# Prevalence and pathological findings of cholelithiasis in goats

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**Key words:**
goat, cholelithiasis, prevalence, bacteriology, pathology.

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Received: 11 January 2012
Accepted: 27 May 2012

***Abstract:***

**BACKGROUND:** Gallstones are concretions that form in the biliary system. There are two major types of gallstones (pigment and cholesterol), which seem to form due to distinctly different pathogenic mechanisms. **OBJECTIVES:** The aim of this study was to determine the prevalence and chemical composition of gallstones in goats. Bacteriologic analysis and pathological findings were reviewed. **METHODS:** The study was carried out on 411 goats at Juneghan abattoir in Chaharmahal-Bakhtiari province of Iran. **RESULTS:** Gallstones were found in the gallbladder of 5 goats (1.2%). Biliary calculi were more frequent in adult goats ($p<0.05$). No significant difference was seen between male and female goats with gallstones. Chemical analysis of the gallstones revealed 4 goats with pigment (bilirubin) stones and 1 goat with cholesterol stones. Chemical composition of bile in these goats was evaluated. Bacteriologic analysis of the bile in the affected goats revealed bacteria (*Escherichia coli* and *Salmonella* spp.) in 3 goats. Microscopic examination of gallbladders revealed cystic glands, necrosis and atrophy of mucosal layer, edema, focal and diffuse infiltration of lymphocytes in submucosal layer, and hypertrophy of smooth muscles in goats with gallstones. **CONCLUSIONS:** It was concluded that the prevalence of both types of gallstones in goats are low. Cholelithiasis can cause chronic inflammation of the gallbladder but it is not likely to become clinically significant.

## Introduction

Choleliths or gallstones are concretions that form in the biliary system, usually the gallbladder. Biliary tract diseases in large animals are rare. Cholelithiasis has been described in horses, cattle, sheep and pigs; however, it does not seem to be recognized as a clinical problem in sheep (Gerros, 2009). There are two major types of gallstones (pigment and cholesterol), which seem to form due to distinctly different pathogenic mechanisms. Pigment stones are composed of large quantity of bile pigments, along with less amounts of cholesterol and calcium salts. Cholesterol stones can be almost pure cholesterol or mixtures of cholesterol and substances such as mucin (Johnston and Kaplan, 1993). Biliary stone formation begins with the precipitation or aggregation of normally soluble components of bile. Other mechanisms involved in the pathogenesis include ascariasis, ascending biliary infection or inflammation, biliary stasis, changes in bile composition, and presence of a foreign body (Gerros, 2009).

The formation of biliary calculi is frequently reported as a cause of cholestasis in humans and has been reported in horses and cattle (Johnston et al., 1989). Choleliths in the gallbladder usually do not become clinically significant unless they migrate and obstruct the extrahepatic bile ducts (Cullen, 2007). Obstruction of the bile ducts or common bile duct by biliary calculi is a rare occurrence in farm animals. The etiology and pathogenesis of cholelithiasis in horses is uncertain. Retrograde bacterial infection from the small intestine is considered probable. Culture of liver biopsy material yielded *E. coli* and Bacteroides vulgatus from only a small number of affected animals (Radostits et al., 2007). The pigment gallstones are more frequently detected in sheep and they are associated with high total bilirubin concentration in the bile (Cavallini et al., 1991). Amazingly, the fetal sheep gallbladder is capable of containing concentrated bile that can produce gallstones. These stones are composed of calcium palmitate and pigment (Wood et al., 1974).

The clinical signs associated with cholelithiasis in the large animals are anorexia, weight loss, low milk yield, chronic intermittent diarrhea, recurrent attacks of sever abdominal pain, alimentary tract stasis, pain on percussion over the liver, pyrexia, jaundice, recumbency, depression and coma. (West and Hogg, 1988; Johnston et al., 1989; Radostits et al., 2007). Hyperammonemic hepatic encephalopathy and photosensitization are other less common features of cholelithiasis. A subclinical presentation, caused by partial obstruction of the biliary tree, may be recognized only on postmortem examination (Gerros, 2009). The lack of observational models for non-induced cholelithiasis in animals had contributed to the absence of epidemiologic studies, which have been done in human beings (Cavallini et al., 1991). In view of the paucity of information on cholelithiasis in goats, this paper reports the prevalence and composition of choleliths in this species. Bacteriologic analysis and histopathological findings were reviewed.

