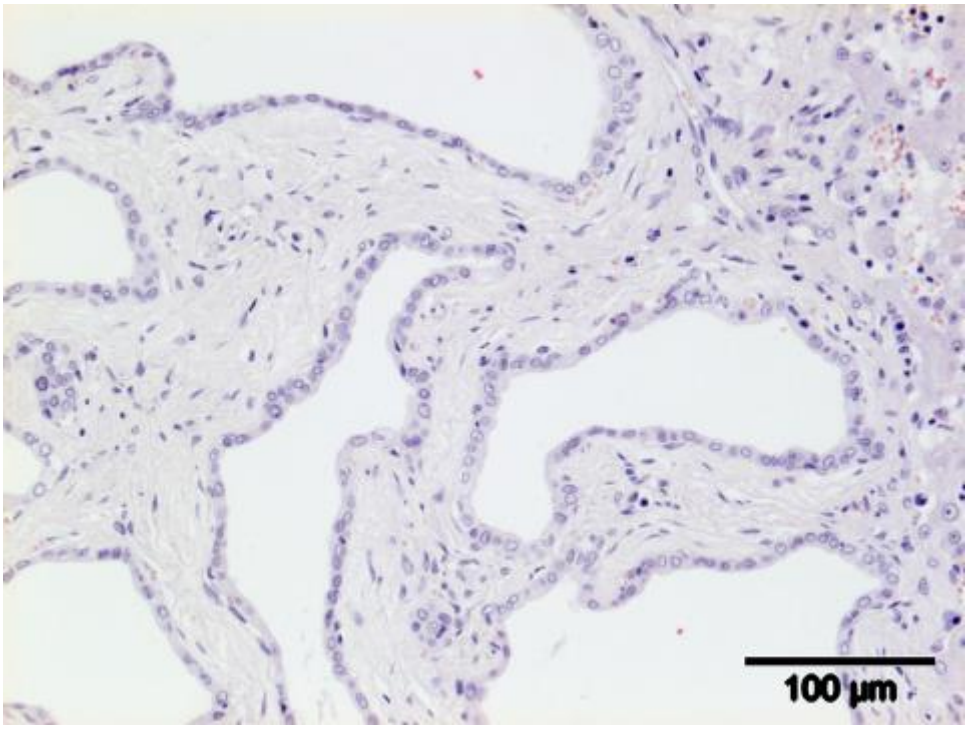

Fig. 6. Cat. Adult type polycystic liver disease. Fibrous tissue with large irregularly formed cystic bile ducts lined with a single layer of epithelium. HE.

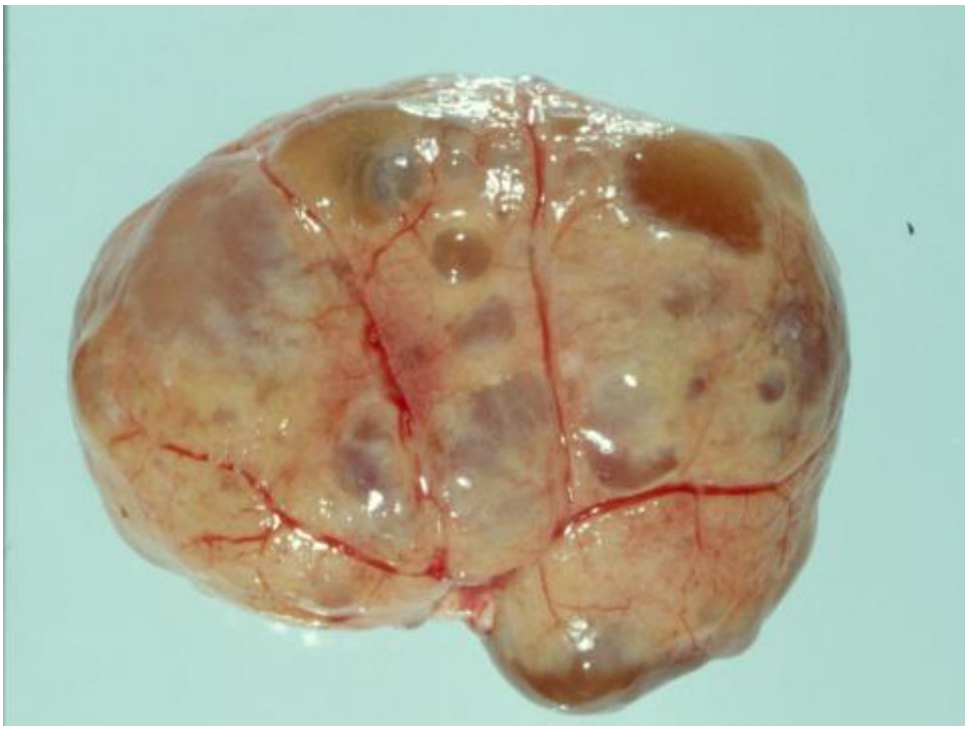

Fig . 7. Cat. Adult type polycystic kidney disease. Multiple rounded cysts of varying size throughout the kidney.

In **dogs** various forms of congenital cystic disease of the liver have been described: **1) congenital hepatic fibrosis** [41,42], microscopically characterized by moderate to marked portoportal bridging fibrosis with irregularly formed often slightly dilated bile ducts,which may extend in the surrounding parenchyma, and hypoplastic portal veins and arteriolar proliferation (Fig. 8); the disease may be associated with macroscopically recognisable cysts which may contain mucinous material or inspissated bile[42] , **2) adult type polycystic liver disease** with multiple non-communicating, rounded unilocular and multilocular cysts containing a clear watery fluid (Fig. 9) and microscopically lined by a single layer of epithelium; sometimes, also Von Meyenburg complexes are present [13] (Fig. 10), and **3) congenital dilatation of hepatic and large intrahepatic bile ducts**, morphologically identical to Caroli disease (Fig. 11, 12), and microscopically characterized by dilated, irregularly formed large bile ducts lined with columnar epithelium (Fig . 13). The lumen may contain inspissated mucin, bile and/or calcareous material (Fig. 14) [13,14,43-45].

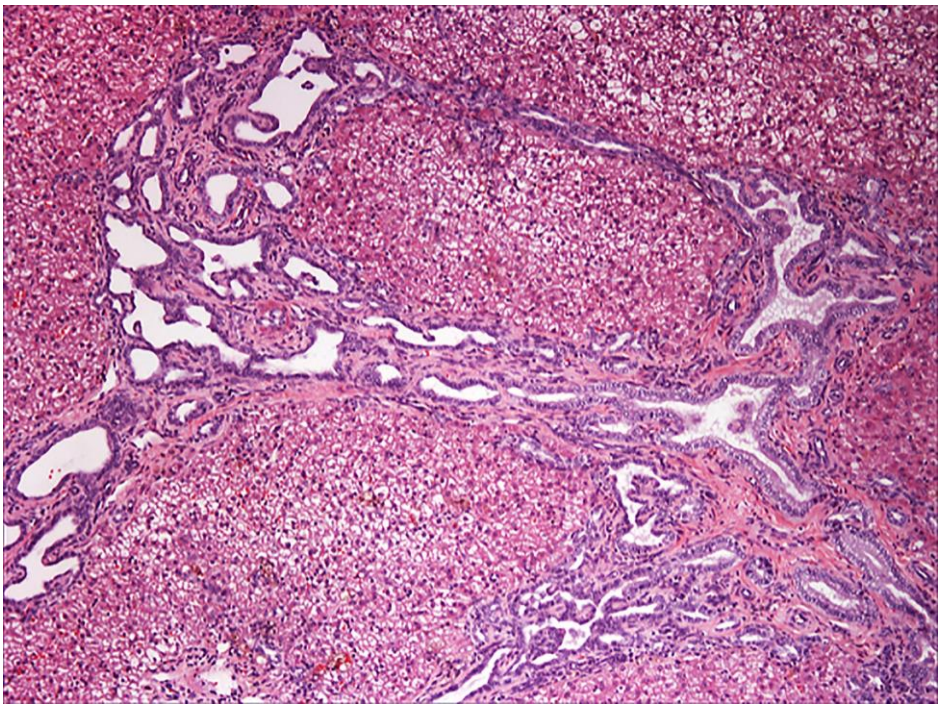

Fig. 8. Dog. Congenital hepatic fibrosis with portoportal bridging fibrosis and irregularly formed, often dilated bile ducts. (Brown et al[41])

Fig. 9. Dog. Adult type polycystic liver and kidney disease with multiple rounded cysts in the right lateral liver lobe and in the kidneys (Van den Ingh and Rothuizen[13])

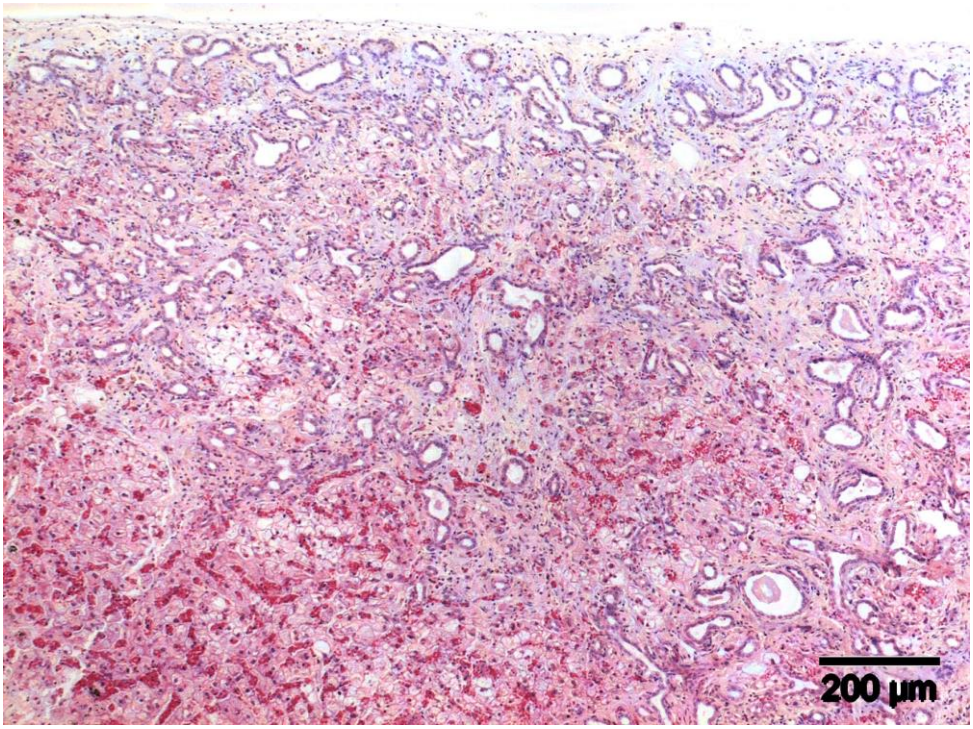

Fig. 10. Dog. Von Meyenburg complex characterized by focal fibrosis and microscopically small, irregularly formed dilated bile ducts. HE.

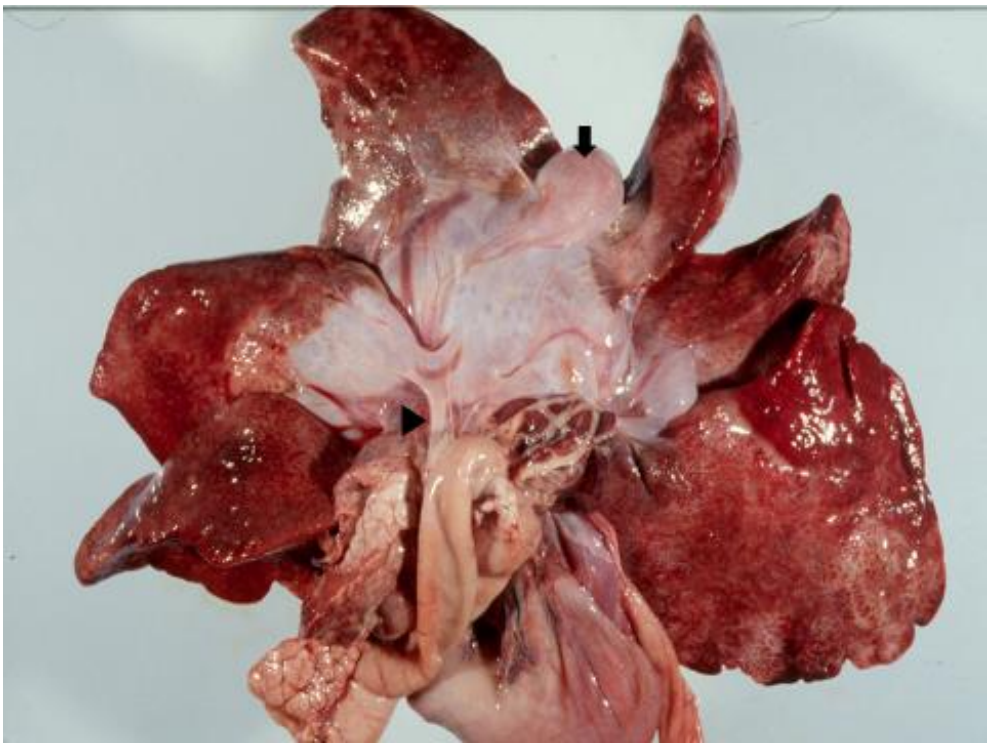

Fig. 11. Dog. Congenital dilatation of the hepatic and the large intrahepatic bile ducts. The gallbladder (arrow) and the common bile duct (arrowhead) are normal in size. (Görlinger et al [14]).

