# Ultrasonographic diagnosis of hepatic coccidiosis in rabbits

Echografische diagnose van levercoccidiose bij konijnen

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Eimeria (E.) stiedae is a protozoan parasite causing hepatic coccidiosis in rabbits. It mostly infects younger animals and causes nonspecific signs like stunted growth, weakness, dehydration, diarrhea and anorexia. Macroscopically, the liver has a typical appearance. It is enlarged, showing firm yellow-white lesions on the surface. These lesions are enlarged bile ducts filled with bile and necrotic debris. Although all diagnostic tools for hepatic coccidiosis in live animals are currently impracticable or inconclusive, ultrasound might be useful for the diagnosis of hepatic coccidiosis. However, the appearance of liver changes associated with E. stiedae on ultrasonography has poorly been described in the literature.

In this study, ex-vivo ultrasound of 24 livers was performed, i.e. the livers of two healthy rabbits and 22 livers of rabbits with suspected liver coccidiosis. Hyperechoic lesions of variable size and shape were found in all affected livers. In some of these livers, other signs of hepatic disease were detected: heterogenous liver parenchyma, appearance of hepatomegaly with round edges, gallbladder sludge and thickening of the gallbladder wall.

### **SAMENVATTING**

*Eimeria stiedae* is een parasitair protozoair organisme dat levercoccidiose veroorzaakt. Het leidt vooral tot ziekte bij jongere konijnen en veroorzaakt daarbij niet-specifieke symptomen, zoals zwakte, diarree en anorexie. Post mortem kent het een duidelijke pathologie, i.e. een vergrote lever en stevige geel-witte laesies. Deze laesies zijn vergrote galgangen gevuld met gal en necrotisch materiaal.

Alhoewel de technieken om levercoccidiose te diagnosticeren tot op heden omslachtig of nietsluitend zijn, kan echografie mogelijk een goed diagnostisch middel zijn. In de literatuur werd het echografisch uitzicht van levercoccidiose tot nu toe echter zeer weinig beschreven.

In dit onderzoek werden 24 levers beoordeeld met behulp van echografie, twee van gezonde konijnen en 22 van konijnen verdacht van levercoccidiose. Al deze levers vertoonden hyperechogene laesies die varieerden in grootte en vorm. Daarnaast vertoonden enkele levers ook andere tekenen van leverziekte, i.e. heterogeen parenchym, leververgroting met afgeronde randen, galblaas met neerslag en een verdikte galblaaswand.

### INTRODUCTION

Coccidiosis, caused by the protozoan parasite *Eimeria*, is commonly found in young rabbits. *Eimeria* is a pathogen that most often causes lesions and disease in animals kept in crowded and/or unsanitary

conditions, for example in breeding facilities (Buseth and Saunders, 2015). There are multiple coccidia (*Eimeria*) species causing intestinal coccidiosis in rabbits, and mixed infections can occur (Duszynski and Couch, 2013). In total, 16 *Eimeria* species can infect rabbits (Harcourt-Brown, 2014). *Eimeria stiedae* is

the only species causing hepatic coccidiosis. It can infect any rabbit, but clinical presentation occurs most often in young rabbits, up to the post-weaning period (five to eight weeks). The signs are nonspecific, i.e. stunted growth, weakness, dehydration, diarrhea (watery to mucoid, sometimes bloody) and anorexia (Redrobe, 2013). Especially in young rabbits, it can occasionally be fatal (Gomez-Bautista et al., 1987). According to Sivajothi et al. (2016), adults can become carriers of the disease, and thereby be the source of infection.

Transmission of *Eimeria stiedae* occurs after rabbits ingest sporulated oocysts. Once ingested, oocysts hatch in the duodenum and travel via the mesenteric lymph nodes to the liver, where they enter the epithelial cells of the bile ducts (Varga, 2014). After multiple asexual schizogony cycles, sexual reproduction takes place and oocysts develop (Oglesbee and Jenkins, 2012). The bile duct cells burst and the oocysts are released via the bile into the intestines (Varga, 2014). A schematic overview of the lifecycle of *Ei*-

meria stiedae can be found in Figure 1. The reproduction of *E. stiedae* causes compression of the liver cells, leading to large areas of necrosis and damage. This leads to disturbances in all the liver functions, causing the clinical signs as described earlier (Jing et al., 2016). Hepatomegaly can occur because of the excessive proliferation of the bile duct epithelium (Al-Naimi et al., 2012).

