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Diagnosis of liver flukes in cows – a comparison of the findings in the liver, in the feces, and in the bile

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Summary

Percutaneous ultrasound-guided cholecystocentesis and aspiration of bile were attempted in 176 cows. These same procedures were performed in another 100 cows immediately after slaughter. The bile samples were examined microscopically for large and small liver fluke eggs. In addition, a fecal sample from each cow was examined for liver fluke eggs. The findings of both groups were summarized, and the results of the fecal and bile sample examinations were compared. In all cows the liver was examined for flukes, and the results were used as a reference. Of 41 cows in which adult flukes were found in the liver, 28 had *F. hepatica* eggs in fecal samples and 40 had *F. hepatica* eggs in bile samples. Of 204 cows in which no adult flukes were found in the liver, 23 had *F. hepatica* eggs in fecal samples and 27 had *F. hepatica* eggs in bile samples. The sensitivity of the determination of *F. hepatica* eggs in fecal and bile samples was 68 and 98%, respectively. The negative predictive values for fecal and bile examination were 93 and 99%, respectively.
Of 49 cows in which adult flukes were observed in the liver, 13 had *D. dendriticum* eggs in fecal samples and 44 had *D. dendriticum* eggs in bile samples. Of 176 cows in which no adult flukes were found in the liver, 19 had *D. dendriticum* eggs in fecal samples and 49 had *D. dendriticum* eggs in bile samples. The sensitivity of the deter

Vergleichende Untersuchungen auf Fasziolose und Dikrozöliose in Galle, Kot und Leber beim Rind

Bei 176 Kühen wurde versucht, die Gallenblase unter Ultraschallkontrolle zu punktieren und Galle zu aspirieren. Bei weiteren 100 Kühen wurde die Gallenblase unmittelbar nach der Schlachtung punktiert. Die Galle wurde mikroskopisch auf Eier des kleinen und grossen Leberegels untersucht. Parallel dazu wurde von jedem Tier eine Kotprobe auf Leberegeleier untersucht. Die Ergebnisse beider Gruppen wurden zusammengefasst und die Eibefunde in Galle und Kot miteinander verglichen. Als Referenz diente der Leberegelbefund in der Leber.
F.-hepatica-Eier konnten im Kot bei 28 und in der Galle bei 40 von 41 Kühen mit positivem und bei 23 bzw. 27 von 204 Kühen mit negativem Leberegelbefund nachgewiesen werden. Die Sensitivität betrug für den *F.-hepatica*-Einachweis im Kot 68% und für denjenigen in der Galle 98%. Die negativen prädiktiven Werte betrugen für Kot und Galle 93% und 99%.
In bezug auf *D.-dendriticum* gelang der Einachweis im Kot bei 13 und in der Galle bei 44 von 49 Kühen mit positivem und bei 19 bzw. 49 von 176 Kühen mit negativem Leberegelbefund in der Leber. Die Sensitivität betrug für den *D.-dendriticum*-Einachweis im Kot 27% und in der Galle 90%. Die negativen prädiktiven Werte betrugen für Kot und Galle 81% und 96%.

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mination of *D. dendriticum* eggs in fecal and bile samples was 27 and 90%, respectively. The negative predictive values for fecal and bile examination were 81 and 96%, respectively.
The results of this study indicate that the examination of bile is clearly a more reliable method of diagnosing liver fluke infections than microscopic examination of feces.

Key words: **cattle – liver flukes – diagnosis – feces – bile**

Die Untersuchungen haben gezeigt, dass der Einachweis in der Galle der Kotuntersuchung deutlich überlegen ist.

