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Short communication

Liver flukes promote cholelithiasis in sheep

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ABSTRACT

The main objective of this study was to investigate whether cholelithiasis in sheep is related to parasitism or other commonly observed disorders such as liver abscesses. Additionally, the features of the observed biliary calculi are described. The livers of 254 randomly selected clinically healthy adult dairy sheep were used. All visible concretions in the bile were considered as stones. Based on the macroscopical examination, 60 livers were normal, 40 were parasitized with *Fasciola hepatica*, 42 were parasitized with *Dicrocoelium dendriticum*, 28 were parasitized with both *D. dendriticum* and *F. hepatica*, 40 livers had abscesses and 44 had hydatid cysts. Biliary calculi were detected in 40 livers. Twenty livers had pigment stones and 20 livers had cholesterol stones. The percentage of cholelithiasis was significantly higher in livers parasitized with flukes compared with the others ($P < 0.05$) and the parasitoses with *F. hepatica* and *D. dendriticum* were proven to be significant risk factors ($P < 0.05$) for the presence of cholelithiasis in sheep.

1. Introduction

Cholelithiasis in sheep is not commonly observed in clinical practice. However, it is referred that biliary calculi are frequently detected in slaughtered animals and there is evidence in the literature suggesting that the prevalence of cholelithiasis in some countries is high among clinically healthy sheep (Petruzzi et al., 1988; Cavallini et al., 1991). In almost all the available reports, the calculi are observed in the gallbladder and are classified as pigment stones (Petruzzi et al., 1988; Cavallini et al., 1991).

The pathogenesis of biliary calculi formation in sheep is currently unknown. Some researchers have suggested that gallstone formation is related to high total bilirubin concentration in the bile and the presence of deconjugating bacteria (Cavallini et al., 1991). However, there is no reference correlating cholelithiasis with liver diseases and especially liver parasitosis. In human medicine, the presence of small, multiple intrahepatic stones is associated, among others, with liver parasitism and especially with chronic fasciolosis (Valero et al., 2003). Furthermore, it has been observed that cholelithiasis is the most common accompanied disease in patients with liver hydatid cysts (Mergen et al., 2007).

As parasitism with liver flukes of *Fasciola hepatica* and *Dicrocoelium dendriticum* and the presence of hydatid cysts are quite common in sheep, at least in Greece (Theodoropoulos et al., 2002; Christodoulopoulos et al., 2008) where the present study was carried out, it is possible that these conditions may act as predisposing factors for cholelithiasis in sheep. The purpose of this research was to investigate whether calculi formation is related to parasitism or other commonly observed disorders, such as liver abscesses. A complementary objective of the study was

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to describe the features (type and location) of the biliary calculi detected.

2. Materials and methods

Experimental design: The livers of 254 clinically healthy adult dairy sheep were randomly selected prior to slaughter in 4 visits to the local abattoir of Karditsa in Greece. Immediately after slaughter, the livers were collected, labelled and examined macroscopically, and any observed lesions were recorded. Afterwards, the gallbladder and the major bile ducts were opened with a scalpel and the presence of calculi was recorded. All visible concretions in the bile were considered to be stones. The calculi were divided into gallbladder stones and bile duct stones, according to their anatomical location (Kim et al., 2003). Based on their external appearance, the white to yellow mulberry shaped calculi were classified as cholesterol stones (CS) (Kim et al., 2003) and the small, dark (black and brown), hard ones as pigment stones (PS) (Stewart et al., 2002).

According to the macroscopical lesions recorded, the livers were divided into the following six groups: (i) the normal group consisted of the livers without any macroscopical lesion, the (ii) Fh, (iii) Dd and (iv) FD groups of those parasitized with *F. hepatica, D. dendriticum*, and both *F. hepatica* and *D. dendriticum*, respectively, (v) the group (Hc) which consisted of livers that had hydatid cysts and finally (vi) the Ab group which consisted of the livers that had abscesses.

Statistical analysis: Fisher's exact test was run to compare the percentages of cholelithiasis for the various pathological liver conditions. The relative risk (RR) of cholelithiasis associated with the liver diseases considered was estimated using binary Logistic Regression Analysis (SPSS® 15). The model was run forward stepwise with probability for entry in the model $P < 0.05$ and the Normal group was used as the reference variable. The Hosmer–Lemeshow goodness-of-fit statistic was used to determine whether the model adequately reflects the data.