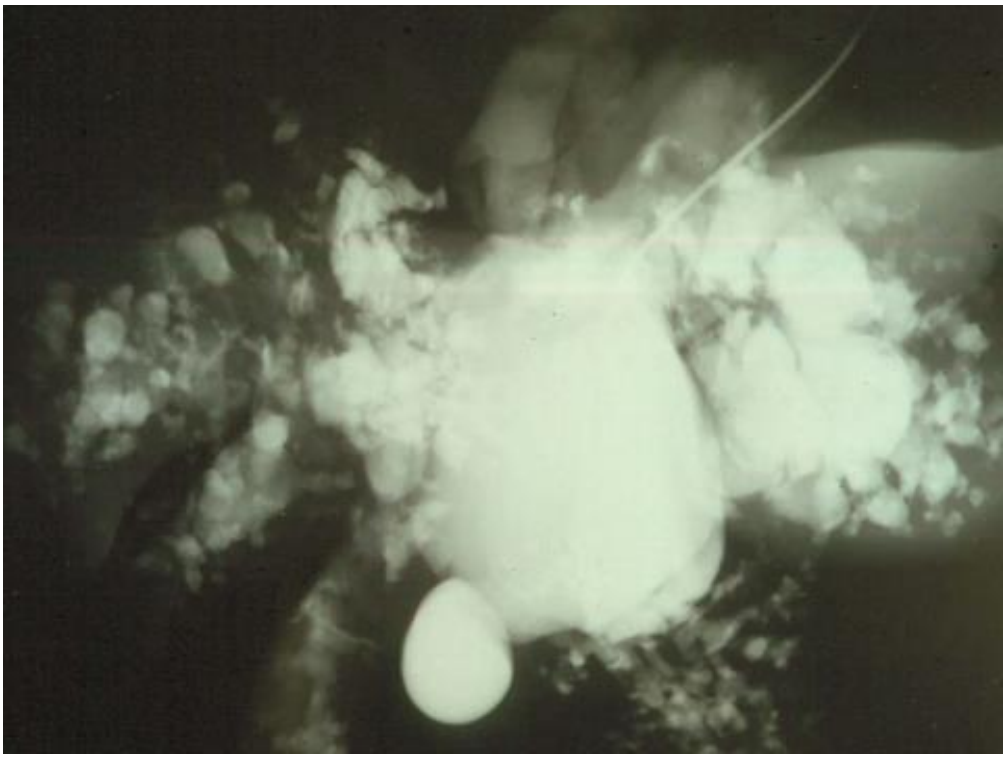

Fig. 12. Dog. Congenital dilatation of the hepatic and the large intrahepatic bile ducts. The gallbladder is normal in size. Postmortem retrograde cholangiography.

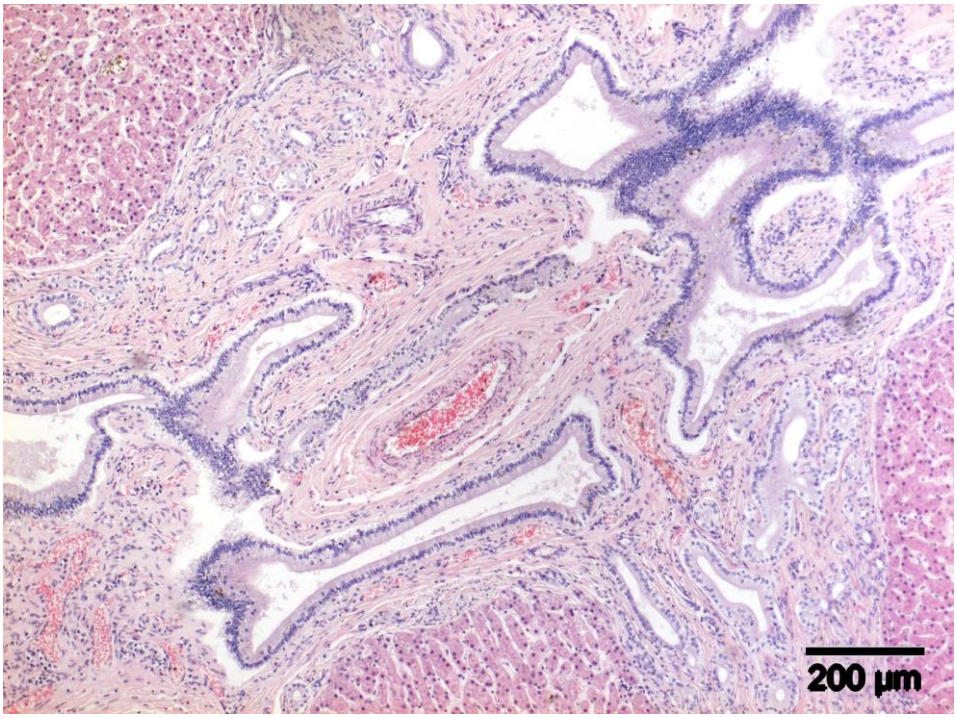

Fig. 13. Dog. Congenital dilatation of the hepatic and the large intrahepatic bile ducts. Fibrotic portal area with dilated irregularly formed bile ducts lined with columnar epithelium. HE.

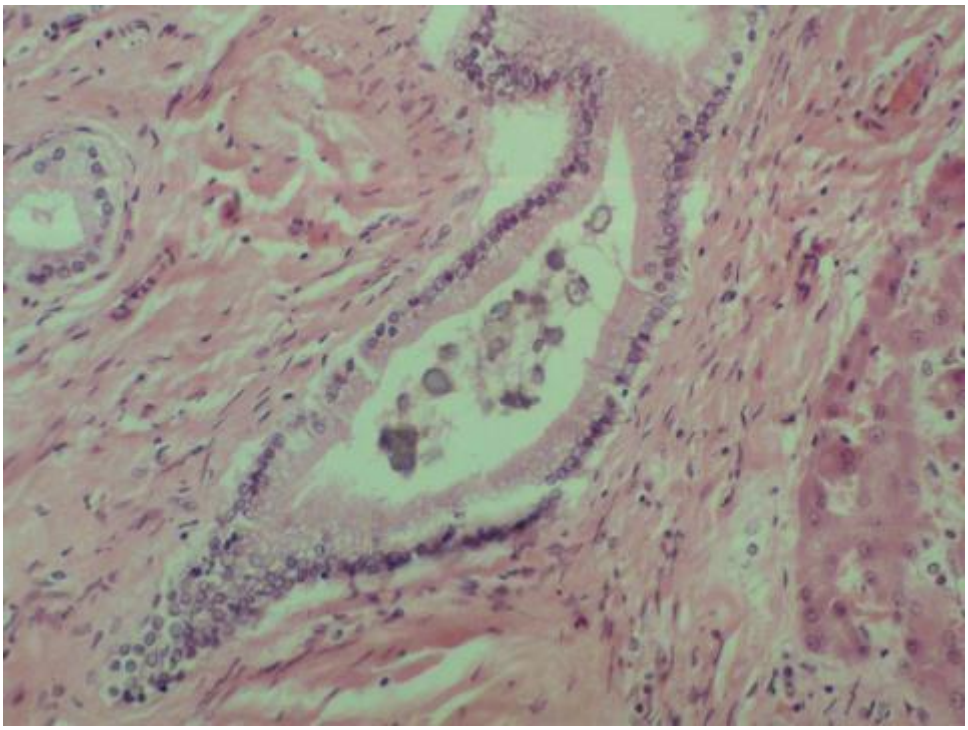

Fig. 14. Dog. Congenital dilatation of the hepatic and the large intrahepatic bile ducts. Large intrahepatic bile duct with columnar epithelium and in the lumen calcareous material. HE.

Adult type polycystic liver disease in the dog was always accompanied by renal polycystic disease with multiple rounded, unilocular or multilocular cysts [13] Congenital dilatation of the hepatic and large intrahepatic bile ducts was always associated with polycystic kidney disease type I, characterized by diffuse involvement of the kidneys with dilatation of collecting ducts, thus producing a radial cystic pattern from papilla to cortical surface. (Fig. 15) [13,14,44,45,46]. In congenital hepatic fibrosis there was no evidence of polycystic kidney disease in the reported cases.

A predisposition for the Boxer dog was seen for congenital hepatic fibrosis[42] and was regularly (27%) associated with gallbladder agenesis and other hepatic abnormalities (vide infra).Congenital dilatation of the hepatic and large intrahepatic bile ducts associated with polycystic kidney disease type I was diagnosed in two litters from 2 matings between the same pair of West Highland White terriers affecting 4/6 respectively 3/5 pups as well as in 3 pups from 2 litters of Cairn terriers from the same sire and in 2 Golden retriever littermates [43-43-45] suggesting an autosomal recessive mode of inheritance.

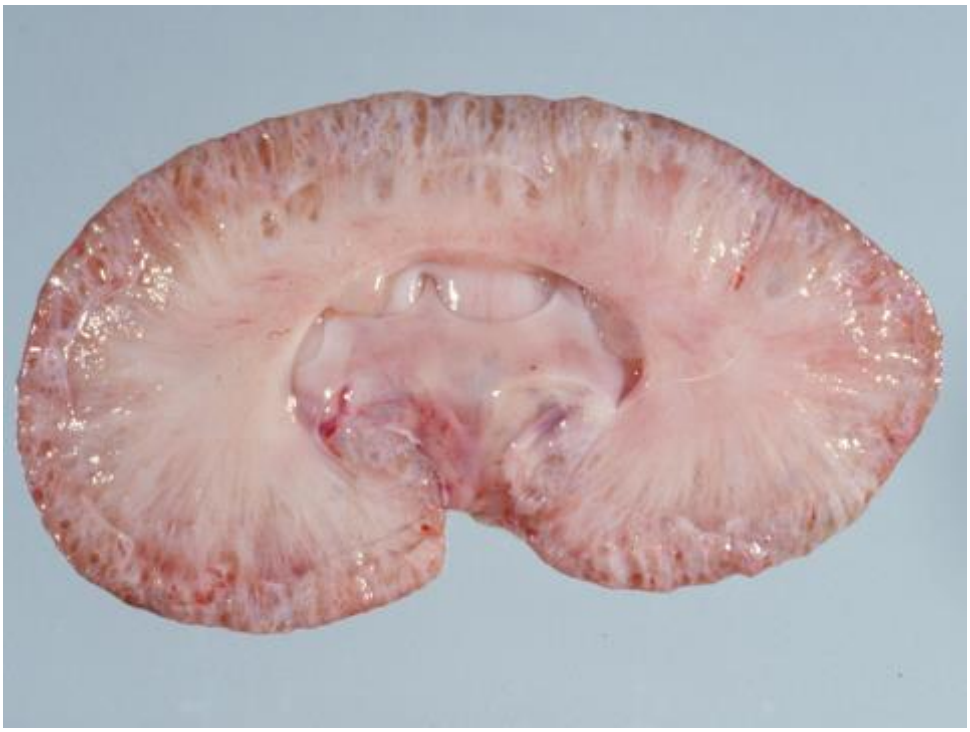

Fig. 15. Dog. Cut surface of the kidney of same dog as fig.11. Fusiform radially arranged cysts and fibrosis are present throughout the cortex with marked fibrosis of the medulla. (Görlinger et al [14]).

3 Biliary atresia

Biliary atresia is a very rare congenital anomaly in domestic animals and has been described only once in a dog.[16] The lesion was characterized by atresia of the common bile duct at the transition between the common bile duct and the hepatic ducts (Fig. 16) suggesting non-fusion of the cranial or hepatic and the caudal or cystic anlage of the bile ducts. The animal was jaundiced since birth. At post mortem examination there was complete absence of ductal structures at the site of the atresia. The liver showed histologically severe porto-portal bridging fibrosis with extensive biliary proliferation reminiscent of congenital hepatic fibrosis. This might indicate a combined anomalous development of extrahepatic and intrahepatic bile ducts in this dog, which corresponds with the occurrence of a ductal plate anomaly in 20-40 % of human cases with extrahepatic biliary atresia.[17, 18, 34]

CHOLESTASIS AND CHOLATE-STASIS

1 Cholestasis (bilirubinostasis) is defined as impaired bile flow accompanied by the accumulation in the blood of components normally secreted in the bile (among others bile acids, conjugated bilirubin, cholesterol). [4] Morphologically cholestasis (bilirubinostasis) is characterized by the presence of bile in the hepatic parenchyma and can be recognized as bile plugs in the canaliculi, phagocytosed bile (plugs) in Kupffer cells / macrophages and rarely as bile granules in the cytoplasm of hepatocytes (Fig. 17, 18).[4] Cholestasis is easily recognized in cytological smears and frozen sections, but due to the paraffin embedding procedure often markedly less in paraffin sections of the same animals, particularly in cats.