Schlüsselwörter: **Rind – Leberegel – Diagnose – Kot – Galle**

Introduction

Fascioliasis and dicrocoeliasis in cattle have a worldwide distribution, and chronic infections can result in decreased meat and milk production and impaired fertility. Parasitological examination of feces is currently the standard method of diagnosing fascioliasis (Malone and Craig, 1990; Bürger, 1992). However, there are disadvantages to this method: fluke eggs cannot be demonstrated before the end of the prepatent period (Bürger, 1992), the number of eggs passed into the feces varies from day to day and even during one day, and eggs may be passed irregularly in batches into the feces (Düwel and Reisenleiter, 1990; Malone and Craig, 1990). According to Boray (1969) only approximately 30% of the eggs passed into the feces can be demonstrated. Other indirect methods of diagnosing fascioliasis such as ELISA and determination of the level of the bile duct enzyme gamma glutamyl transferase are highly unreliable (Malone and Craig, 1990). Diagnosis of dicrocoeliasis is more difficult than that of fascioliasis; the routinely used fecal examination is not very suitable for demonstrating *D. dendriticum* eggs (Bürger, 1992).
It is apparent that additional methods should be investigated to improve the diagnosis of liver fluke infection. It is known that liver fluke eggs accumulate in the gallbladder, and examination of bile obtained after slaughter is a more reliable method of demonstrating fluke eggs than examination of feces (Lemmermöhle, 1973; Hauser, 1977). Obviously, from a diagnostic point of view, there is little sense in examining bile at *post mortem*. However, until recently, cholecystocentesis in live cows was only possible if at all during laparotomy. Recent studies have demonstrated that in cattle the gallbladder can be visualized clearly by ultrasonography (Braun, 1990) and percutaneous ultrasound-guided cholecystocentesis and aspiration of bile can be safely performed (Braun and Gerber, 1992). In addition, liver fluke eggs were demonstrated in the bile via microscopy, and it was assumed that examination of bile was a more reliable method of diagnosing liver fluke infection than examination of feces. The goal of this study was to investigate this further by determining the sensitivity and specificity of bile and fecal examination for liver fluke eggs and by comparing the results using the liver findings at *post mortem* as a reference.

Animals, materials and methods

Ultrasonography, puncture of the gallbladder, and collection of bile in 176 cows and heifers

The examinations were performed in 176 cows and heifers of the Swiss Braunvieh, Simmental, and Holstein breeds (Wolfensberger, 1993). The animals were eight months to 16 years old (mean ± SD, 5.6 ± 3.1 years). The animals were slaughtered within three weeks of cholecystocentesis. Ultrasonography was performed according to a described method (Braun, 1990), using a linear real-time scanner (Dynamic Imaging, Concept 2000, Pameda AG, Münchenstein, Switzerland) on the right side of the abdomen while cows were standing. The hair over an area between the 8th and 12th intercostal spaces was clipped and shaved. After application of transmission gel, each intercostal space was scanned by use of a 3.5-MHz linear transducer, beginning dorsally and progressing ventrally. Initially, the texture of the liver, hepatic and portal veins, visceral and diaphragmatic surface of the liver, and the gallbladder was examined.
Percutaneous cholecystocentesis was performed according to a described method at the location where the organ was best visualized via ultrasonography (Braun and Gerber, 1992). Animals were not sedated. The region for percutaneous cholecystocentesis was disinfected and anesthetized locally using 10 ml of 2% lidocaine. A spinal needle with a stylet (20 gauge × 3.5 inch, 0.90 × 90 mm, Terumo Corp, Cosanum Ancilla AG, Schlieren, Switzerland) was introduced and guided by ultrasonography through the abdominal wall and the liver toward the gallbladder wall. The needle was advanced to the gallbladder wall, and with a light thrust, was pushed through. The end of the needle within the gallbladder was usually visible in ultrasonograms. The stylet was removed and, using a syringe, 10 to 15 ml of bile was aspirated. Ten milliliters of isotonic NaCl solution was infused in the gallbladder to stir up liver fluke eggs, which normally sediment to the lowest point within the gall-

bladder. Immediately after this, 10 ml of bile was again aspirated for demonstration for liver fluke eggs.

Puncture of excised gallbladders in 100 cows and heifers after slaughter

In 100 Swiss Braunvieh cows and heifers, centesis was performed in gallbladders excised immediately after slaughter. The procedure was carried out by introducing a spinal needle into gallbladders still attached to the liver, analogous to ultrasound-guided puncture. Ten milliliters of isotonic NaCl solution was infused in the gallbladder, and 15 ml of bile was aspirated immediately thereafter.