Hepatic coccidiosis results in typical post-mortem findings. Macroscopically, the liver is enlarged and shows firm yellow-white lesions on the surface, which constitute enlarged bile ducts filled with bile and necrotic debris; the size may vary. The nodular lesions have an abscess-like aspect, sometimes with a fibrous capsule (Figures 2 and 3). The gallbladder is often grossly enlarged (Meredith and Rayment, 2000). Lesions resulting from hepatic coccidiosis can stay evident in the liver for many years (Varga, 2014). Microscopically, the bile duct epithelium is hyperplastic and the bile ducts themselves are enlarged. There is often fibrosis mixed with infiltration of inflamma-

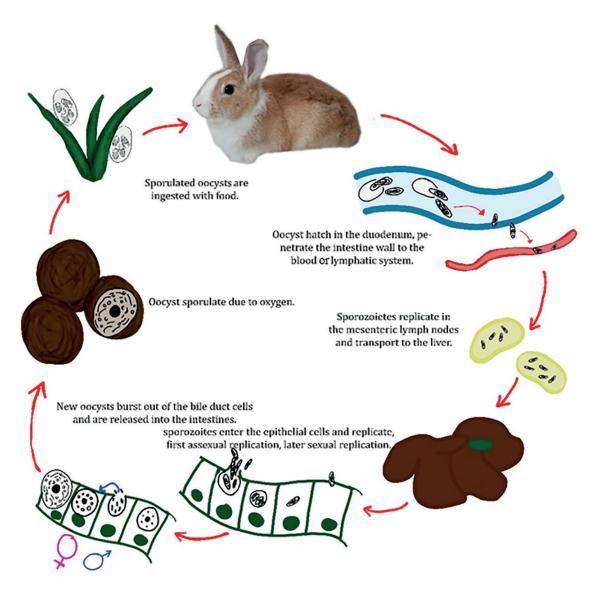


Figure 1. Lifecycle of *Eimeria stiedae*.

tory cells (eosinophils, lymphocytes and plasma cells) (Al-Mathal, 2008). Within the epithelium, the oocysts can be demonstrated (Turner et al., 2018).

As hepatic coccidiosis gives nonspecific clinical signs, the differential diagnosis comprises for example metabolic diarrhea, bacterial dysbiosis or hepatitis (Redrobe, 2013). Most commonly, coccidiosis is diagnosed by fecal examination, with fecal flotation. However, finding oocysts is not sufficient to confirm the diagnosis; they need to be specified and differentiated from intestinal and nonclinical species. Identification needs to be done on sporulated oocysts. This is rather complicated because of the great variability in size and shape within the same species (Licois, 2004). The oocyst of *Eimeria stiedae* does show a typical extra membrane, called a veil, which none of the coccidia that can be detected in rabbits, develop. However, this veil can only be visualized using electron microscopy (Ball et al., 2014). Oocysts of E. stiedae can also be demonstrated in a wet mount of bile (Turner et al., 2018). While bile samples can be obtained by percutaneous ultrasound guided cholecystocentesis in dogs and cats (Savary-Bataille et al., 2003), the authors could not find any description of this technique for rabbits.

Other tools such as blood examination are not specific enough to diagnose hepatic coccidiosis (Melillo, 2007; Meredith and Rayment, 2000; Oglesbee and Jenkins, 2012; Jenkins, 2008). Laboratory methods like polymerase chain reaction (PCR) and enzymelinked immuno sorbent assay (ELISA) are being studied with variable success, and are currently not available for veterinary use (El-Ghany, 2020).

Ultrasonographic findings so far described for hepatic coccidiosis can include hepatomegaly, thickened bile duct walls, alteration of the texture of the liver and/or enlarged hepatic lymph nodes (Reese, 2011a) and ascites (Jing et al., 2016). Çam et al. (2008) also found a dilated gallbladder, increased echogenicity of the liver and hyperechogenic appearance of the intrahepatic vessel walls. However, the description of most of these findings is limited in the literature. The aim of the current study was to more extensively describe the ultrasonographic appearance of hepatic coccidiosis in rabbits by means of ex vivo ultrasound of livers of rabbits with confirmed liver coccidiosis.