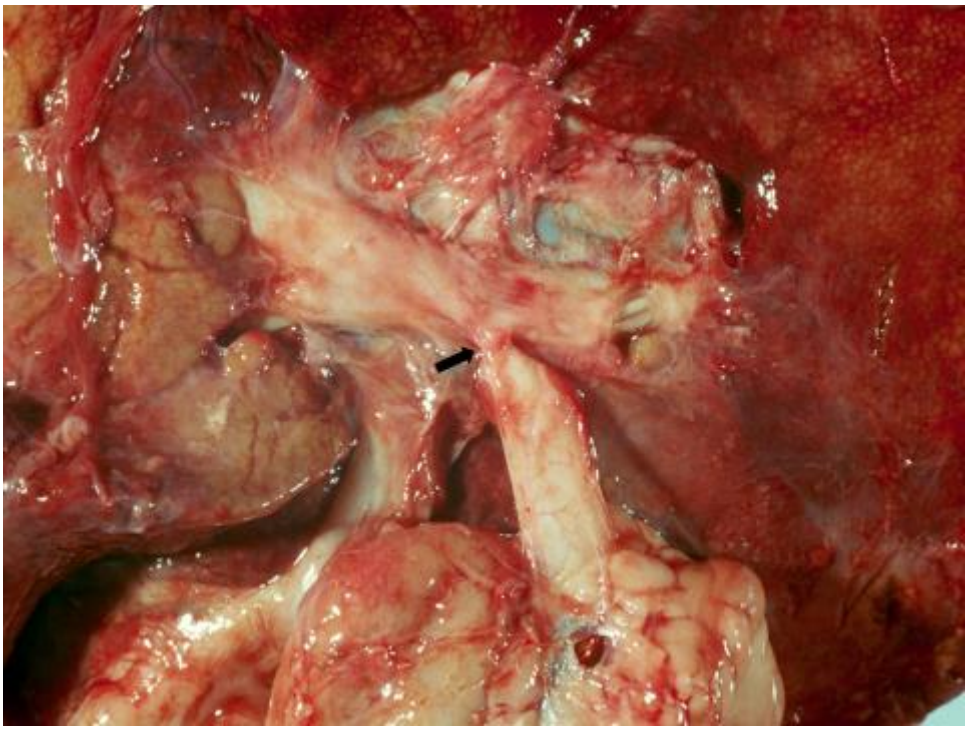

Fig. 16. Dog. Extrahepatic biliary atresia. Atresia of the common bile duct (arrow) near the hilus of the liver. (Schulze et al [16]).

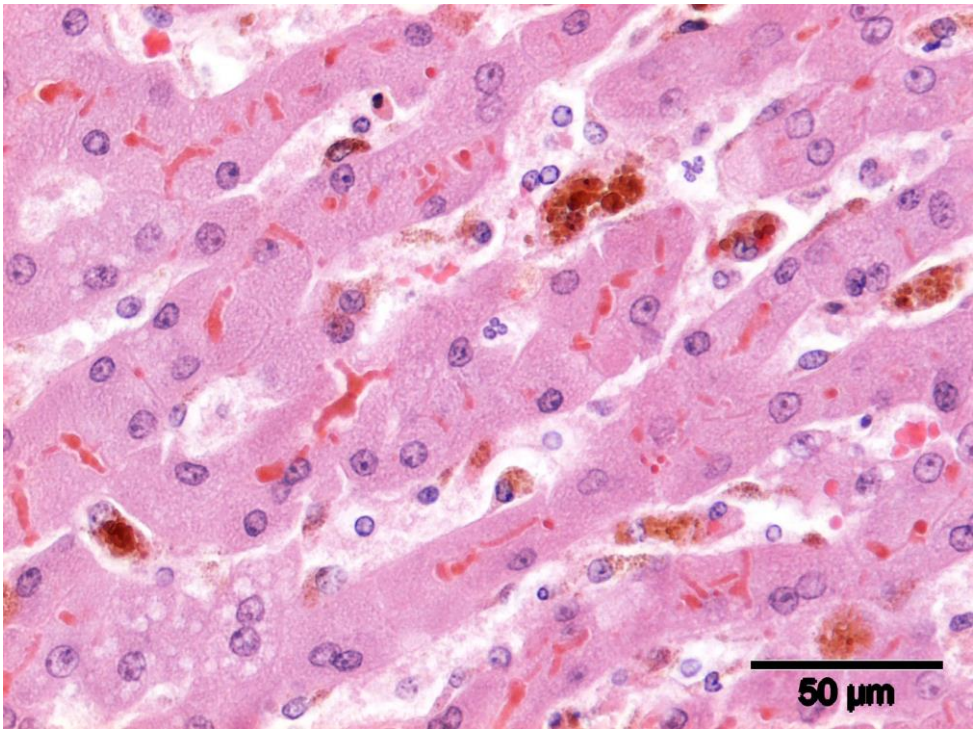

Fig. 17. Dog. Cholestasis associated with haemolytic anaemia. Bile plugs in the canaliculi and haemosiderin in Kupffer cells. HE.

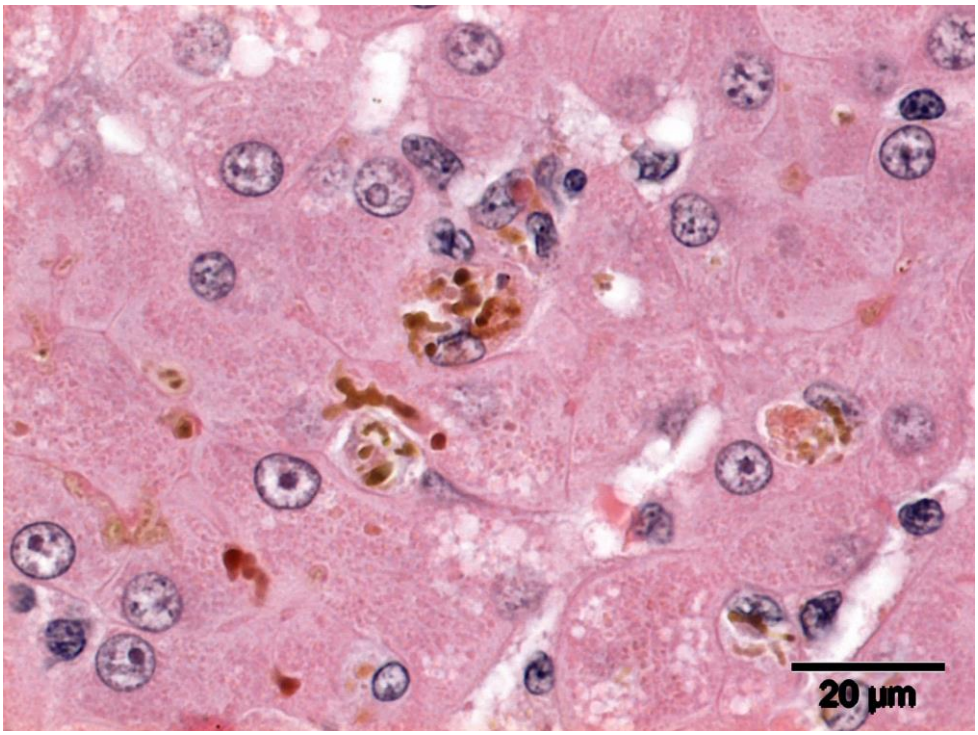

Fig. 18. Dog. Cholestasis. Bile plugs in the canaliculi and phagocytosed bile plugs in the Kupffer cells. HE.

2 Intrahepatic cholestasis

Intrahepatic cholestasis is associated with a wide spectrum of liver diseases. In general, microscopic lesions apart from the cholestasis are related to the primary hepatic disease, but cholestasis may be the only histological abnormality.[19]

3 Extrahepatic cholestasis

Extrahepatic cholestasis can be associated with intraluminal obstruction (gall stones, mucinous cystic hyperplasia) or luminal constriction (neoplasia or inflammatory processes} of the extrahepatic biliary tract, and occasionally a large intrahepatic duct, and results in stasis of bile and dilatation of the bile ducts proximal to the obstruction (Fig. 19).

The characteristic microscopic lesions are related to the leakage of bile from the bile ducts into the connective tissue of the portal areas. In the **acute stage** this results in enlarged oedematous portal tracts with a neutrophilic portal infiltrate and often, a degenerative and proliferative reaction of the bile duct epithelium (Fig. 20). Whereas cholestasis is a constant feature in acute extrahepatic cholestasis, bile infarcts, i.e. foci of hepatocellular necrosis due to insudation of extracellular bile (Fig. 21), although seen in other species, are mostly absent in dogs and cats. The **chronic stage** of extrahepatic cholestasis is characterized by enlarged portal areas with fibrosis, bile duct proliferation, periductal concentric fibrosis and an inflammatory infiltrate with pigment-laden macrophages, lymphocytes, plasma cells and neutrophils (Fig. 22, 23). Histologic evidence of cholestasis may be present or absent. In longstanding cases porto-portal bridging fibrosis and eventually biliary fibrosis may develop.[19]

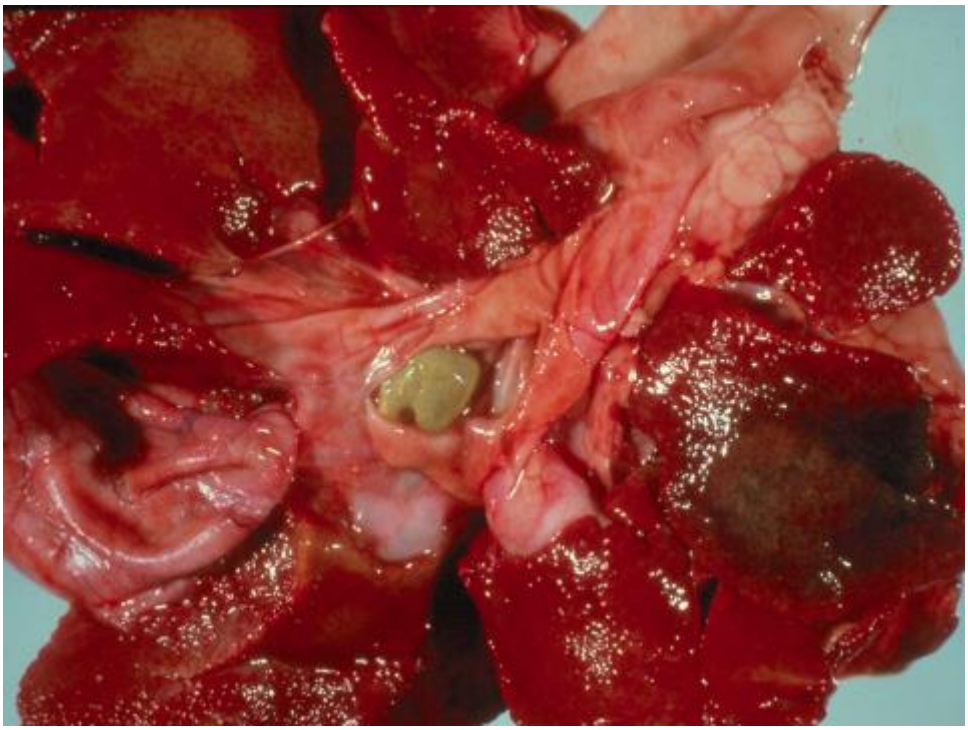

Fig. 19. Dog. Extrahepatic cholestasis due to a gall stone with dilatation of the extrahepatic bile ducts.

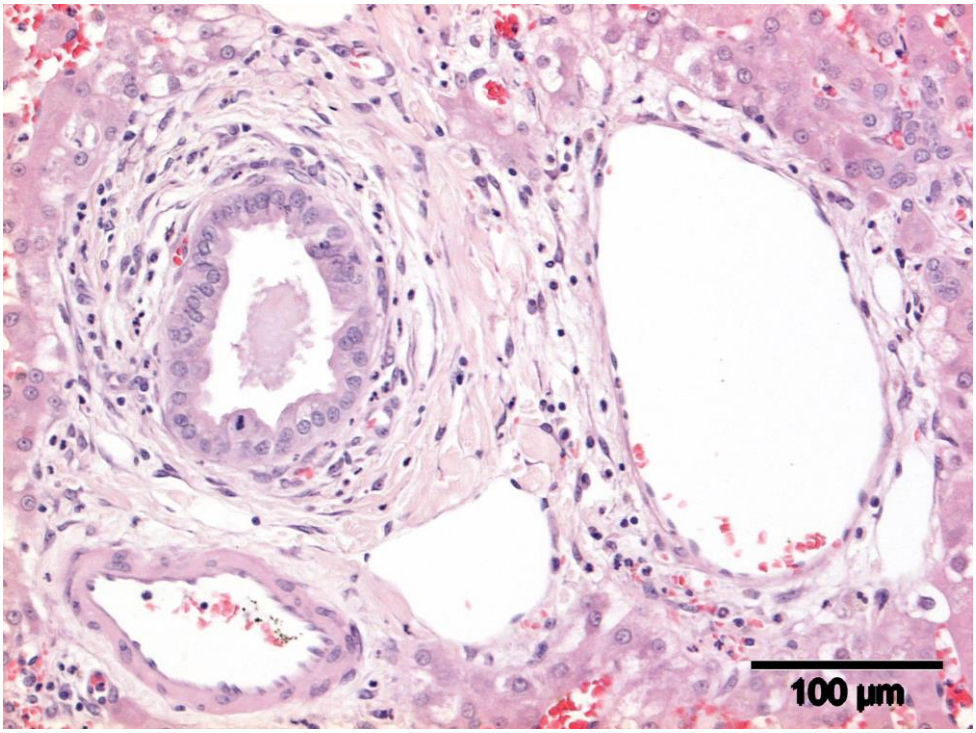

Fig. 20. Dog. Acute extrahepatic cholestasis. Enlarged oedematous portal area with a neutrophil portal infiltrate and slight proliferative reaction of the biliary epithelium. HE.