Examination of bile for liver fluke eggs

Bile was poured into conical centrifuge tubes, which were placed in a refrigerator (4 °C) over night. Then the sediment was aspirated by use of a Pasteur pipette, placed on a glass slide, and examined at 10 × magnification for liver fluke eggs.

Fecal sampling

In each cow, a fecal sample was obtained from the rectum using a clean rectal glove. The feces were placed in a plastic container and stored in a refrigerator (4 °C) until examined.

Examination of fecal samples for *F. hepatica* eggs

The fecal samples were examined according to a modified version of the method described by Happich and Boray (1969). Six grams of feces and approximately 30 ml of water were mixed in a porcelain pestle to form a homogeneous suspension. This suspension was filtered through a sieve with a pore size of 300 µm into a 250 ml beaker. The sieve was rinsed with a stream of water so that there was 200 ml of liquid in the beaker. The liquid was allowed to sit for three minutes to aid sedimentation. Then the supernatant was discarded until a small opaque amount of sediment remained; the latter was resuspended by adding water. This procedure was repeated twice. After discarding the supernatant a third time, the sediment was stained with two to three drops of 1% methylene blue stain, placed in a 50 ml Corning culture flask, and examined with a stereoscopic microscope at 50 × magnification.

Examination of feces for *D. dendriticum* eggs

Fecal samples were examined for *D. dendriticum* eggs according to the method of Schmidt (1971). Four grams of feces and 30 ml of saturated zinc chloride solution (specific gravity 1.3) were mixed in a pestle until a homogeneous suspension was formed. This was poured through a sieve with a pore size of 300 µm into a 100 ml graduated cylinder. The sieve was rinsed with 30 ml of zinc chloride solution. The suspension of feces was mixed by blowing air through it via a 5 ml pipette. Both sides of a counting chamber (Therapogen-Zählkammer, Sharp & Dohme GmbH, Munich, Germany) were filled with the suspension and it was examined for *D. dendriticum* eggs at 100x magnification using a light microscope.

Examination of the liver and abdominal cavity at slaughter

All cows were inspected by official meat inspectors according to state law after slaughter (Eidgenössisches Veterinäramt, 1976). The liver was examined for adult liver flukes, and the liver, gallbladder, and adjacent peritoneum were examined for lesions attributable to cholecystocentesis. The liver was examined macroscopically and by deep palpation. In addition, the liver was cut once through the bile ducts of the quadrate lobe and once through the bile ducts of the caudate process.

Statistical analysis

The findings in the liver, feces, and bile were compared. The findings in the liver were used as a reference. For evaluation, the results of both groups of cows were summarized into one group because it was assumed that the number of liver fluke eggs in the bile was not influenced by slaughter. Statistical calculations were performed by means of a calculation program, according to the method of Norusis (1990). The relative frequencies were calculated. The prevalence, sensitivity, accuracy and negative predictive value were calculated in order to determine the diagnostic potential of each of the examined methods (Thomas, 1988).

Results

Ultrasonographic examination

Ultrasonographically, the gallbladder appeared as a typical fluid-filled vesicle of variable size as earlier described (Braun, 1990; Braun and Gerber, 1992). The gallbladder was visualized from the eighth, ninth, tenth or eleventh intercostal space in 162 of 176 cows and was successfully punctured in 156 of these cows. The distance between the body surface and the gallbladder varied from 0.7 to 8.3 cm (3.0 ± 1.28). At slaughter, no lesions attributable to cholecystocentesis were found in the gallbladder or peritoneum.

Comparison of findings in fecal samples and in the liver with regard to *F. hepatica*

F. hepatica eggs were found in the feces of 28 of 41 cows with adult flukes in the liver and in the feces of 23 of 204 cows with no adult flukes in the liver. In all other cows there were no fluke eggs demonstrated in the feces (Table 1).

Comparison of findings in bile samples and in the liver with regard to *F. hepatica*

F. hepatica eggs were found in bile samples of 40 of 41 cows with adult flukes in the liver and in bile samples of 27 of 204 cows with no adult flukes in the liver. In all other cows there were no fluke eggs found in the bile (Table 1).

