Prevalence, risk factors, and biochemical markers in dogs with ultrasound-diagnosed biliary sludge

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Abstract

Regarded as an incidental finding, biliary sludge is often diagnosed in dogs on abdominal ultrasound. The aims of the present study were to assess the risk factors, biochemical markers and ultrasonographic findings and to estimate the prevalence and influence of different breeds, sexes, and ages on biliary sludge in dogs. Results demonstrate that the prevalence of biliary sludge is high, especially in senior dogs. The biochemical markers did not have a significant correlation with biliary sludge, and the type of diet was not considered to be the major risk factor. Hepatomegaly was frequently observed on the ultrasound scan of affected animals and of dogs on different systemic drugs and with cardiopathies, which have been referred to as risk groups for the development of inspissated bile.

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1. Introduction

There are few reports about diseases of the biliary vesicle in dogs (Kirpensteijn et al., 1993; Besso et al., 2000; Holt et al., 2004; Ward, 2006; Romero et al., 2008), however, biliary sludge is frequently identified in dogs during abdominal ultrasonography performance (Brömel et al., 1998; Bandyopadhyay et al., 2007), being considered an incidental finding (Mannion, 2006).

On microscopy, biliary sludge has been defined as a mixture of precipitated crystals, glycoproteins, proteins, cell debris, and mucin. Its composition varies, but cholesterol monohydrate crystals, calcium bilirubinate, and other calcium salts are the most common components (Ko et al., 1999; Jüngst et al., 2006).

On ultrasound, sludge appears as low-level echoes without acoustic shadowing (Jüngst et al., 2006) and with gravity-dependent motility (Besso et al., 2000).

In humans, it is considered abnormal (Quinn and Cook, 2009) and associated with long fasting periods, long-term administration of total parenteral nutrition, pregnancy, bone marrow or solid organ transplantations, gastric surgeries, rapid weight loss, and ceftriaxone and octreotide therapies (Abey-surija et al., 2010). Its natural course ranges from complete resolution to progression to gallstone disease (Jüngst et al., 2006) and its complications include biliary colic, cholangitis, and acute pancreatitis (Abey-surija et al., 2010).

In dogs, thickened bile can be associated with diseases, but it is a frequent finding among clinically healthy older dogs (Quinn and Cook, 2009). To date, its clinical importance remains unknown (Brömel et al., 1998; Nyland et al., 2005; Quinn and Cook, 2009).

The present study sought to estimate the prevalence of biliary sludge, the influence of age, breeds and sex on its development, and to assess risk factors, biochemical factors, and ultrasonographic findings in dogs, irrespective of their health status.

2. Materials and methods

Biliary sludge was diagnosed by ultrasonography using a Mindray DC-6 Vet color Doppler console, with convex multifrequency (5, 6.5 and 8 MHz) and linear multifrequency (5, 10 and 12 MHz) transducers.

The prevalence of sludge was determined, retrospectively, examining files from the Division of Imaging Diagnosis of PETLAB (Porto Alegre, RS, Brazil) between January and September 2010. From those 1021 cases, we examined the biliary sludge prevalence in different breeds, ages and sex. One hundred dogs, regardless of their health status, were randomly selected for blood sampling and application questionnaire during admission at the laboratory. In this study we evaluated risk factors, biochemical markers, and ultrasonographic findings with or without biliary sludge.
The 100 dogs randomly selected, whose owners have agreed to answer the questionnaires, underwent ultrasound examination, blood collection and were examined in right and left decubitus positions for proper evaluation of biliary sludge formation. Abdominal findings were recorded and bile contents were measured based on the presence of echodense material, graded as follows: 0+ absent, 1+ mild, 2+ moderate and 3+ severe (Fig. 1).

An adapted questionnaire (Sallander et al., 2001) containing questions on the type of diet, age, breed, sex, reproductive stage, body habitus, diseases, and use of medications was used to investigate risk factors in these 100 dogs.

After the abdominal ultrasound, a blood sample was collected by venipuncture of the patients’ jugular or cephalic vein, and kept in a vacuum red-top tube (4 mL), protected from the light, for analysis of biochemical markers.

2.1. Laboratory tests

The samples of the 100 dogs were centrifuged (at 2500 rpm per 10 min) after the blood clot retraction, 30 min after collections. The separated sera were kept in Eppendorf tubes, protected from the light, and stored at −20 °C until analysis (according to manufacturer’s instructions).