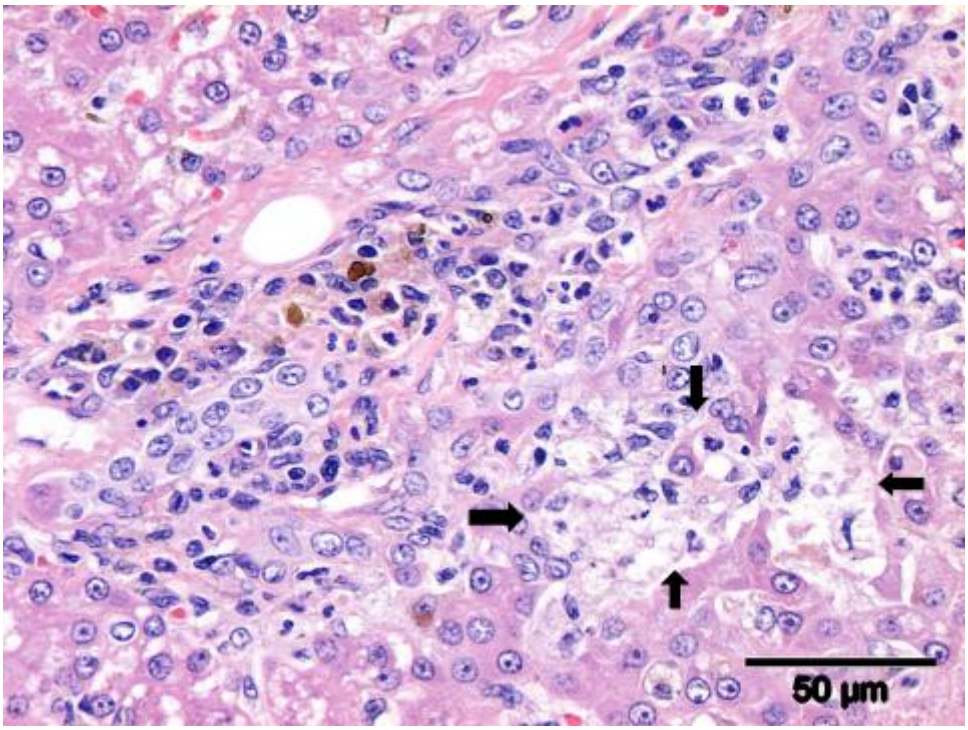

Fig. 21. Dog. Subacute extrahepatic cholestasis with a mixed portal inflammatory infiltrate and slight biliary proliferation. A bile infarct is present in the periportal parenchyma (arrowheads). HE.

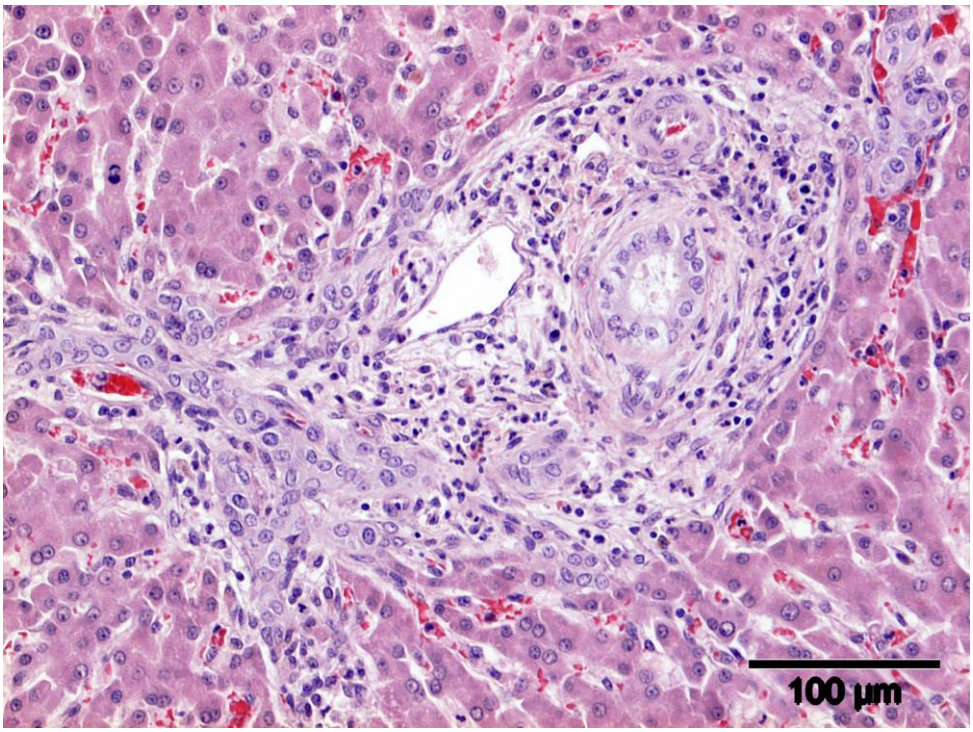

Fig. 22. Dog. Chronic extrahepatic cholestasis with fibrosis, biliary proliferation and a mixed portal inflammatory infiltrate. HE.

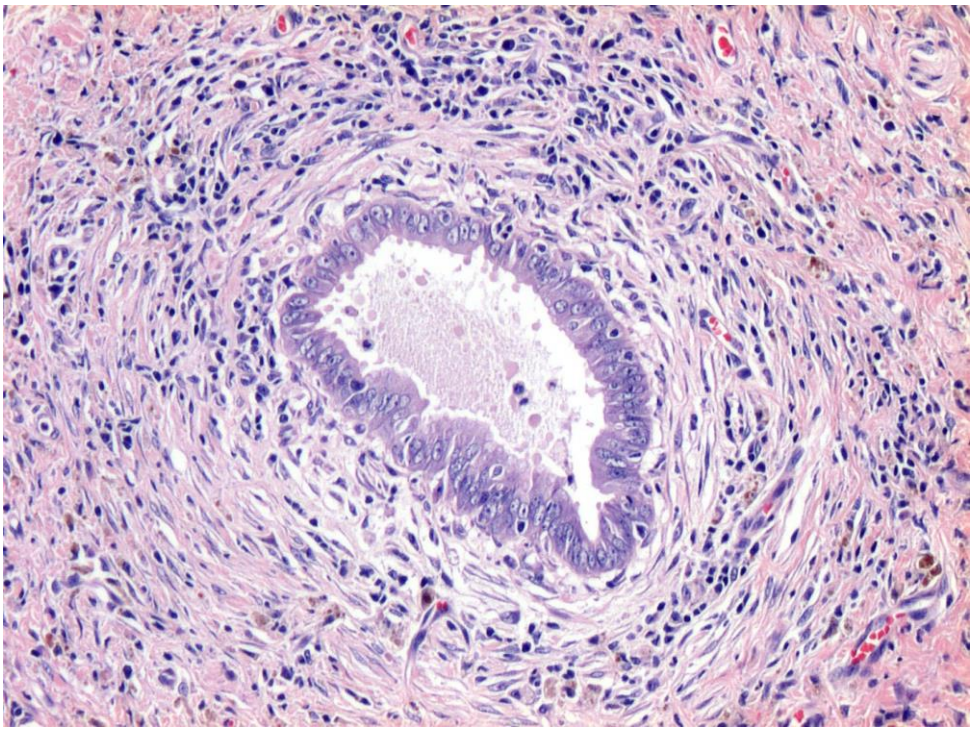

Fig. 23. Dog. Chronic extrahepatic cholestasis with periductal concentric fibrosis and a mixed inflammatory infiltrate. HE.

4 Cholate-stasis

Cholate-stasis is a morphological entity well recognized in man and horses with chronic cholestatic liver diseases. The lesion is thought to be caused by the retention of bile acids in the hepatocytes, preferentially seen in the periportal region and often associated with ductular proliferation. The lesion is characterized by rounded swollen and pale hepatocytes with a distinct cell border and centrally located, often copper containing granules (Fig . 24, 25).[4] It is a rare finding in dogs with chronic liver disease and is particularly seen in lobular dissecting hepatitis.

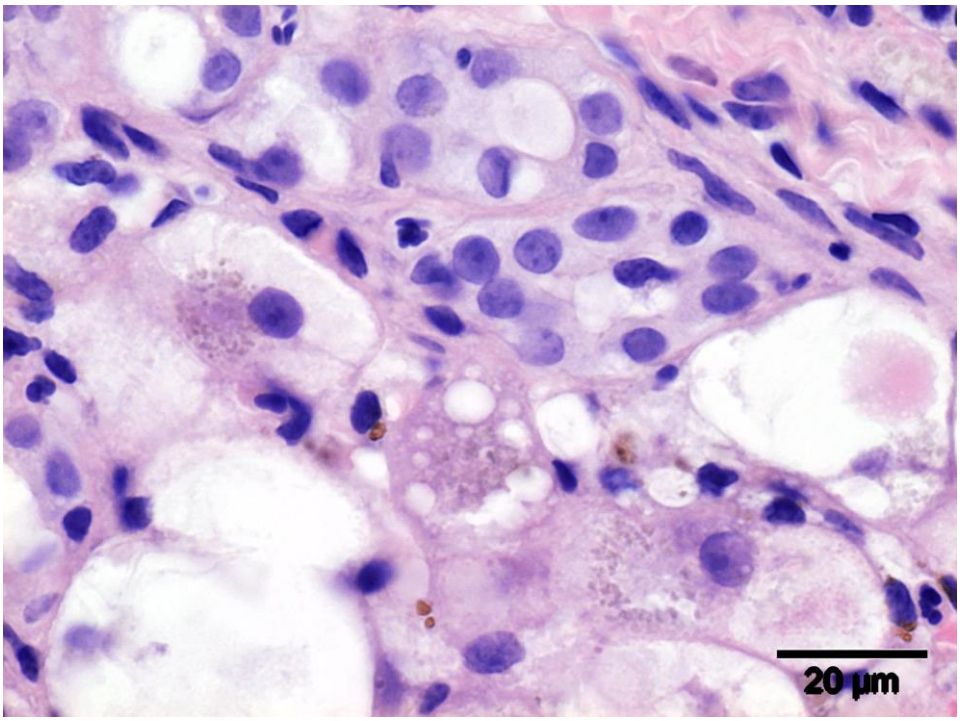

Fig. 24. Dog. Cholate-stasis. Swollen, pale hepatocytes with centrally located copper containing granules. HE.

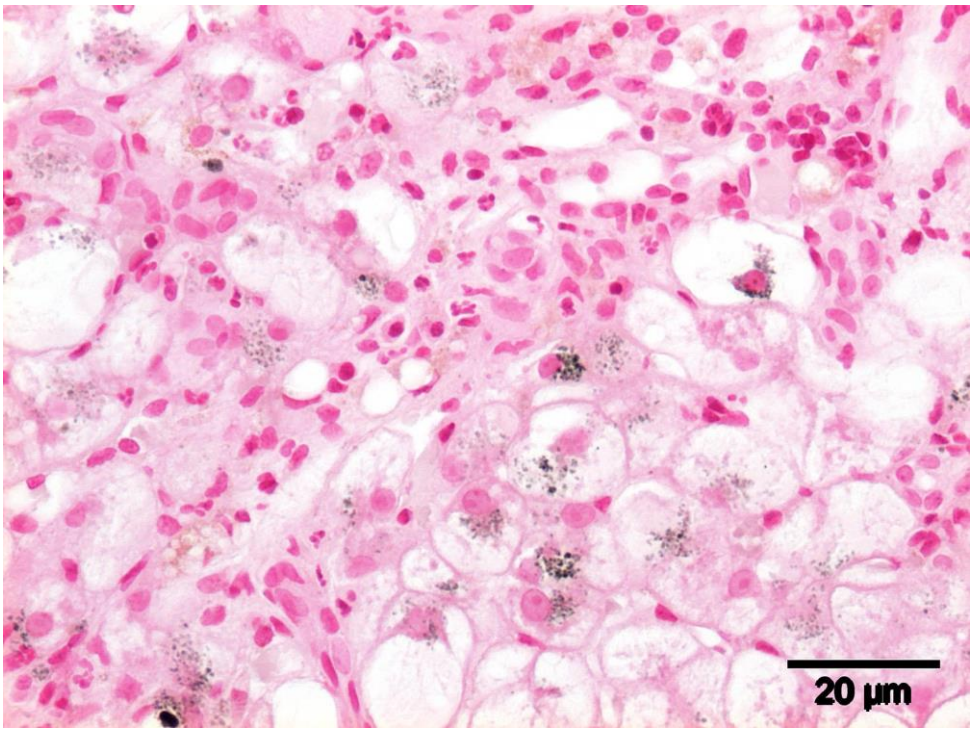

Fig . 25. Dog. Cholate-stasis. Swollen, pale hepatocytes with centrally located copper containing granules. Rubeanic acid stain for copper.

CHOLANGITIS

Cholangitis can be differentiated as follows:

1. Neutrophilic cholangitis
2. Lymphocytic cholangitis
3. Destructive cholangitis
4. Chronic cholangitis associated with liver fluke infestation

1 Neutrophilic Cholangitis

Neutrophilic cholangitis (syn: suppurative or exudative cholangitis / cholangiohepatitis) is the most common type of cholangitis, which is frequently seen in cats and rarely in dogs and believed to result from ascending bacterial infection from the intestine particularly by Escherichia coli, Enterococcus spp and Clostridium spp.[20-22,46,47,54] The lesion is histologically characterized by the presence of neutrophils in the lumen and,or epithelium of the bile ducts. In the acute stage the lesion is often associated with the presence of oedema and neutrophils in the portal areas (Fig. 26, 27, 28). The neutrophilic inflammation may extend into the hepatic parenchyma (cholangiohepatitis) and incidentally even result in hepatic abscesses. In the chronic stage the lesion is often associated with the presence of a mixed inflammatory infiltrate in the portal areas consisting of neutrophils, lymphocytes and plasma cells and possibly fibrosis and bile ductular proliferation.