3. Results

Of the 254 livers that were used in the present study, 60 were found to be macroscopically normal, 40 were parasitized with *F. hepatica*, 42 were parasitized with *D. dendriticum*, 28 were parasitized with both *D. dendriticum* and *F. hepatica*, 40 livers had abscesses and 44 had hydatid cysts.

Biliary calculi were detected in 40 livers (15.8%). Gallbladder stones were found in 14 out of 40 livers (35%), bile duct stones in 20 out of 40 (50%), both bile duct and gallbladder stones in 6 out of 40 (15%). Concerning their type, the calculi were classified as CS in 20 out of 40 (50%) livers and as PS in the other 20 cases (50%). All gallbladder stones were CS whereas the bile duct stones were PS. In cases that both bile duct and gallbladder stones were detected, all the stones were CS. The different forms and the location of the calculi detected in each group are summarized in Table 1.

As shown in Table 2, the percentage occurrence of cholelithiasis was significantly higher in the Fh, Dd and FD groups compared to the Normal, Hc and Ab groups ($P < 0.05$). Furthermore, the animals in the Fh, Dd and FD groups were at higher risk for the presence of cholelithiasis ($P < 0.05$). The calculi obtained from the Normal and FD groups were all classified as CS. All the stones detected in the Fh group were PS, whereas of the 18 livers with cholelithiasis in the Dd group 12 had CS and 6 had PG.

4. Discussion

One of the objectives of the present study was to describe the features (anatomical location and type) of the biliary calculi in sheep presenting cholelithiasis. For this reason, the livers of 254 randomly selected clinically healthy sheep were evaluated. The survey results proved that choleliths can be formed not only in the gallbladder but also in the bile ducts of sheep. There is no such evidence in the literature, since in previous studies only the gallbladder was inspected for the presence of stones (Petruzzi et al., 1988; Cavallini et al., 1991). This helps explain why the percentage of livers with cholelithiasis recorded here is higher in comparison with previous reports (Petruzzi et al., 1988; Cavallini et al., 1991).

In all cases of cholelithiasis in sheep discussed in the literature (Petruzzi et al., 1988; Cavallini et al., 1991; Khaki, 2005), the biliary calculi detected were classified as pigment stones. In the present study, however, apart from pigment stones, cholesterol stones were found in half of the cases; to the authors' best knowledge this is the first report of cholesterol stones in sheep. The pathogenesis of pigment and cholesterol stone formation in sheep is currently unknown. In human medicine, it is nowadays accepted that pigment stone formation is associated with the presence of bacteria (Stewart et al., 2002) and that cholesterol stone formation is related to bile stasis (Kim et al., 2003).

The main goal of the present study was to investigate whether biliary stone formation is related to the most commonly observed liver pathological conditions. The percentage of cholelithiasis was significantly higher in livers parasitized with liver flukes in comparison with normal livers and those with abscesses or hydatid cysts. Based on the population examined, it was proved that parasitism with *F. hepatica*, *D. dendriticum* and with both *F. hepatica* and *D. dendriticum* significantly increases the risk of cholelithiasis 32, 44 and 20 times, respectively.

The lithogenic capacity of *F. hepatica* is well documented in rats (Valero et al., 2000; Valero et al., 2003) and cases of lithiasis, concomitant with choric fasciolosis, are frequent in human medicine (Yilmaz et al., 2004; Mas-Coma et al., 2005; Kabaalioglu et al., 2007). Furthermore, in cases of fasciolosis in human and rats, the biliary calculi detected are typically described as pigment stones (Valero et al., 2006), as at the present study. Based on the pathogenesis of pigment stones, it can be supported that the formation of pigment stones in sheep with fasciolosis is due to the presence of bacteria in the biliary tree. In accordance to this point of view is the observation that advanced fasciolosis is associated with bacterobilia in rats (Valero et al., 2006).

There is no evidence in the literature to indicate that *D. dendriticum* infection is related to cholelithiasis. The present study revealed that the parasitosis with *D. dendriticum* is a significant risk factor for cholelithiasis in