The following biochemical variables were analyzed: albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total proteins, bilirubins, cholesterol, triglycerides, and calcium. All analyses were performed using an automated spectrophotometer (Advia 1650, Siemens) for bilirubin and calcium (Dade Behring and Labtest reagents, respectively) and Metrolab DR-1600, Labquest and Celm (Labtest reagents) for the other parameters.

2.2. Statistical analysis

The SPSS 10.0 statistical package was used for the analysis, and Pearson’s correlation and regression analysis were employed for qualitative data, with statistical significance accepted at p < 0.05, i.e., with a 95% confidence interval.

The odds ratio (OR) and 95% confidence intervals (95% CI) were used for all data and inserted into a spreadsheet (Excel for Windows 2007). OR values between 1.5 and 3.5 were considered to have a small correlation, while those between 3.5 and 9.0 and those greater than 9.1 were considered to have moderate and high correlation, respectively, for biliary sludge outcome and previous exposure to the analyzed factor. Significant OR values were those which 95% CI did not exceed 1 whereas negative OR values were regarded as protective factors.

Owing to the large discrepancy of the results obtained for the serum biochemistry (Table 1), the statistical analysis was based only on odds ratio. The dogs were divided into two groups: those exposed to the risk factor, whose results were higher than the reference values for the species (Kaneko et al., 2008), and those not exposed to the risk factor, whose values were within the reference range.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sludge</th>
<th>Absence</th>
<th>Total</th>
<th>Odds ratio</th>
<th>CI</th>
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<td>6</td>
<td>14</td>
<td>1.6</td>
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<td>Unexposed</td>
<td>39</td>
<td>47</td>
<td>86</td>
<td></td>
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<tr>
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<td>Exposed</td>
<td>6</td>
<td>11</td>
<td>17</td>
<td>0.55</td>
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<tr>
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<td>42</td>
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<td></td>
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<td>30</td>
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<td>29</td>
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<td></td>
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<tr>
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<td>4</td>
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<tr>
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<tr>
<td></td>
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<tr>
<td>Total</td>
<td></td>
<td>47</td>
<td>53</td>
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</tr>
</tbody>
</table>

* Confidence interval.

2.3. Research ethics committee

The study protocol was approved by the Committee on Animal Research of Universidade Federal do Rio Grande do Sul – CEUA/UFRGS-RS, Brazil, protocol number 19733/2010.

3. Results

The prevalence of biliary sludge was 34.9% (356 out of 1021 dogs). No significant difference was observed between males and females when dogs with and without biliary sludge were compared. Of 393 males (38.5%) and 628 females (61.5%), 38.0% (253) of males and 62.0% (412) of females did not have biliary sludge and 39.3% (140) of males and 60.7% (216) of females presented with sludge.

With respect to age, 53 dogs were excluded because their ages could not be determined. Of the assessed dogs, 23.8% (243) were young (0–4 years), 31.9% (326) were middle-aged (5–9 years) and 39.1% (399) were old (10 years or older). Thickened bile was predominant among senior dogs, (p < 0.001), of which 56.5% (201) had biliary sludge, compared to 9.6% (34) among young dogs and 30.9% (110) among middle-aged dogs (Fig. 1).
Fifty-five breeds were identified. The most frequent ones were: Beagle (29), Cocker Spaniel (44), Labrador (36), Lhasa Apso (38), Pinscher (37), Poodle (180), Schnauzer (39), Teckel (51), Yorkshire (65), and mixed breed (191). Biliary sludge was more predominant in Beagle (5.1%/18), Cocker Spaniel (6.2%/22) and Poodle (23.3%/83) (p < 0.001).

In the 100 dogs randomly selected, biliary sludge was present in 47 and absent in 53.

As to the presence of echodense material, it was mild in 21% and moderate in 21% of the dogs. Only 5% of the dogs had high rates of biliary sludge. Out of the five animals which have shown a severe amount of sludge, three of them were senior and two were middle aged dogs. It was observed a considerable increase on ALP levels in three of these dogs. However, in one of them, it was found a concomitant increase of the ALT.

No statistically significant difference was noted for the analyzed biochemical parameters (Table 2). Despite these results, ALP in dogs with and without biliary sludge revealed mean values greater than the reference standards, but dogs with biliary sludge had higher means than those without it (426.49 U/L and 223.91 U/L, respectively).