The lesions occur in varying intensity and may affect the liver diffusely as in severe disease or show an irregular distribution with only limited numbers of portal tracts affected. Not all cases show the above typical changes; there may only be non-specific reactive hepatitis (Chapter 7).

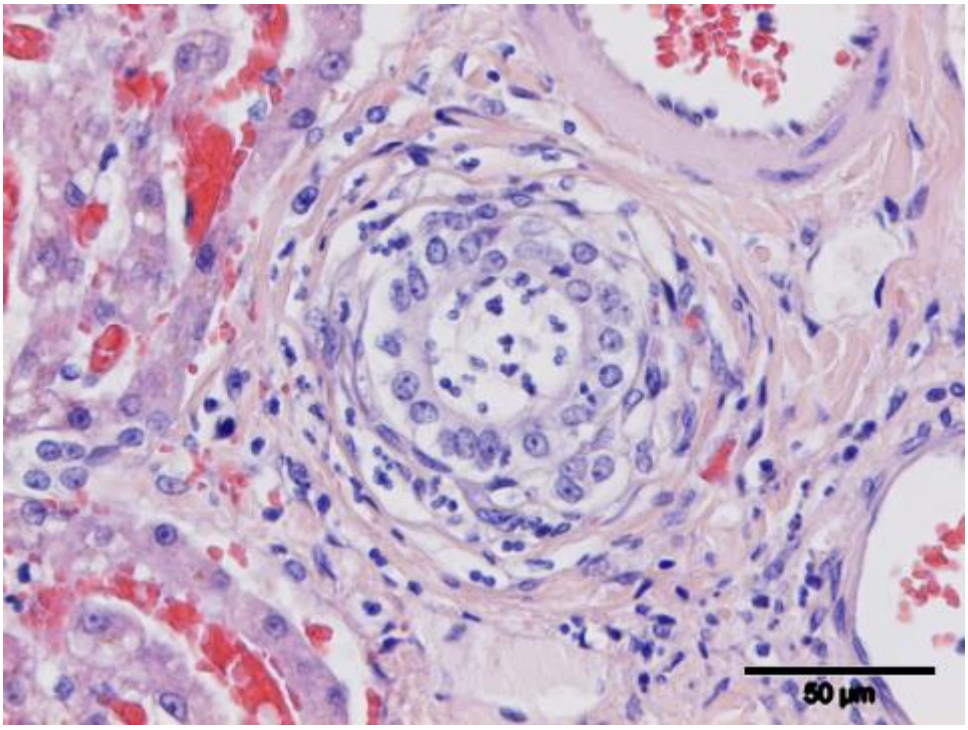

Fig . 26. Cat. Neutrophilic cholangitis. Neutrophils in the lumen and epithelium of the bile duct and extension of neutrophils in the portal stromal tissue. HE.

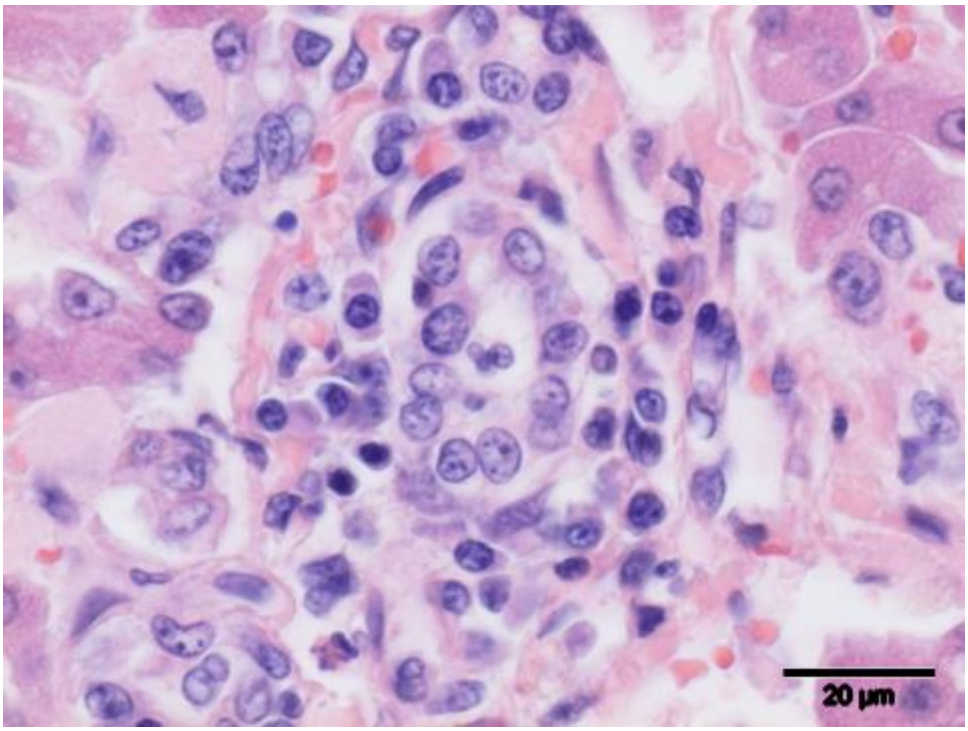

Fig. 27. Cat. Neutrophilic cholangitis with a neutrophil in the lumen of the bile duct and some lymphocytes and plasma cells in the portal stromal tissue. HE.

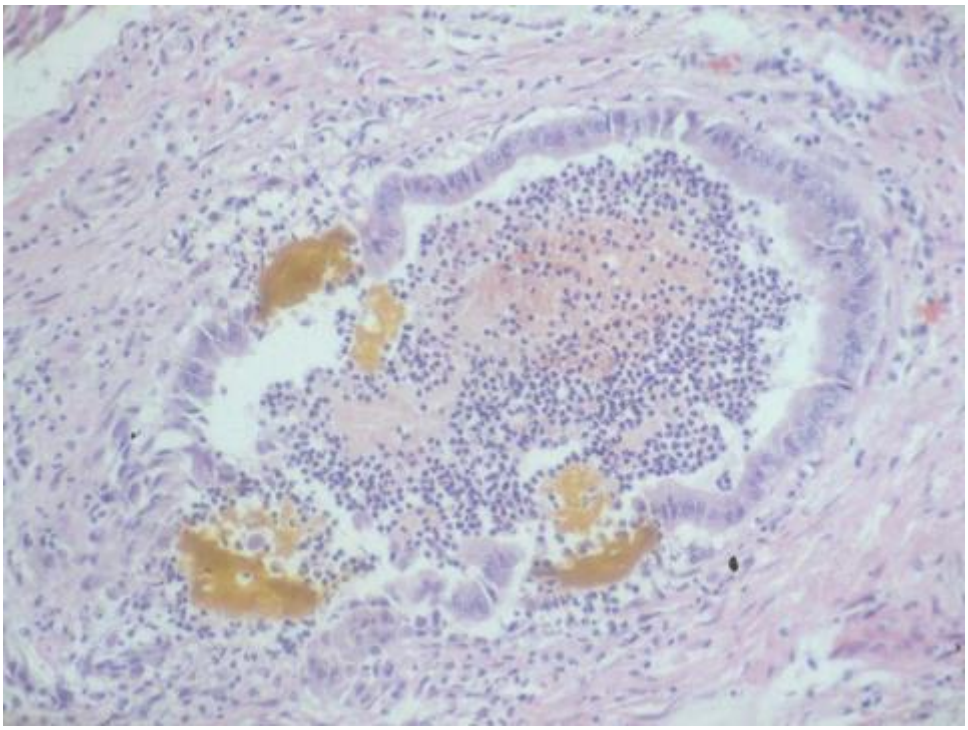

Fig. 28. Dog. Neutrophilic cholangitis with local ulceration of the the epithelium and extension of the inflammation and exsudation of bile in the surrounding fibrous tissue. HE.

2 Lymphocytic Cholangitis

Lymphocytic cholangitis (syn: lymphocytic cholangiohepatitis, lymphocytic portal hepatitis, non-suppurative cholangitis) [20-23] is a rather common disease in cats with an unknown aetiology and pathogenesis. This is usually a very slowly progressive and extremely chronic disease. The disease is characterized by a consistent infiltration of small lymphocytes in and restricted to the portal areas, often associated with variable portal fibrosis and biliary proliferation (Fig. 29,.30). Lymphocytes centering around the bile ducts or present in the biliary epithelium and sometimes destruction of the biliary epithelium may be seen but are not a specific hallmark of the disease. Apart from lymphocytes some plasma cells and even follicular structures as well as eosinophils and lipogranulomas may be present[48]. Well differentiated lymphocytic lymphoma may be difficult to discriminate from lymphocytic cholangitis and ancillary testing may be required.

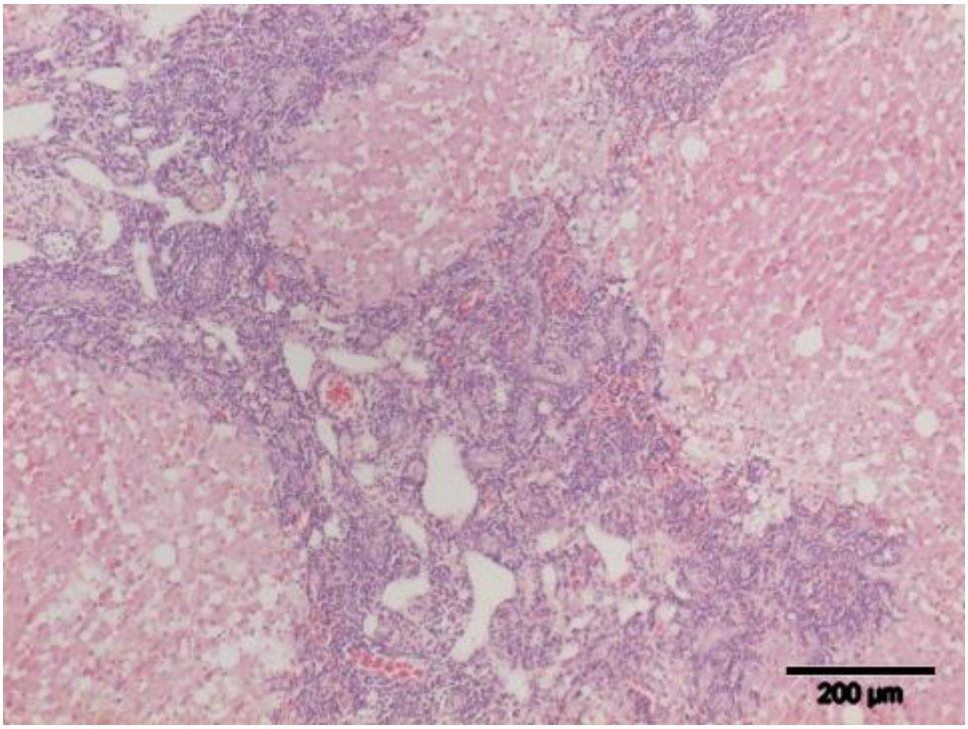

Fig. 29. Cat. Lymphocytic cholangitis with porto-portal bridging. HE.

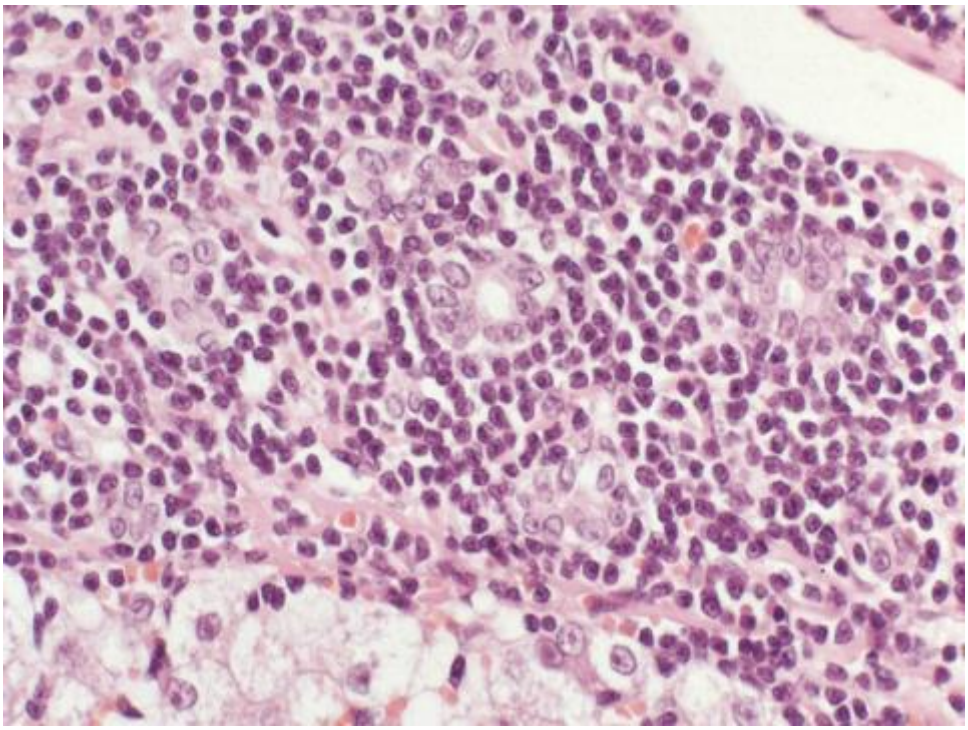

Fig. 30. Cat. Lymphocytic cholangitis. Marked infiltration of small lymphocytes in the portal area, biliary proliferation and foamy macrophages (bottom). HE.