Table 1: Demonstration of Fasciola hepatica *eggs in feces and in bile in relation to the identification of adult liver flukes in the liver of 245 cows*

	Cows with adult F. hepatica		Cows with no adult F. hepatica	
Variable	Fluke eggs found	No fluke eggs found	Fluke eggs found	No fluke eggs found
Feces	28 (68%)	13 (32%)	23 (11%)	181 (89%)
Bile	40 (98%)	1 (2%)	27 (13%)	177 (87%)

Comparison of the findings in fecal samples and in the liver regarding *D. dendriticum*

D. dendriticum eggs were found in the feces of 13 of 49 cows with adult flukes in the liver and in the feces of 19 of 176 cows with no adult flukes in the liver. In all other cows, there were no fluke eggs found in the feces (Table 2).

Comparison of findings in bile samples and in the liver regarding *D. dendriticum*

D. dendriticum eggs were found in bile samples of 44 of 49 cows with adult flukes in the liver and in bile samples of 49 of 176 cows with no adult flukes in the liver. In all other cows, there were no fluke eggs in bile samples (Table 2).

Table 2: Demonstration of Dicrocoelium dendriticum *eggs in feces and in bile in relation to identification of adult liver flukes in the liver of 225 cows*

	Cows with adult *D. dendriticum*		Cows with no adult *D. dendriticum*	
Variable	Fluke eggs found	No fluke eggs found	Fluke eggs found	No fluke eggs found
Feces	13 (27%)	36 (73%)	19 (11%)	157 (89%)
Bile	44 (90%)	5 (10%)	49 (28%)	127 (72%)

Reliability of the examination of feces and bile for *F. hepatica* eggs

The prevalence of adult *F. hepatica* infections was 17% (Table 3). The sensitivity or the probability that cows with adult *F. hepatica* in the liver will be correctly diagnosed based on the results of examination of feces or bile was 68% for fecal examination and 98% for examination of bile. The negative predictive value or the probability that an animal, in which no fluke eggs were found in the feces or in the bile, did not have fascioliasis was 93% for fecal examination and 99% for examination of bile. The accuracy or the percentage of correct results was 85% for fecal examination and 89% for examination of bile. The specificity and the positive predictive value were not determined because there are no false positive findings in either the fecal or the bile examinations.

Table 3: Reliability of fecal and bile examination in terms of diagnosis of Fasciola hepatica *and* Dicrocoelium dendriticum *infection*

Variable	Prevalence (%)	Sensitivity (%)	Negative predictive value (%)	Accuracy (%)	Specificity (%)	Positive predictive value (%)
F. hepatica						
Feces	17	68	93	85	NA	NA
Bile	17	98	99	89	NA	NA
D. dendriticum						
Feces	22	27	81	76	NA	NA
Bile	22	90	96	76	NA	NA

NA = Not applicable

Reliability of the examination of feces and bile for *D. dendriticum* eggs

The prevalence of *D. dendriticum* eggs in bile and in feces was 22% (Table 3). The sensitivities of the fecal and bile examinations were 27 and 90%, respectively. The negative predictive values for the fecal and bile examinations were 81 and 96%, respectively. The accuracy of both examinations was 76%.

Discussion

The results of this study reinforce those of Braun and Gerber (1992) who reported that percutaneous ultrasound-guided cholecystocentesis is a safe procedure in cattle. However, in this study, the gallbladder could not be ultrasonographically visualized in 14 cows, and cholecystocentesis could not be performed in six cows even though the gallbladder could be imaged. This is in contrast to the earlier study (Braun and Gerber, 1992) in which the gallbladder was ultrasonographically visualized and punctured in all cows. A possible explanation is that the conditions for examination of these 20 cows, in which visualization and/or cholecystocentesis were not possible, were not ideal. The cows were either in the owner's barn or at the slaughterhouse and resisted hand-

ling; local anesthetic could not be used in cows prior to slaughter. These circumstances hindered successful cholecystocentesis. In the study of Braun and Gerber (1992), local anesthetic was always used, fractious cows were sedated, and when the gallbladder was too small, the cow was re-examined the following day prior to feeding. The latter is recommended when examining cows with very small gallbladders because in cows that are not fed, there is no reflex emptying of the gallbladder; the gallbladder remains full and usually can be visualized and punctured easily. It is important to note that before aspiration of a bile sample, isotonic NaCl solution must be infused in the gallbladder to stir up the liver fluke eggs, which normally sediment to the lowest point in the gallbladder.