### MATERIALS AND METHODS

#### Post-mortem ultrasound of rabbit livers

The livers of 24 rabbits were included in the study. Two livers of healthy rabbits were used as a control, which were derived from a different study. The other 22 livers were suspected of hepatic coccidiosis based on the macroscopic aspect. Two suspected livers originated from necropsy of pet rabbits, the other twenty were derived from a slaughterhouse. The rabbit livers where kept refrigerated until ultrasound could be

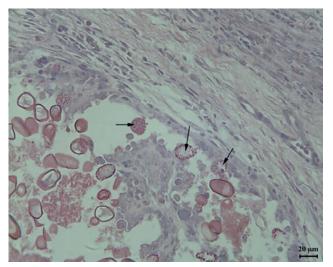


Figure 2. Histopathological aspect of a hepatic coccidiosis infected liver on HE staining. The arrows indicate a great number of sporangium, merozoite and gametocyte (Jing et al., 2016).



Figure 3. *Eimeria stiedae* oocysts in bile on 40x10 amplification.

conducted, which was always within 24 hours post mortem.

Ultrasound was performed with a Philips Epic 7 machine. The liver was placed in a container with water and scanned with a microconvex transducer of 5-8 MHz or linear transducer of 4-18 MHz without applying pressure. Images were saved for later interpretation and description.



Figure 4. Ultrasound of a control rabbit. P: Liver parenchyma; GB: Gallbladder; W: Water and A: Mirror artefact.

### Microscopic confirmation

If the gallbladder was present, the bile was collected with a 21-gauge needle and a syringe. A droplet of bile was placed on a glass slide for microscopic examination. If the gallbladder was absent due to the slaughter house process, a cytological smear of the nodules was made on a slide. A piece of a nodule with a diameter of approximately 2 mm was cut off and put on a glass slide. The tissue was softly squashed and spread out over the slide by using a second slide.

The native bile or cytology of the liver nodules were examined for *Eimeria stiedae* oocysts under the light microscope at 10x10 amplification.

### **RESULTS**

### **Control group**

The two livers of the control group had an unremarkable ultrasonographic appearance. The liver parenchyma had a homogeneous echotexture of normal



Figure 5. Visceral side of a mildly affected liver.

echogenicity. The liver was subjectively normal in size with sharply defined edges. The gallbladder was moderately filled with anechoic bile, without sludge and with a normal thickness of the wall (Figure 4).

### Hepatic coccidiosis group

Macroscopic examination

All 22 livers obtained, were suspected of liver coccidiosis, based on the macroscopic aspect of the nodules in the liver. The livers showed nodules with a mean diameter of approximately 5 mm, white to yellowish in color. The nodules were either hard in consistency or had a more fatty, soft content. The nodules were divided over all liver lobes. The number of nodules differed greatly. The livers showed a normal, brown-red parenchyma; however, they differed in size. Certain, especially severely infected, livers were enlarged with rounding of the margins. The bile present in the gallbladders differed in color from dark green to golden-yellow. The gallbladder was still present in 18 out of 22 cases (Figures 5 and 6).

### Ultrasound

Ultrasound showed lesions in all livers. Subjectively, the lesions were more numerous on ultrasound compared to the macroscopic appearance. The severity of ultrasonographic involvement correlated well with the macroscopic appearance. The most characteristic lesions were nodules of varying shape and diameter, ranging from 1 to 7 mm. Most lesions were round to ovoid; however, some lesions had a more irregular geometrical shape. The lesions were mostly well-defined. In severe cases, some ill-defined lesions with tendency to confluence were noted. The appearance of the lesions varied from homogeneously hyperechoic, heterogeneously hyperechoic to 'target-like' appearance characterized by a hypoechoic center and hyperechoic rim (Figures 7, 8, 9). Rarely, small hyperechoic foci suggestive of mineralization were noted. Few nodules deformed the hepatic capsule.

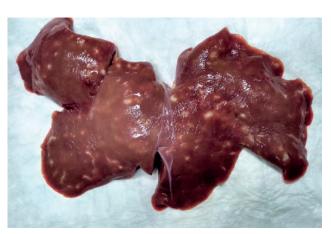


Figure 6. Diaphragmatic side of a severely affected liver.

In most cases, the liver parenchyma between the nodules was normal; however, in two out of 22 cases, it was diffusely heterogenous. Some livers were enlarged with rounding of the edges. In nine cases, echoic sediment was present in the gall bladder (Figures 10 and 11), in three cases, its wall was irregular, thickened and/or hyperechoic.