## Materials and Methods

The study was carried out on 411 native breed of goats (290 males and 121females) at Juneghan abattoir in Chaharmahal-Bakhtiari province of Iran from November 2009 to June 2010. Before slaughter, an antemortem examination was done on all goats during which their age and gender were evaluated. A division of young (≤2 years, n=270) and adult (>2 years, n=141) goats were done after inspection of their teeth. After slaughtering the goat, the gallbladder was removed and the surface and some sections of the liver were examined macroscopically. A short incision was made in the gallbladder by use of a scissors. In sterile conditions, a sample of bile was collected by a swab for bacterial culture. Then the gallbladder was opened and its contents passed through a filter (fourfold gauze) to collect the gallstones. A sample of bile was also collected from the goats with gallstones for chemical analysis. Number, diameter, weight, color, consistency and chemical composition of gallstones were de-termined. Gallstones were analyzed for the determin-ation of cholesterol (CHOD-PAP), bilirubin (DCA), calcium (arsenazo) and phosphorous (phosphometric UV) concentrations. Measurements were per-formed by commercial kits (Parsazmoon, Tehran, Iran) using an automated biochemical analyzer (Biotecnica, Targa 3000, Roma, Italy) in accordance with the instructions by the manufacturers. Oxalate was determined (enzymatic method) with a com-mercial kit (Darman Kav, Tehran, Iran) using the spectrophotometer (Clima, Madrid, Spain). Bile samples were analyzed by the same methods using Hitachi 704 autoanalyzer (Tokyo, Japan). Tissue specimens from gallbladders with gallstones were fixed in neutral buffered 10% formalin, processed, and sectioned. Tissue sections were stained with hematoxylin-eosin, and prepared for histopathological examination.

The SPSS version 16 statistical package was used for data analysis. The chi-square and Fisher's exact tests were used for comparing the groups, and $p<0.05$ was considered to be significant.

## Results

Five of the 411 goats (1.2%) had 3 to 10 gallstones in their gallbladders. The clinical sings associated with cholelithiasis were not observed in the affected goats. Necropsy examination revealed no visible findings in the liver of goats with gallstones. Number, diameter, weight, color, consistency and chemical composition of gallstones are shown in tables 1 and 2.

Table 1. Characteristics of the goats and their gallstones.

| Goat | | | Gallstone | | | | |
|---|---|---|---|---|---|---|---|
| No. | Gender | Age (year) | No. | Diameter (mm) | Total weight (g) | Color | Consistency |
| 1 | Female | 4 | 8 | 2 | 0.02 | Green | Soft |
| 2 | Male | 3 | 4 | 1 | 0.03 | Brown | Soft |
| 3 | Female | 1 | 10 | 2 | 0.01 | Green | Semi hard |
| 4 | Female | 4 | 6 | 3 | 0.01 | Black | Semi hard |
| 5 | Male | 2 | 3 | 3 | 0.02 | Dark green | Semi hard |

Table 2. Chemical composition of gallstones in the goats.

| Goat | Gallstone | | | | |
|---|---|---|---|---|---|
| No. | Bilirubin (%) | Cholesterol (%) | Oxalate (%) | Calcium (%) | Phosphorous (%) |
| 1 | 41 | 15 | 4 | 25 | 5 |
| 2 | 20 | 11 | 6 | 49 | 14 |
| 3 | 12 | 38 | - | 30 | 20 |
| 4 | - | 85 | - | 10 | 5 |
| 5 | 24 | 34 | - | 32 | 10 |

Chemical analysis of the gallstones revealed 4 goats with pigment (bilirubin) stones and 1 goat with cholesterol stones. Bacteriologic analysis of the bile in 5 goats with gallstones revealed bacteria (*Escherichia coli* and *Salmonella* spp.) in 3 goats. Concentrations of total and direct bilirubin, cholesterol, calcium and phosphorous in the bile of the goats with gallstones are shown in table 3. The gallstones were more frequently found in adult than in young goats ($p<0.05$). No significant difference was seen between male and female goats with gallstones. Microscopic examination of gallbladders revealed cystic glands, necrosis and atrophy of mucosal layer, edema, focal and diffuse infiltration of lymphocytes in submucosal layer, and hypertrophy of smooth muscles in goats with gallstones.

