FOOD/FARMED ANIMALS

Dicrocoeliosis in sheep in England and Wales: under diagnosed and misdiagnosed?

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SUMMARY

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We report four cases of dicrocoeliosis in sheep of untraced origin at an abattoir in Wales in 2015. Liver presentation ranged from severe, with extensive disseminated fibrosis and small bile duct hyperplasia and ectasia, to mild, characterised by occasional small white lesions on the subcapsular surface. Incising the liver revealed black fluid containing Dicrocoelium dendriticum. In another case, from North West England, adult parasites were found in the gall bladder-there were no liver lesions—and eggs were present in faeces. The cases demonstrate that this condition may appear in sheep in UK outside the known endemic areas of western Scotland. Surveillance is therefore necessary but only achievable through accurate diagnosis. Investigators must be aware that lesions can appear similar in some cases to those caused by other parasites, especially Fasciola hepatica and Cysticercus tenuicollis, and that coprological methods to detect sheep nematode eggs are insensitive for this parasite.

BACKGROUND

Dicrocoelium dendriticum, the Lancet fluke, affects ruminants in many parts of the world, causing impaired liver function and economic losses (Otranto and Traversa 2002, Otranto and Traversa 2003). This parasite has a wide host range that includes many wildlife species and is common in sheep flocks in many European countries, including France, Italy and Greece with prevalences of up to 100 per cent recorded (reviewed by Ducháček and Lamka 2003, Manga-Gonzalez and others 1991). In contrast, transmission foci of ovine dicrocoeliosis in the UK appear very restricted, historically recognised as existing mainly on the islands off the west coast of Scotland and in some parts of western mainland Scotland (Tarry 1969). As far as we are aware, the only reports south of the scottish border appear in a communication from the Ministry of Agriculture and Food, some 50 years ago (Rowlands 1963) and concerned autochthonous transmission in Anglesey and Cumbria. More recently however, cases have been reported in feral and indigenous goats in North Devon (Cranwell and others 2010). According to textbooks, severe infections manifest as numerous small areas of fibrosis on the liver surface with cirrhosis occurring in the more extreme cases (Taylor and others 2016). Diagnosis of dicrocoeliosis can be confirmed by finding tiny, thin lancet-shaped flukes (approx. 5-12 mm x 1.5-2.5 mm) in the small bile ducts and in the gall bladder.

Although ovine dicrocoeliosis is a disease known to veterinarians and meat inspectors in parts of Europe, staff working in UK abattoirs will be largely unfamiliar with its presentation, thus compromising surveillance and recognition of possible new authochthonous foci of transmission. For these reasons, here we report various types of liver lesions in four sheep at a Welsh abattoir. We also report a case of a sheep grazing in North West England in which there were no obvious liver lesions, but parasites were found in the gall bladder and eggs in faeces.

CASE PRESENTATION

The four infected sheep livers were coincidental findings in a single routine collection of some various tissues that had been rejected at slaughter in an abattoir in northeast Wales in March 2015 and subsequently used in teaching and training by Liverpool University's Institute of Veterinary Science. The carcases had been passed as fit for human consumption; consequently it is assumed that none of the animals had been emaciated or had shown signs of general disease. The four livers exhibited variable severity of cholangiohepatitis with changes linked to the observed burden of intralesional D. dendriticum. In one case (Fig 1a) the presentation was of classical severe disease: the subcapsular surface showing disseminated white linear (1mm-10mm x 1-3 mm) to round (3-5 mm in diameter) mildly raised areas and lesions throughout appeared as short branched cords. When the liver was cut, these lesions revealed thickened, small-sized bile ducts; the consistency of the liver was variably firm to highly friable and exhibited sharp edges. When the cut organ was compressed, small bile ducts consistently oozed thick black viscous fluid (Figs 1b and 2c) containing copious clumps of lancet flukes, some of which were found to be degenerated. These clumps were not easily recognisable as aggregates of small parasites in the first instance, and it was only when dispersed and cleaned in a little water that D. dendriticum could be confirmed, under low power microscopy (Fig 1c). Other livers (eg, Fig 2a,b) were far less severely affected and had fewer of the lesions described above. In these cases the overall liver architecture and subcapsular surface was largely unaffected; however, isolated/multifocal round fibrotic cyst-like lesions were evident, which also revealed lancet flukes when cut (Fig 2d-e).