## Microscopical confirmation

On all but one liver, *Eimeria* oocysts could be found either in the bile or in a cytological smear. Only liver number 4 was inconclusive, due to the thickness of the smear. Most livers contained a large number of oocysts; liver 22 showed a subjectively smaller number of oocysts in the bile. The oocyst were not sporulated to be identified as *Eimeria stiedae;* however, this is the only species that can be found in the liver and bile (Redrobe, 2013).

### **DISCUSSION**

In this study, a clear correlation between the presence of *Eimeria* oocysts, the macroscopic nodules and the ultrasonographic lesions was found. In all 22 of the affected livers, ultrasonographic abnormalities were seen, all in the form of nodules.

The lesions on ultrasound differed greatly in size and shape. Grossly, they can be divided into three types: homogenously hyperechoic, heterogeneously hyperechoic and target-like (with a hyperechoic center and hypoechoic rim). Most of the lesions were well defined and round to ovoid; however, some showed more of an amorphous to even geometrical shape. The most severely affected livers often showed ill-defined lesions with a slight confluence to the surrounding tissue. Assuming all these lesions are caused by hepatic coccidiosis, a way to explain the different types of nodules might be the different stages of the disease. Therefore, a comparison of the ultrasonographic lesions to the macroscopic view of E. stiedae is performed. As the nodules, which are macroscopically visible, are enlarged bile ducts (Meredith and Rayment, 2000), it makes sense that those on ultrasonography are too. An infection can macroscopically lead to nodular, abscess-like lesions. In some cases, these have a fibrotic capsule (Meredith and Rayment, 2000). Nodules can also be surrounded by inflammatory cells. The damaged liver parenchyma is later replaced by fibrous tissue (Pakandl, 2009). The change from active infection to fibrotic tissue can be an explanation for the different kinds of lesions seen in the liver. The target-like nodules with hyperechoic wall could be abscess-like lesions as a result of an active infection, the hyperechoic rim being the fibrotic capsule. As fibrotic tissue appears more hyperechoic on ultrasound, the amorphous hyperechoic lesions could be from a more progressed stage of infection. Singla et al. (2000) described that foreign body granulomas

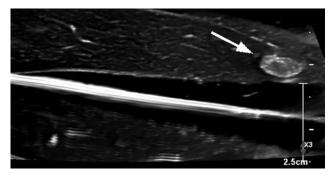


Figure 7. A rounded nodule with a target like lesion (arrow). Liver parenchyma between the nodules is normal.

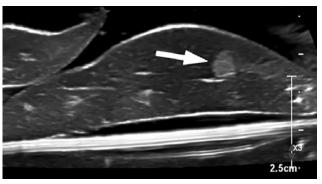


Figure 8. Single round to amorphous hyperechoic lesion (arrow). Liver parenchyma around the nodule is normal.

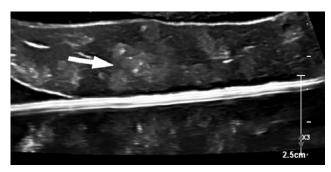


Figure 9. Heterogenous nodule (arrow).

can occur when the continuity of the bile duct epithelium is broken. Therefore, *Eimeria* might similarly act as foreign bodies. This might cause the more geometric, ill-defined and confluent hyperechoic lesions. However, further research is necessary to confirm this and to relate ultrasonographic findings to histologic changes.

Abnormalities of the gallbladder as seen in this study, have been described in the literature by Çam et al. (2008). In 50% of the present gallbladders, sludge was present. Gallbladder sludge is considered an abnormality in rabbits and might indicate starvation, just like bile stasis (Loff et al., 1999). The presence of sludge could be explained by the fact that these rabbits suffered from anorexia due to hepatic coccidiosis (Redrobe, 2013). Another sign of hepatic coccidiosis is cholestasis (Meredith and Rayment, 2000), which can also lead to gallbladder sludge. A hyperechoic

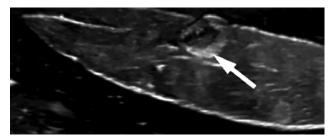


Figure 10. Gallbladder with thickened wall (arrow) and moderate amount of sludge.