Fourteen dogs whose fasting was inappropriate were excluded from cholesterol and triglyceride measurements. These analyses were not significantly different, but patients with biliary sludge had higher means for both analyses (134.17 mg/dL for triglycerides and 225.46 mg/dL for cholesterol), compared with dogs without biliary sludge (89.56 mg/dL for triglycerides and 223.46 mg/dL for cholesterol).

All patients showed bilirubin levels within normal range.

Regarding dietary habits, there was no correlation with eating frequency, intake of human food and of homemade food, commercial feed, or association of both. Patients fed treats such as rawhide chews, biscuits, among others, did not reveal a higher frequency of biliary sludge (p = 0.015).

No difference was found between body habitus and reproductive stage in the analyzed groups.

Animals on systemic drugs showed a higher prevalence of biliary sludge (p = 0.022).

Thickened bile was associated with hepatomegaly in 61.8% (21) (p = 0.034) of the 47 dogs diagnosed with sludge on ultrasonographic examination. With respect to concomitant diseases, 82.4% (14) (p = 0.001) had cardiopathies.

The odds ratio for the variables in the random sample had a weak or no correlation with the risk factors and biochemical parameters analyzed. For cardiovascular diseases, there was a moderate correlation with the presence of biliary sludge (OR: 7.07; 95% CI = 1.88–26.62).

4. Discussion and conclusions

These results highlight the high prevalence of biliary sludge in dogs with different medical conditions, as well as the absence of influence of gender for the presence of biliary sludge in dogs, once the prevalence in males and females was similar as seen in previous reports (Brömel et al., 1998; Bandyopadhyay et al., 2007).

The influence of age is evident in this study and it corroborates with other studies (Brömel et al., 1998; Quinn and Cook, 2009). The mechanisms which senior dogs are most affected are not yet elucidated, but it is possible to assume that due to advanced age, it might occur lower emptying velocity of vesicle or the higher frequency of sludge in these dogs could be associated to higher incidence of other disorders that cause biliary composition and flow rate alterations.

The assessment of breed distribution due to some diseases varies from region to region because of local breeding preferences, thus limiting a comparison between different studies as well as the assertion that some breeds are more susceptible to biliary sludge formation. Unlike it was previously reported, there was a higher prevalence in Beagle, Cocker Spaniel and Poodle breeds (Bandyopadhyay et al., 2007).

In humans, one of the most common complications due to biliary sludge is the formation of gallstones (Jüngst et al., 2006). In dogs, despite the high prevalence of biliary sludge, there are few reports on biliary lithiasis (Kirpensteijn et al., 1993). It has been proposed that mucocele formation in dogs is resulted by sludge progression. (Besso et al., 2000; Ward, 2006), however, there are few studies about this disturb as well (Besso et al., 2000; Romero et al., 2008). Considering the low prevalence of severe bile thickenings diagnosed in this study, it is possible to confirm the hypothesis that biliary sludge can barely bring about more serious complications like mucocele and biliary lithiasis. However, in order to better evaluate the consequences of biliary sludge in the long run, it would be necessary to conduct a prospective study in which dogs diagnosed with sludge would be monitored for several years to assess possible outcomes and alterations in blood biochemistry over time.

With the aim of evaluating the pathological status of the biliary sludge, it would be interesting to perform an ultrasonographic evaluation for dogs displaying cholestasis already identified by clinical findings and alterations of biochemical markers to allow a comparison between the degree of gravity of biliary sludge echographically assessed and the gravity of the cholestasis assessed for biochemical markers (e.g. ALP, GGT, bilirubin, cholesterol) then, seeking to identify more precisely which factors are a risk for the development of a more serious sludge that could evolve to cholelithiasis or mucocoele.

No biochemical markers were detected for biliary sludge. Bile retention is the strongest stimulus for the rapid production of alkaline phosphatase (Watson and Bunch, 2010). Although no significant difference was observed for ALP levels, affected dogs showed higher mean values, but this enzyme, used as cholestasis marker, is not liver-specific and is found in other tissues as well.
(Hoffmann and Solter, 2008); in addition, its levels can increase with the use of (exogenous or endogenous) corticosteroids, cephalosporins, barbiturates, phenobarbital, phenylbutazone, phenothiazines, tetracyclines, thiabendazole, and halothane (González and Silva, 2006). There were no evidences of bone affections in none of the animals in this study. This suggests no influence of bone alkaline phosphatase (BAP), nevertheless, to regard it as a marker of thickened bile, healthy dogs not submitted to drug treatments and presenting biliary sludge should be evaluated; unlike the present study, in which dogs with different concomitant diseases and submitted to different drug treatments were assessed.