The case from North West of England was an adult female Valais black nose sheep previously imported from Switzerland and grazed on a small holding (no



FIG 1: (a) Severe infection with Dicrocoelium dendriticum; (b) severely infected liver, cut to show clumps of parasites in small bile ducts; (c) clumps of cleaned parasites from small bile ducts.

other details unavailable). The animal had neurological signs and was submitted to the VLA laboratories in Shrewsbury to investigate the cause of death. There was no evidence of any lesions in the liver, but small numbers of adult *D. dendriticum* were found incidentally in the gall bladder. *D. dendriticum* eggs could be detected in faeces using a standard improved modified McMaster technique, but using zinc sulphate greatly improved sensitivity of the assay.

DISCUSSION

This occasionally zoonotic fluke has a very complex life-cycle that involves a land snail and an ant as intermediate hosts. Young D. dendriticum enter through the opening of the common bile duct, and sometimes through the portal circulation of the final host-they do not migrate through the parenchyma but remain in the small bile ducts where they develop. The maturing flukes irritate the bile duct surfaces by means of buccal stylets that results in hyperplasia and ectasia of parasitised ducts and at times local fibrosis of the immediately surrounding parenchyma, as shown here. The lesions described could be mistaken for fibrous changes following migration of Fasciola hepatica or indeed other common forms of fibrosis and postnecrotic scarring described by Taylor and others (2016) for F. hepatica. Furthermore, black material from cut lesions could be confused with the dark metabolic products seen in bile ducts and produced by adult F. hepatica. Diagnosis at the abattoir level is, then, challenging and made more so by the fact that the isolated or multifocal fibrotic cyst-like lesions we describe might be misdiagnosed

as small developing or degenerated *Cysticercus tenuicollis*, or possibly *Echinococcus granulosus*.

In the laboratory, the McMaster egg flotation technique on faeces from the case from North West England was imperfect and showed that D. dendriticum eggs will sink quickly when sodium chloride or sucrose (two saturated salts commonly used in parasitology labs to quantify gastrointestinal nematode eggs) is used. Indeed, a preliminary experiment showed that lancet fluke egg counts were initially 400 eggs per gram but reduced to 100 eggs per gram after leaving in these solutions for several minutes. When saturated zinc sulphate with a high specific gravity (1.3-1.45) was used, there was a significant increase in egg count to 2300 eggs per gram. Egg counts remained at this level in the assay and without collapse of the egg structure (Personal communication, Claire Corfield, Parasitology Dept, APHA Shrewsbury) as occurs with F. hepatica eggs under the same conditions. Sedimentation techniques used for F. hepatica and paramphistomes have also been shown to be inefficient and insensitive for Dicrocoelium eggs due to the low numbers of eggs usually excreted and their small size (Otranto and Traversa 2002).

In conclusion, dicrocoeliosis can be diagnosed in sheep in England and Wales but is challenging at the abattoir and laboratory postmortem levels and during faecal analysis. Differential identification of lesions mainly concerns *F. hepatica*. We have recently seen a case of *D. dendriticum* and *F. hepatica* coinfection, again in Wales, the details of which we will report in due course.



FIG 2: (a) Fibrotic lesion on surface of infected liver; (b) round fibrotic lesion on surface of infected liver; (c) cut surface of liver showing clumps of parasites within bile ducts; (d) cut surface of area of fibrosis involving small bile ducts and neighbouring parenchyma showing parasites in a paracentral small bile duct; (e) cut surface of liver showing parasites within bile ducts.

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