The statistical attributes, prevalence, sensitivity, negative predictive value, and accuracy were used to analyse the diagnostic importance of the examination of feces and bile. The specificity and the positive predictive value were not determined because it was found that there were false negative results in the hepatic examination, which was used as a standard. Of 204 cows with no adult flukes in the liver, 23 had *F. hepatica* eggs in fecal samples and 27 had *F. hepatica* eggs in bile samples. Similarly, of 176 cows with no adult flukes in the liver, 19 had *D. dendriticum* eggs in fecal samples and 49 had *D. dendriticum* eggs in bile samples. These cows were not treated for fascioliasis or dicrocoeliasis between the time of sampling and slaughter, and it is unlikely that spontaneous expulsion occurred during this same time period, because the majority of cows were slaughtered a few days after sampling. It was also highly unlikely that there was a confusion or contamination of the samples during collection or examination. This means that the cows were incorrectly assessed by the meat inspector. Considering the large size of the liver, and that it was cut in only two places during inspection, it is possible to understand why an incorrect diagnosis may have been made in mild cases of liver fluke infection. This is similar to the findings of Neuhaus and Six (1964) who reported that of 510 slaughtered cows, 39% had *F. hepatica* eggs and 21.8% had *D. dendriticum* eggs in the gall bladder. However, adult *F. hepatica* or *D. dendriticum* were found in the liver of only 6.5% of these cows. Lemmermöhle (1973) also considered the findings of the fecal examination and determined that examination of bile is more sensitive than that of the feces, which in turn is more sensitive than inspection of the liver at slaughter.

Examination of bile is a more sensitive method of diagnosing dicrocoeliasis than examination of feces. In other words, the probability that a cow with adult flukes in the liver will be correctly diagnosed, is 98% based on examination of bile and 68% based on fecal examination. Of the cows with fascioliasis, only 2% were not diagnosed by examination of bile, whereas 32% were not diagnosed by examination of feces. More profound was the difference in sensitivities of the two tests with regard to *D. dendriticum*. The sensitivity of fecal examination was only 22%; that for examination of bile was 90%. Thus, for diagnosing *D. dendriticum* infection, examination of bile is a highly sensitive test whereas examination of feces can be considered worthless in individual animals (Feinstein, 1975). Bürger (1992) also reported that the present method of examining feces is not suitable for diagnosing dicrocoeliasis; there may be false negative results because eggs are released from the gallbladder irregularly in batches. Only 50 to 60% of animals with fascioliasis are diagnosed by the sedimentation technique (Boch and Supperer, 1983). Similar results were reported by Braun and Gerber (1992) who compared the findings of fecal and bile examination in 20 cows. In that study, *F. hepatica* eggs, were found in bile samples of seven cows and in fecal samples of only four cows. *D. dendriticum* eggs were found in bile samples of 12 cows and not at all in fecal samples. The difference in the sensitivities can be attributed to the fact that *F. hepatica* eggs can be easily differentiated from methylene blue-stained fecal particles (Eckert, 1992) because of their yellow colour and their considerable length of 130 to 145 µm (Thienpont et al., 1979). In contrast, *D. dendriticum* eggs are relatively difficult to identify. They are substantially smaller in length (38 to 45 µm) and they are dark brown. Thus, they are difficult to recognize microscopically in fecal samples but easily identified in bile.

In the future, the examination of feces will remain the method of choice for diagnosing liver fluke infection. This applies particularly to diagnosis on a herd basis where there is a greater probability of diagnosing liver fluke infection by examining many animals. Percutaneous ultrasound-guided cholecystocentesis and microscopic examination of bile are the most important aids for verifying the diagnosis in a cow which is suspected of having fascioliasis or dicrocoeliasis, but has non-specific clinical symptoms and negative results of fecal examination. This is particularly important because not only direct demonstration of eggs by fecal sedimentation but also indirect methods such as the ELISA, using serum, and determination of the level of the bile duct enzyme, gamma glutamyl transferase, are highly unreliable (Malone and Craig, 1990). Bile particularly appears to be the ideal medium for identifying *D. dendriticum* eggs. Currently, there is no diagnostic test for identifying fascioliasis and dicrocoeliasis as sensitive as the microscopic examination of bile. It is now possible to apply this test via percutaneous ultrasound-guided cholecystocentesis which is a rapid, easy, and low risk technique. Thus, microscopic examination of bile provides a substantial improvement of the present methods used for diagnosis of liver fluke infection. Furthermore, this method can be used for scientific purposes. For serological monitoring of fascioliasis, it would be more meaningful to use the results of examination of bile as a reference because this test is more sensitive than examination of feces.