In a similar study with two groups of dogs (with and without hepatobiliary diseases), no differences were observed as to AST, ALT, ALP, and total bilirubin, compared to dogs with and without biliary sludge (Brömel et al., 1998).

As already mentioned for humans, the biliary thickening is considered a precursor of biliary calculosis (Ko et al., 1999; Jüngst et al., 2006). In attempt to elucidate the pathogenesis of biliary calculus affection, various studies have assessed the specific role of dietary components as potential risk factor for gallstone formation (Paumgartner and Sauerbruch, 1991). Some of these studies included the intake of some types of fatty acids, cholesterol, fiber, carbohydrates, alcohol, and some vitamins and minerals (Paumgartner and Sauerbruch, 1991; Cuevas et al., 2004).

In dogs, the role of dietary in the formation of sludge is not known, however, it is known that the canine bile has low cholesterol and free calcium concentration, which limit the gallstone development (Fossum, 2005). In attempt to seek answers for the large prevalence of biliary sludge in dogs, it has been assessed the types of 100 patients dietary, identifying a mild correlation between the onset of the disease and food abuse with appetizers.

Tackling into account the results in human studies, it may assume that the high prevalence of sludge in animals which were receiving systemic drugs should be assessed with precaution because, despite existing human reports on the influence of some antibiotics related to the formation of biliary thickening (Lopez et al., 1991), the veterinary literature do not mention the association between drugs and biliary thickening.

In the present study, the names of the drugs used were not disclosed; only information on whether they were used sporadically or continuously was provided. Then, further studies testing different medicines with periodic ultrasonographic assessment are necessary.

Most of the dogs affected by sludge had cardiopathies; in addition, the ultrasonographic examination revealed that most of them suffered from hepaticomegaly as well. Hepaticomegaly could originate from passive congestion. This finding often indicates cardiac other than liver diseases (Nylund et al., 2005).

Hepatic congestion of cardiac origin may predispose cholestasis and in counterpart, facilitating the development of biliary sludge, as suggested in this report.

Cholestasis plays an important role in biliary sludge formation. Black and viscous material microscopically identical to sludge was induced 3 days after cystic duct ligation in 18 healthy dogs (Bernhoff et al., 1983), but there was a high prevalence of thickened bile without evidence of hepatobiliary diseases diagnosed by ultrasonography and/or serum biochemistry (Brömel et al., 1998).

In this study, the fact that we observed patients with biliary sludge related to cases of hepaticomegaly is probably associated with the higher prevalence of cardiovascular diseases in this group rather than with primary hepatobiliary disorders. The possible causes for the association between thickened bile and cardiovascular diseases were not determined and should be further investigated. However, as cardiovascular diseases are found in senior dogs (Carr, 2004), a correlation between age, cardiovascular disease and biliary sludge is suggested.

In this study, it was not possible to somewhat correlate the presence of sludge with the type of dietary, as well as establishing possible blood markers. Nevertheless, due to a variety of speculated data and the lack of work about this issue, this study serves as a contribution for further researches involving biliary sludge in dogs to determine the necessity of treatment and prevention as this is a condition frequently diagnosed in canine. Besides confirming previous findings in the veterinary literature, as well as a bigger predisposition of senior dogs and the absence of gender preference, this study still raises the question about the association of sludge with determined disorders and type of dietary. We may also assume that, unlike humans, which sludge can cause serious complications, in dogs, the progression of sludge to severe disease hardly occurs, nevertheless, it should not be ignored and the treatment must be considered.

In order to obtain more accurate information about the influence of these factors on the development of thickened bile, it would be necessary to carry out case-control studies that assess healthy dogs with and without sludge submitted to different controlled diets or to the use of specific drugs. Regular ultrasonographic assessments to determine when biliary sludge formation/dissolution occurs, besides additional tests such as microscopic analysis of the sludge and post-mortem histopathological analysis of the gallbladder wall, since the risks of performing this examination in vivo would not justify its application for research, it may contribute to shed some light upon sludge formation and its clinical significance in dogs.

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References