3 Destructive Cholangitis

Destructive cholangitis in dogs [24] is characterized by destruction and loss of the bile ducts in the smaller portal areas with subsequent inflammation (pigment laden macrophages, neutrophils and/or eosinophils) and eventually portal fibrosis (Fig. 31). It has been postulated to result from an idiosyncratic reaction to drugs particularly sulphonamides. However, viral infection e.g. canine distemper (Fig. 32.) and toxic insults may also be associated with destruction of biliary epithelium. Destructive cholangitis usually causes very severe cholestasis and icterus. It is the only form of intrahepatic cholestasis which is so severe that acholic feces can be seen. Whether destructive cholestasis results in definite destruction and loss of the bile ducts or may recover is unknown.

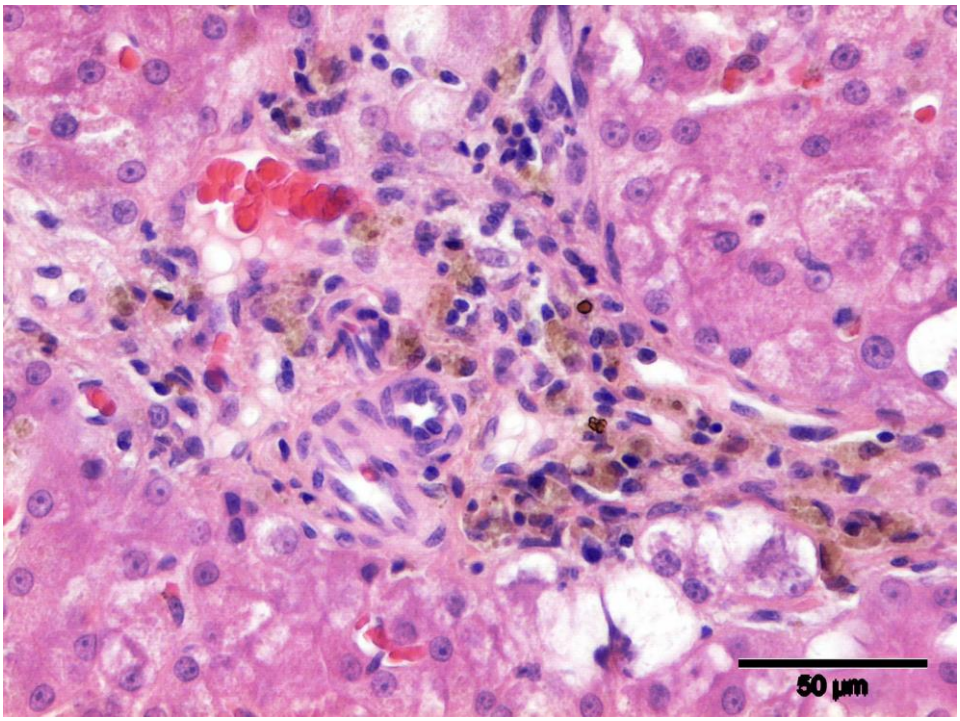

Fig. 31. Dog. Destructive cholangitis. Portal area with absence of bile duct and presence of pigmentladen macrophages. Idiosyncratic reaction to trimethoprim sulfa (TMPS). HE.

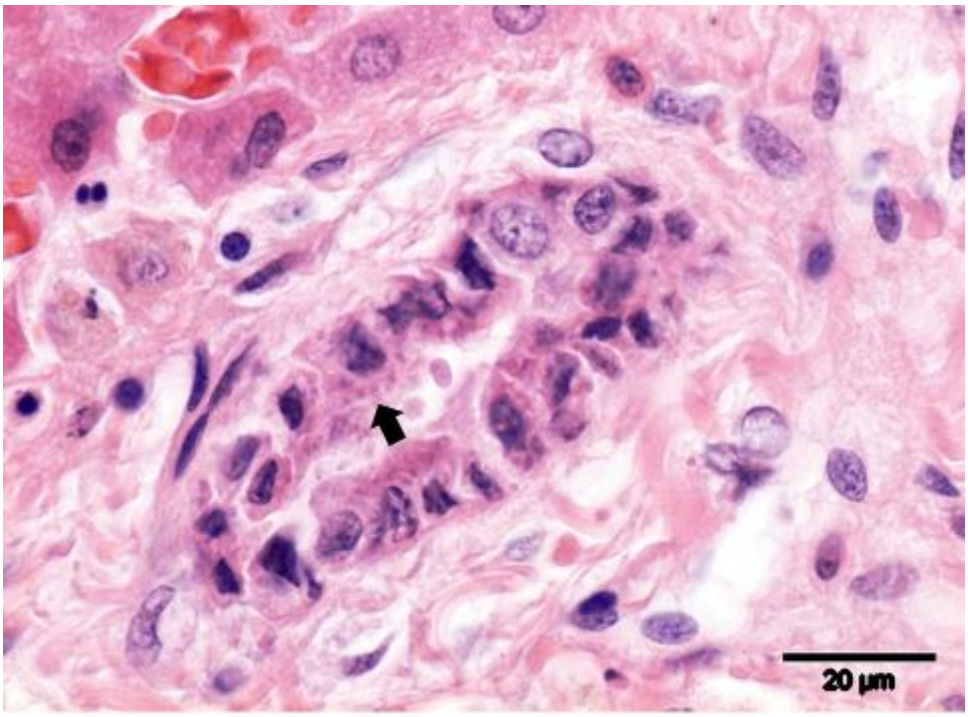

Fig. 32. Dog. Destructive cholangitis with necrosis of the biliary epithelium and some intracytoplasmic viral inclusions (arrow). Canine distemper virus infection. HE.

4 Chronic Cholangitis associated with Liver Fluke Infestation

Chronic cholangitis associated with liver fluke infestation is regularly observed in cats and less frequently in dogs in endemic areas. Infections are caused by members of the family Opisthorchiidae (Table 1), which require two intermediate hosts, the first being water snails and the second a wide variety of fish, in which the metacercariae are encysted. The final host acquires infection by ingestion of raw fish, and the young liver flukes migrate from the intestine to the liver by the bile ducts causing thickening and (cystic) dilatation of the ductus choledochus and large bile ducts (Fig. 33).[25, 26] The lesion is microscopically characterized by dilated larger bile ducts with papillary projections and marked periductal and portal fibrosis (Fig. 34, 35). A slight to moderate inflammation may be seen both within the ducts (neutrophils and macrophages) as well as in the portal areas (neutrophils, lymphocytes and plasma cells) Although eosinophils may be present, they are usually limited in numbers. The number of liver flukes and eggs within the dilated bile ducts varies markedly and regularly only limited evidence of liver flukes or eggs is seen.[25] In cats and dogs, chronic cholangitis due to liver fluke infestation has been associated with the development of intrahepatic and extrahepatic cholangiocellular carcinomas.[25]

Table 1: Species of Opisthorchiidae described in cats and dogs, and their geographic distribution. [26]

Amphimerus pseudofelineus	North, Central and South America
Clonorchis sinensis	China, Japan, Korea, Taiwan
Opisthorchis felineus	Europe, Siberia, Ukraine
Opisthorchis viverrini	South Eastern Asia
Paropisthorchis caninus	India
Metorchis conjunctus	North America
Metorchis albidus	Northern Europe
Metorchis orientalis	South and Eastern Asia
Parametorchis complexum	USA
Pseudamphistomum truncatum	Europe, India

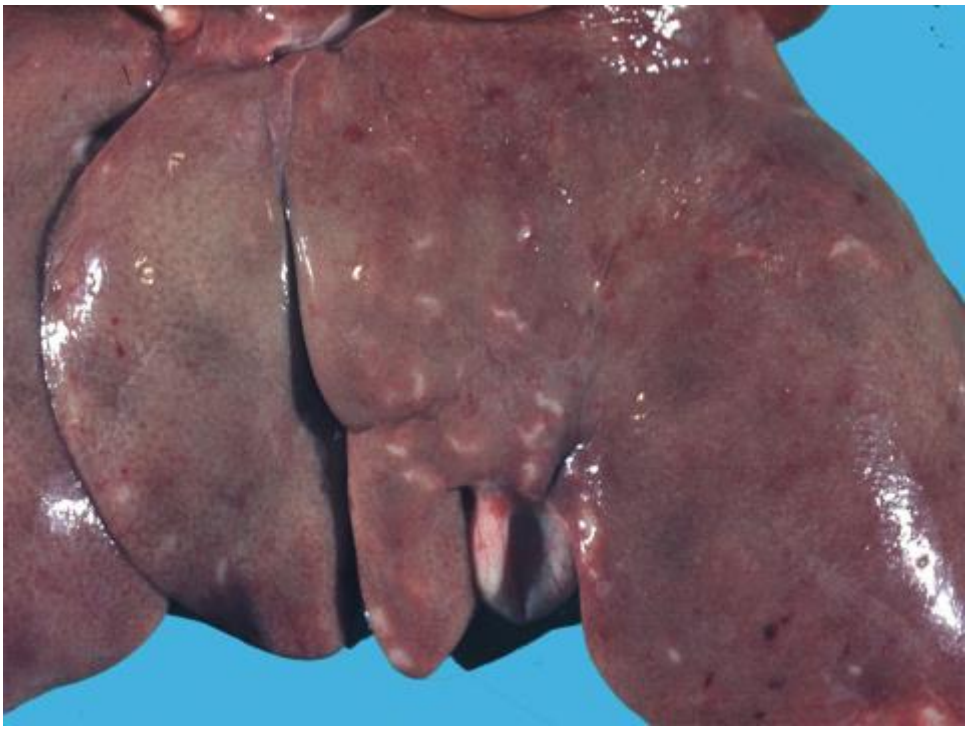

Fig. 33. Cat. Chronic cholangitis due to liver fluke infestation

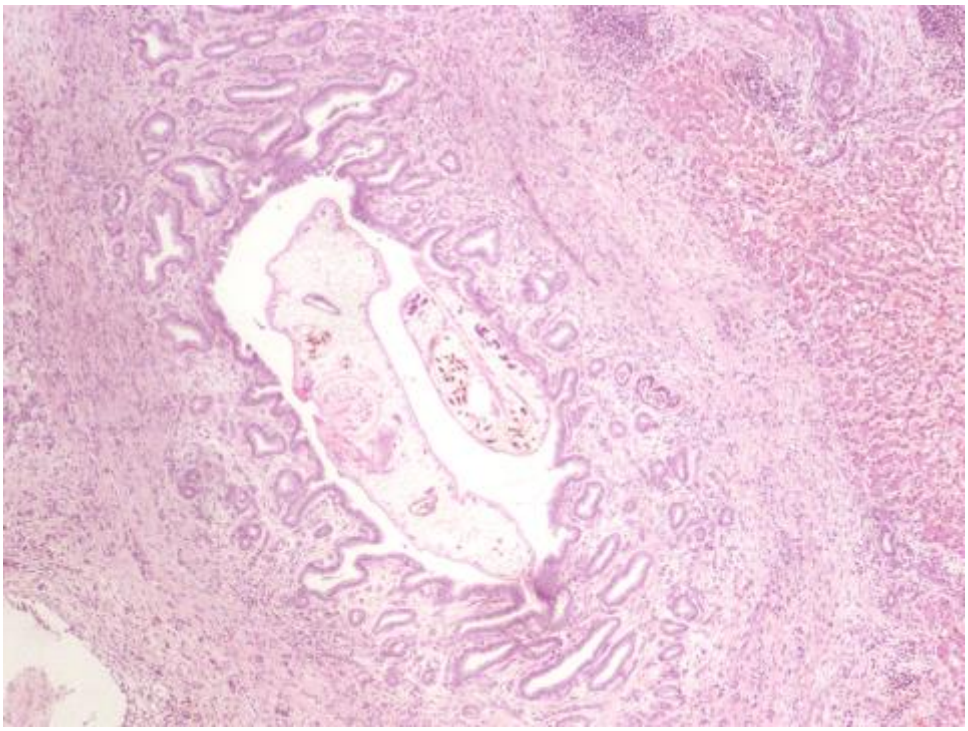

Fig. 34. Cat. Chronic cholangitis due to liver fluke infestation with proliferation of the bile duct epithelium and marked periductal fibrosis. HE.