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Etude comparée sur la fasciolose et la dicrocoéliose dans la galle, les fèces et le foie chez la vache

Un ponction de la vésicule biliaire sous contrôle par ultrasons a été tentée chez 176 vaches afin d'obtenir de la bile. La vésicule biliaire a été ponctionnée chez 100 autres vaches immédiatement après l'abattage. La bile a été examinée au microscope pour des œufs de la petite et de la grande douve. Parallèlement un échantillon de fèces a été examiné pour des œufs de douve chez chaque animal. Les résultats des deux groupes ont été analysés et le nombre d'œufs dans la bile et les fèces ont été comparés. Les observations dans le foie servaient de référence.
Les œufs de F. hepatica ont pu être détectés dans les fèces de 28 vaches et dans la bile de 40 parmi 41 vaches caractérisées par un résultat positif dans le foie et de 23, respectivement 27 parmi 204 vaches caractérisées par un résultat négatif. La sensitivité était 68% pour le détection des œufs de F. hepatica dans les fèces et 98% pour la bile. Les valeurs prédictives négatives étaient 93% pour les fèces et 99% pour la bile.
En ce qui concerne D. dentriticum, le décèlement a été possible dans les fèces de 13 vaches et dans la bile de 44 parmi 49 vaches avec un résultat positif dans le foie et de 19, respectivement 49 parmi 176 vaches avec un résultat négatif. La sensitivité pour la détection des œufs de D. dentriticum était 27% dans les fèces et 90% dans la galle. Les valeurs prédictives négatives étaient 81% pour les fèces et 96% pour la galle.
Cette étude démontre que la détection des œufs dans la galle est nettement supérieure à un examen des fèces.

Analisi comparativa sulla fasciolosi e dicroceliosi nella bile, nelle feci e nel fegato della mucca

In 176 mucche si è cercato di pungere, mediante controllo ultrasonografico, la cistifellea e di aspirarne la bile. In altre 100 mucche la cistifellea è stata punta immediatamente dopo la macellazione. La bile e le feci sono state analizzate microscopicamente alla ricerca di uova di fasciola epatica e di dicrocelium dendriticum. I risultati dei due gruppi sono stati riuniti ed i referti delle analisi fra feci e bile sono stati confrontati. Come riferimento fungeva il ritrovamento di fasciole nel fegato.
Si sono potute rilevare uova di fasciola epatica, nelle feci in 28 casi, e nella bile in 40 casi su 41 mucche risultate portatrici di fasciola epatica nel fegato e in 23 respettivamente 27 casi su 204 mucche risultate non portatrici di fasciole nel fegato. La sensitività dell'analisi per l'accertamento di fasciola epatica nelle feci risultava essere del 68%, mentre che per l'analisi nella bile questa era del 98%. I valori negativi prevedibili erano, per le feci del 98% e per la bile del 99%.
Riguardo a dicrocelium dendriticum si è riusciti ad accertare la presenza di uova nelle feci in 13 casi e nella bile in 44 casi su 49 mucche riscontrate portatrici di dicrocelium nel fegato e in 19 rispettivamente 49 casi su 176 mucche non portatrici di dicrocelium nel fegato. La sensitività dell'analisi per l'accertamento di dicrocelium dendriticum nelle feci risultava essere del 27%, mentre che per l'analisi nella bile questa era del 90%. I valori negativi prevedibili risultavano per le feci del 81% e per la bile del 96%. I risultati dimostrano che l'accertamento delle uova nella bile è molto più affidabile dell'accertamento delle uova nelle feci.

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