wall of the gallbladder was seen in one liver in the present study. Reese (2011a) also described thickened bile duct walls. However, this was not detected during this study. In addition, Cam et al. (2008) described an increased echogenicity of the liver. Hyperechoic liver parenchyma was not seen in the present study; however, this was difficult to judge due to the lack of surrounding organs. An altered echoic texture of the liver parenchyma as described by Reese (2011b) was seen in two out of 22 livers. Parenchyma of these livers was heterogenous instead of homogenous. Mlakar Hrženjak et al. (2021) described two pet rabbits with severe hepatic coccidiosis. In both cases, the liver parenchyma was heterogenous with multiple, poorly defined hyperechoic and irregular shaped lesions. The bile ducts were also dilated in both cases. The second case showed a gallbladder filled with slightly echoic bile and a hyperechoic thickened wall. These findings are very similar to those found in this research. Hepatomegaly is also a sign of hepatic coccidiosis (Reese, 2011a). Hepatomegaly in rabbits is usually defined as a liver that extends clearly outside the costal arch (Reese, 2011b). However, it can also be suspected based on the rounded edges of the liver (Armstrong and Fichera, 2003). Based on these criteria, several cases of hepatomegaly were observed in the present study. Due to the study material, it was impossible to assess the hepatic lymph nodes (Reese, 2011b) and ascites (Jing et al., 2016), which have been described in other studies. Hyperechoic hepatic vessel walls, as described by Cam et al., (2008), were seen in this study.

Differential diagnoses for the ultrasonographic findings include rabbit hemorrhagic disease (RHD), neoplasia, cholangiohepatitis of bacterial origin, septic emboli/abscesses and *Fasciola hepatica*.

The most important differential diagnosis is probably pseudotuberculosis (Reese, 2011b). Yersinia pseudotuberculosis is a bacterium that can cause enteritis and septicemia in both humans and animals. It can be associated with necrosis in the liver and spleen (Najdenski et al., 2003). Microscopically, typical lesions look like multifocal necrosis. Nodules can be up to several centimeters in diameter and are filled with a caseous center (Vetlexicon, 2021). Although most part of this description fits the macroscopical results of the present study, none of the lesions found were larger

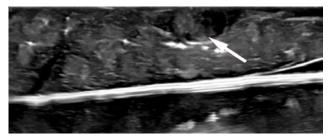


Figure 11. Large amount of nodules with an echoic gall-bladder with echoic sludge (arrow).

than 0.7 cm. This makes *Yersinia* an unlikely cause; however, it cannot fully be excluded. Typically, bacteria can be found on cytologic or histologic examination. If not, Yersiniosis can be excluded. Another important differential diagnosis is neoplasia. From small animal practice, it is known that hepatic neoplasia can give a variety of appearances on ultrasound (Larson et al., 2020). Most forms do not lead to generalized hepatomegaly but only to local enlargements of the liver (Reese, 2011b); however, lymphoma is an exception. Metastasis from uterine adenocarcinomas or mammary carcinomas are commonly detected within the liver (van Zeeland, 2017) and may look as focal lesions with different shapes and sizes. In general, it can be concluded that especially lymphoma and metastasis might give similar lesions as described in the results, and are a likely cause of hepatic nodules on ultrasound. However, it is unlikely that the slaughterhouse rabbits were all affected with the same neoplasia; especially, since tumors more often occur in older animals and are not infectious in nature. In general practice, tumorous lesions might be differentiated on cytological examination following fine-needle aspira-

One of the limitations of this study is the absence of a full abdominal ultrasound, making hepatomegaly, lymphadenopathy, echogenicity and ascites more difficult or impossible to diagnose. Secondly, to exclude the differential diagnoses with certainty, both histology and bacteriology of the samples should have been performed.

For future research, it is advisable to quantify the number of oocysts in the bile as well as the severity of infection visible on the liver both macroscopically and ultrasonographically. Ultrasound examination showed a sensitivity of 100% in the present study; however, it was noted that all livers also showed macroscopic abnormalities. A longitudinal follow-up of rabbits infected with hepatic coccidiosis in the pre-clinical, clinical and post-clinical phase is necessary to judge the progress of the hepatic lesions.

### **CONCLUSION**

In this study, the utility of ultrasound in the diagnosis of hepatic coccidiosis in rabbits is confirmed.

Ultrasonographic abnormalities included hepatomegaly, hepatic nodules with variable size and appearance, biliary sludge and thickening, and irregularity of the gallbladder wall.

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