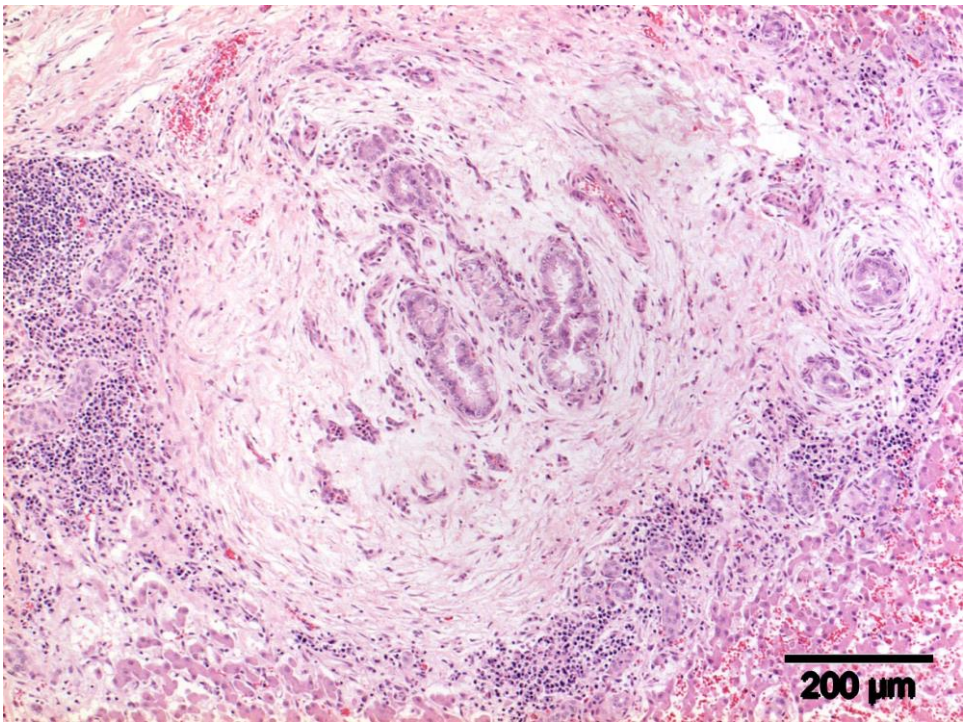

Fig. 35. Cat. Chronic cholangitis due to liver fluke infestation with marked periductal fibrosis, biliary proliferation and peripherally located mononuclear inflammation. HE.

DISORDERS OF THE GALLBLADDER

1 Agenesis and duplication of the gallbladder

Agenesis of the gallbladder is an extremely rare condition in dogs with a preference for small dog breeds i.e. Chihuahua, Maltese dog, Toy Poodle, and for the Boxer dog [27,49],. Most likely it is not associated with developmental failure of the pars cystica, but more likely results from a more complex developmental failure in the embryological development of the liver and bile ducts. In all dogs reported in the literature the condition was characterized by absence of the gallbladder or presence of a vestigial remnant of the gallbladder, dilatation of the common and large hepatic bile ducts without obstruction, congenital hepatic fibrosis with moderate to marked fibrous proliferation, and in about half of the cases by agenesis or hypoplasia of the quadrate or multiple liver lobes.

Duplication of the gallbladder is an incidental finding in cats and is without clinical significance.

2 Mucocele and cystic mucinous hyperplasia.

Mucocele of the gallbladder is increasingly recognized in dogs and characterized by hyperplasia of the epithelium with papillary projections and an increased secretion of abnormally thick mucus (Fig. 36). With extreme mucin production the gallbladder may become markedly extended (Fig. 37) and even may rupture with or without preceding

secondary neutrophilic cholecystitis.[28, 29] In rare cases, gelatinous biliary material is present in the extrahepatic bile ducts and cause extrahepatic cholestasis.[28,55] There is a strong breed predisposition with Shetland sheepdog, American Cocker spaniel, Chihuahua, Pomeranian, Miniature schnauzer and Border terrier at risk[50,51]. Affected dogs have an increased incidence of concurrent endocrinopathy (hyperadrenocorticism in particular, and to a lesser extent, hypothyroidism, prolonged administration of progestatives) or hyperlipidemia [52-54]. These observations suggest a significant influence of both genetic and metabolic factors on disease pathogenesis.

Gallbladder mucocele usually gives a typical ultrasonographic image of a dilated gallbladder with an orange-like radiated structure of the mucinous content (Fig. 38).

Cystic mucinous hyperplasia is recognized as small proliferations in the wall of the gall bladder with papillary proliferation of the epithelium and increased mucus production and has no clinical relevance.

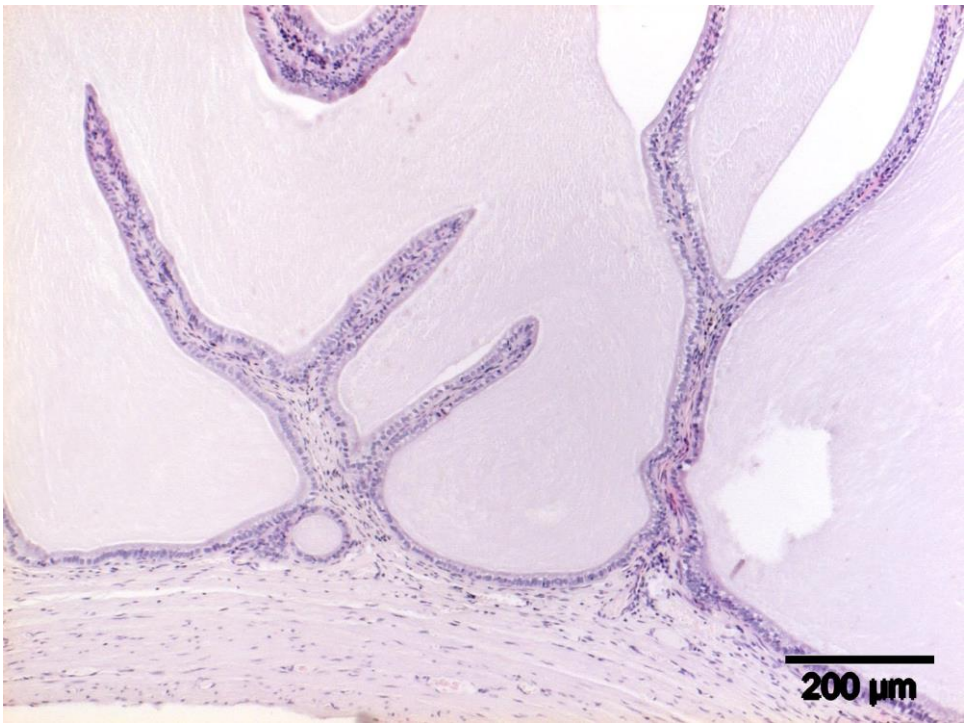

Fig. 36. Dog. Mucocele showing papillary projections of the gallbladder epithelium with secretion of thick mucus. HE.

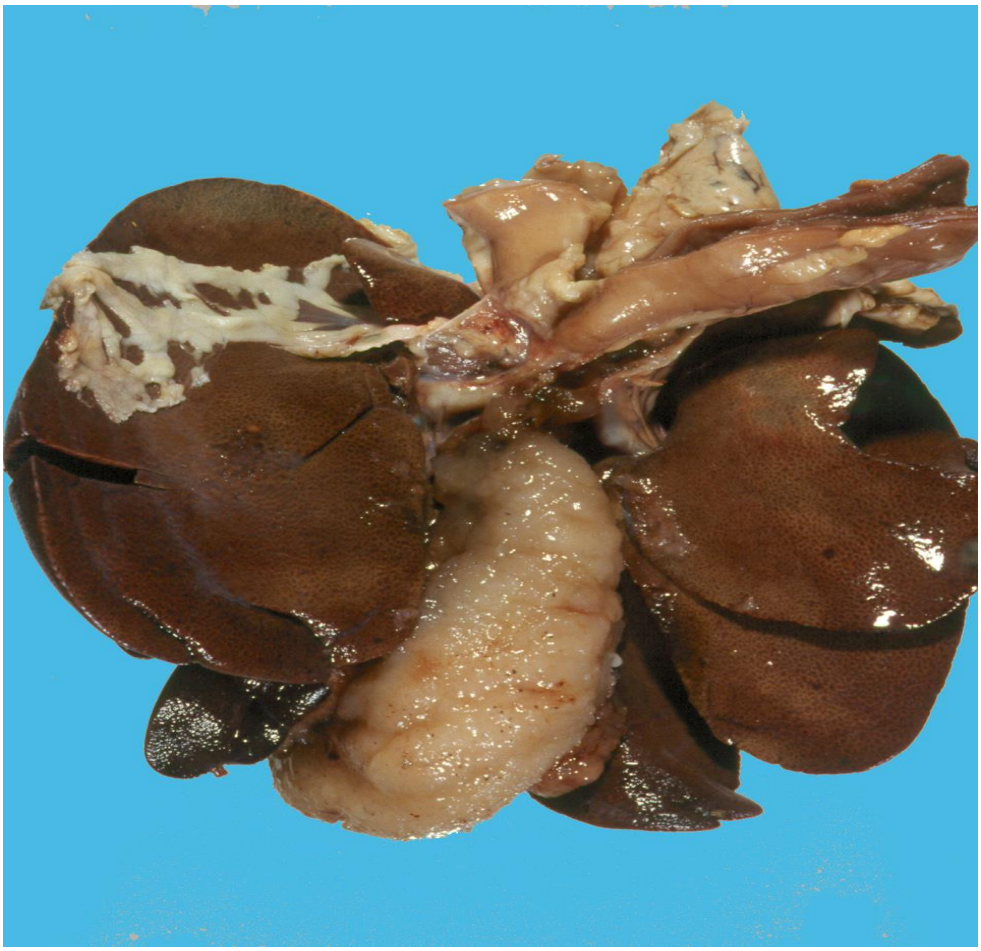

Fig. 37. Dog. Mucocele of the gallbladder. The gallbladder contains a solid mass of mucin.

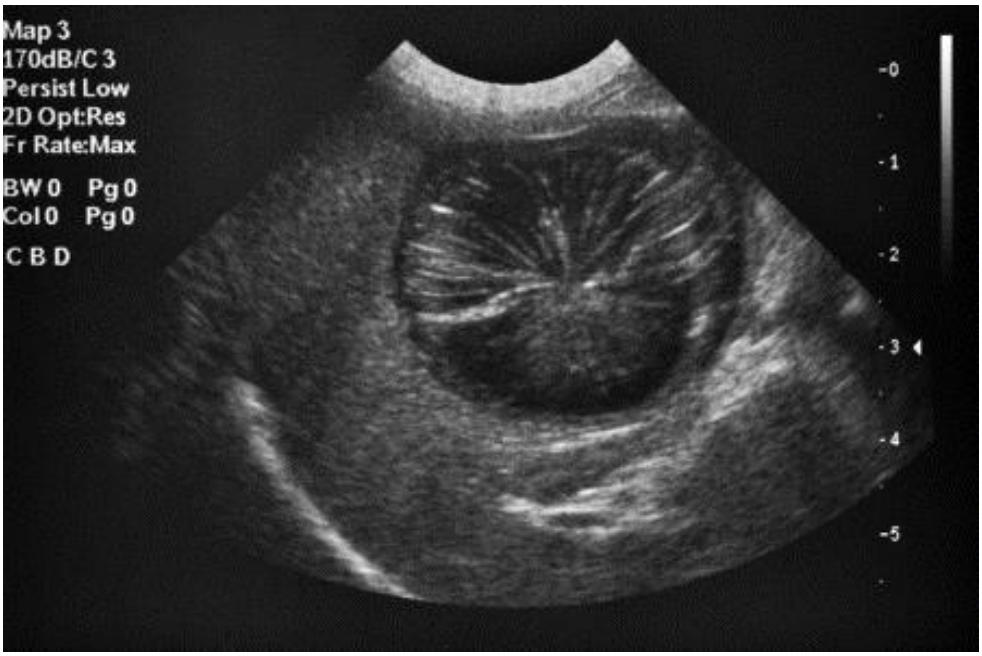

Fig. 38. Ultrasonographic changes in a dog with gallbladder mucocele. The gallbladder is distended and contains thick mucinous material with a typical radiated structure mimicking a cross section of an orange.

3 Cholecystitis

Neutrophilic Cholecystitis is frequently seen in cats and rarely in dogs and in general is associated with ascending bacterial infection from the intestines particularly by Escherichia coli, Enterococcus spp and Clostridium spp[46]. Neutrophilic cholecystitis can be present as a solitary lesion or in combination with neutrophilic cholangitis. The lesion is characterized by the presence of neutrophils in the lumen, the epithelium and/or the wall of the gallbladder (Fig. 39). In the acute stage the lesion can be associated with erosion and ulceration and rarely, depending on the bacterial spp involved, in extensive necrosis of the gallbladder wall or emphysematous cholecystitis[55]. In the chronic stage there is a mixed inflammatory infiltrate consisting of neutrophils, lymphocytes and plasma cells and (possibly) fibrosis.

Lymphoplasmacellular and Follicular Cholecystitis is characterized by the presence of a lymphoplasmacytic infiltrate and, or the presence of lymphoid follicles in the mucosa of the gallbladder (Fig. 40).

4 Infarction of the gallbladder

The gallbladder only has an arterial blood supply provided by the cystic artery, a branch of the hepatic artery. Occlusion of this branch by thrombosis may cause complete or partial infarction of the gallbladder as described in dogs[30]. The lesion is histologically characterized by full thickness necrosis of the wall of the gallbladder without evidence of concurrent cholecystitis (Fig. 41) and incidentally thrombosis of arteries in the wall of the gallbladder. Gallbladder infarction may become more common with intra-arterial application of anticancer treatment via the hepatic artery[56].

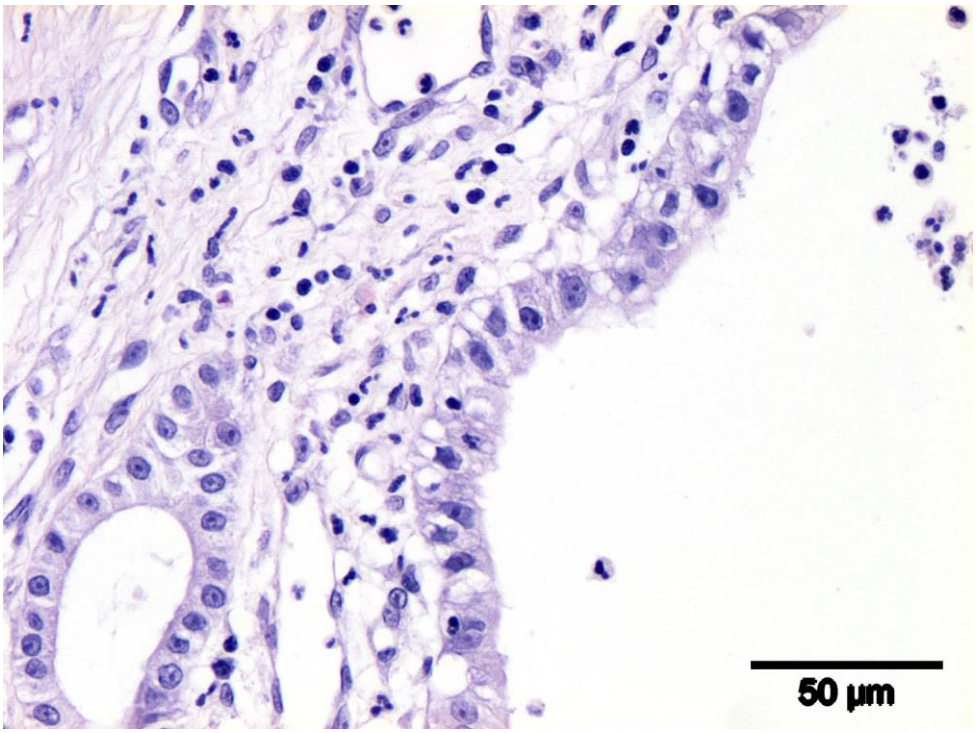

Fig. 39. Dog. Neutrophilic cholecystitis. Neutrophils in the lumen and wall of the gallbladder. HE.

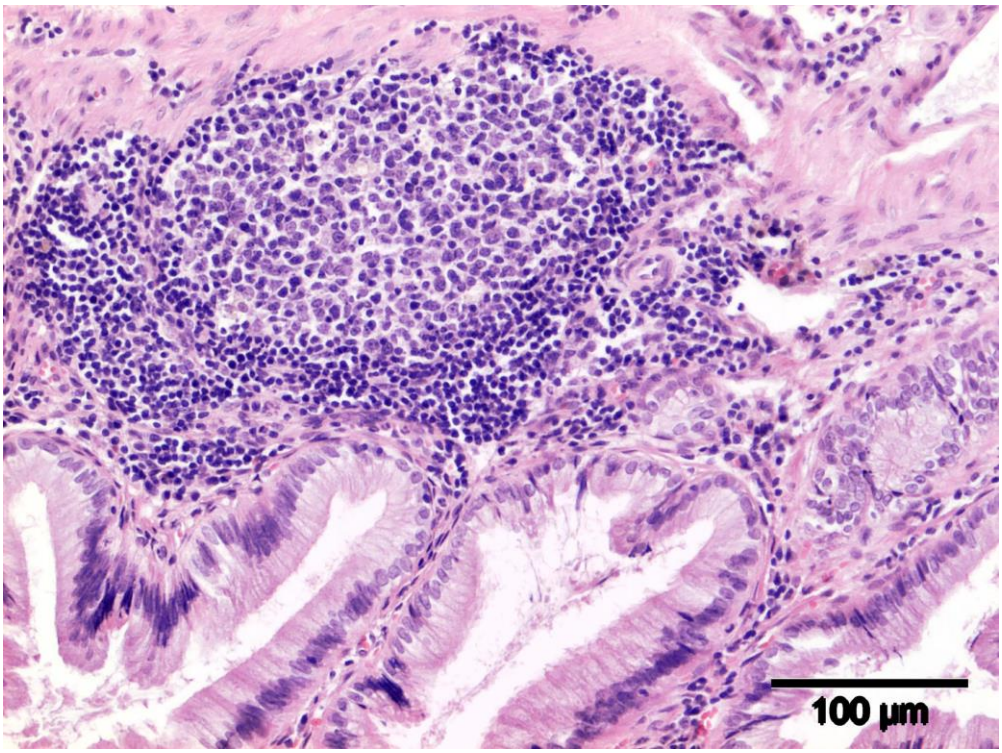

Fig. 40. Dog. Lymphoplasmacellular and follicular cholecystitis. HE.

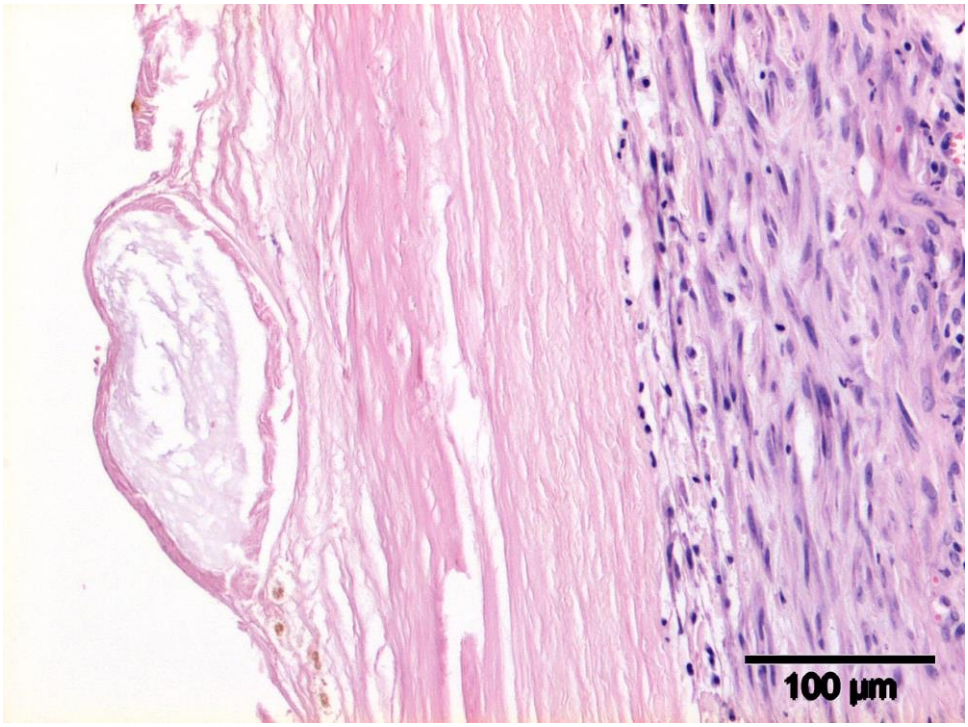

Fig. .41. Dog. Infarction of the gallbladder. Full thickness necrosis of the wall of the gallbladder without inflammation; slight fibroblastic proliferation of the adnexal stroma. HE.

ROLE OF HISTOPATHOLOGICAL EVALUATION, ULTRASONOGRAPHY AND EXAMINATION OF BILE IN THE DIAGNOSIS OF BILIARY DISORDERS

The diagnosis of biliary disorders depends often on a combination of different methods, unlike the parenchymal and neoplastic diseases, which are diagnosed exclusively by histopathological evaluation of the affected tissue. Intrahepatic cholestasis which is associated with many liver diseases is a pure histopathological finding. Cystic liver diseases are usually detected with ultrasonography, but the differentiation between different cystic lesions depends on histopathology. Extrahepatic bile duct obstruction leads to dilatation of the extrahepatic and intrahepatic bile ducts (dilatation of the gallbladder is not an essential feature), and is detected with ultrasonography. In dogs these ultrasonographic findings are diagnostic for extrahepatic cholestasis; the only disease with comparable ultrasound findings is congenital fusiform dilalation of the extrahepatic and intrahepatic bile ducts. However, extrahepatic cholestasis involves the large intrahepatic and extrahepatic bile ducts including the entire common bile duct, whereas in congenital dilalation of the extrahepatic and intrahepatic bile ducts the common bile duct is not affected. These two possibilities can with certainty be differentiated with histopathological evaluation of the liver. In cats, neutrophilic cholangitis, lymphocytic cholangitis, cholangitis due to liver fluke infestation, and extrahepatic bile duct obstruction may have comparable ultrasonographic changes. In all four diseases ultrasonography may show dilatation of the entire biliary tree up to Vater's papilla (Fig. 42). In cats, it is therefore essential to sample liver tissue biopsies for histopathological differentiation of these four diseases. In cats with such lesions it is important to sample bile with puncture of the gallbladder with a thin needle for cytological evaluation and culture. The presence of neutrophils and the identification (and testing of sensitivity for antibiotics) of bacteria in bile are essential to diagnose neutrophilic cholangitis. This form of cholangitis in cats is usually acute without dilatation of the bile ducts and hence without the ultrasonographic changes seen in more chronic cases. Neutrophilic cholangitis does not always produce the typical histopathological changes and the diagnosis may thus be missed when examination is limited to ultasonography and evaluation of a liver biopsy. Evaluation of the bile is required to assess this diagnosis in such cats which present with signs of nausea and vomiting, and may show icterus and increased plasma bile acid and ALT or AP levels.[31-33] The required diagnostic combinations for the biliary disorders of dogs and cats are summarized in Table 2.

Table 2. The roles of histopathology, ultrasonography and bile sampling (cytological evaluation and culture) in the diagnosis of biliary diseases of dogs and cats.

Disorder	Ultrasono -graphy (A)	Histopathology of the liver (B)	Bile cytology (C)	Bile culture (D)	Minimal diagnostic Requirement
Cystic diseases	+ or +/-	+	-	-	A and B
Extrahepatic cholestasis dog	+	+ or +/-	-	-	A
Extrahepatic cholestasis cat	+	+ or +/-	-	-	A,B,C, and D
Neutrophilic cholangitis	+/- or -	+/-	+	+	A,C, and D
Lymphocytic cholangitis	+	+	+/-	-	A,B,C, and D
Liver fluke infection	+	+	-	-	A, B,C and D
Destructive cholangitis	-	+	-	-	B
Gallbladder mucocele	+	-	-	-	A
Gallbladder infarction	+	+*	-	_	A and B

- = no changes demonstrated with this method
+/- = changes may be found, but may also be absent
+ = changes are always demonstrated
* histopathology of the gallbladder required for final diagnosis

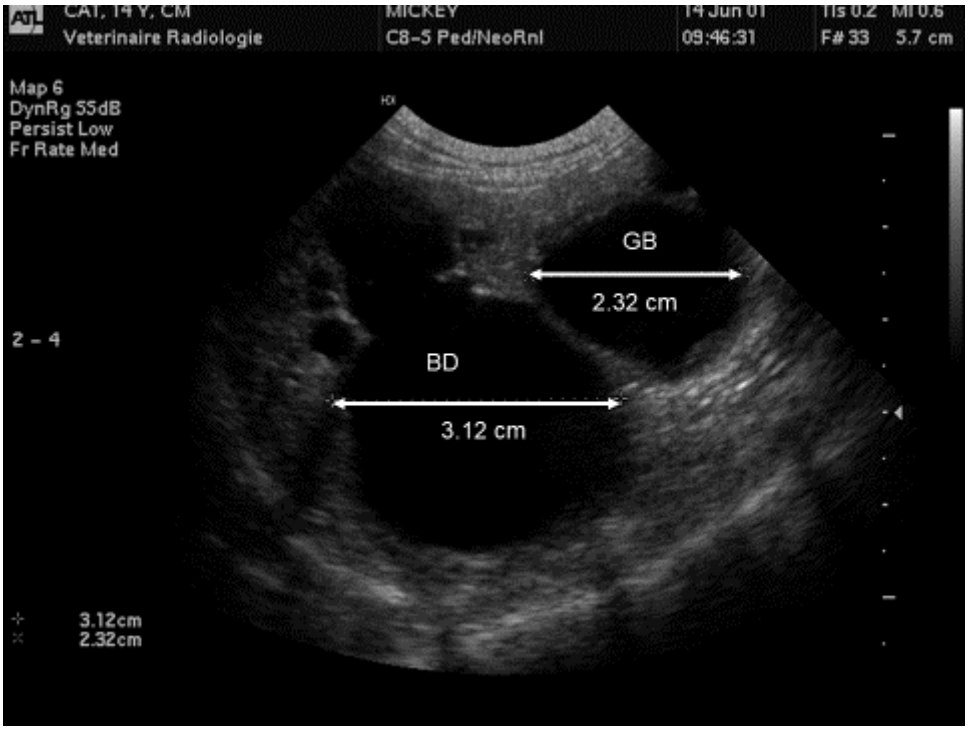

Fig. 42. Ultrasonographic image of a cat with irregularly distended intra- and extrahepatic bile ducts due to lymphocytic cholangitis. Similar changes occur due to extrahepatic cholestasis and liver fluke infestation. GB = gallbladder, BD = bile duct. Permission of Dr. George Voorhout, Dept. Diagnostic Imaging, Fac. Veterinary Medicine, University Utrecht

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