## Discussion

In the present investigation, both types of gallstones were found in goats. The most concentration of cholesterol was recorded in the bile of the goat with cholesterol stones. The key event leading to formation and progression of cholesterol stones is precipitation of cholesterol in bile. Unsterified cholesterol is virtually insoluble in aqueous solutions and is kept in solution in bile largely by virtue of the detergent-like effect of bile salts. This is however a rather precarious situation and several factors can tip the balance in favor of precipitation, including: hypersecretion of cholesterol into bile due to obesity, acute high calorie intake, chronic polyunsaturated fat diet and pregnancy; hyposecretion of bile salts due to impaired bile salt synthesis and abnormal intestinal loss of bile salts; impaired gallbladder function with incomplete emptying or stasis is seen in late pregnancy and due to unknown causes, perhaps associated with neuroendocrine dysfunction. There are clearly important genetic determinants for cholesterol stone formation in human (Johnston and Kaplan, 1993). The most important risk factor for development of pigment stones is chronic hemolysis from almost any cause- this makes sense considering that bilirubin is a major constituent of these stones. Additionally, some forms of pigment stones are associated with bacterial infections. Apparently, some bacteria release glucuronidases that deconjugate bilirubin, leading to precipitation as calcium salts (Johnston and Kaplan 1993). Concretions in the biliary system of cattle are usually a sequel to fascioliasis (West and Hogg, 1988).

The prevalence of gallstones in surveyed goats was 1.2%, which was less than the prevalence in sheep (8.6%) reported by Cavallini et al., 1991. A study on prevalence of cholelithiasis in sheep indicated that 11.7% of sheep had gallstones

Table 3. Chemical composition of bile in the goats with gallstones.

| Goat | Bile | | | | |
|---|---|---|---|---|---|
| No. | Total bilirubin (mg/dl) | Direct bilirubin (mg/dl) | Cholesterol (mg/dl) | Calcium (mg/dl) | Phosphorous (mg/dl) |
| 1 | 11.3 | 7.6 | 39 | 20.5 | 7.8 |
| 2 | 19 | 7 | 59 | 20 | 15 |
| 3 | 16 | 11 | 50 | 12 | 19 |
| 4 | 19.8 | 7 | 80 | 14 | 15 |
| 5 | 14.2 | 6 | 77 | 20 | 17 |

(Petruzzi et al., 1988). Khaki, 2005 reported that the prevalence of gallstones in sheep and cattle slaughtered in Tehran abattoirs in Iran were 4.75% and 5.6%, respectively. A study on the gallbladders of 36 fetal sheep of gestational age 102-147 days revealed a 50% incidence of gallstones (Wood et al., 1974).

In the present investigation, the gallstones were more frequently found in adult than young goats. The risk of gallstones increases with age in human beings. An increase in age may be directly proportional with an increase in cholesterol secretion and saturation (Shaffer, 2006). However, the greater prevalence of gallstones in lambs than in adult sheep has been reported by Cavallini et al.,1991. This finding could be explained in this manner, if the sheep to be slaughtered at a young age and those assigned to be slaughtered at an older age had a different alimentation. Erythrocytolysis also could be attributable to ovine babesiosis, which could be more common in young nonimmunized animals (Cavallini et al., 1991).

The current study shows no significant difference between male and female goats with gallstones. There appears to be no obvious sex difference in animals (West and Hogg, 1988). Cavallini et al.,1991 reported that gallstones are more frequent in female sheep. Female sex hormones are the obvious basis for this gender difference (Shaffer, 2006). Progestrone and also probably estrogen impair gallbladder emptying and are associated with hypersecretion of cholesterol into bile. In addition, estrogen treatment reduces synthesis of bile acids. These proprecipitation factors peak during late pregnancy when the levels of these steroids hormones are at the highest (Johnston and Kaplan, 1993). However, the results of one study indicated higher frequency of gallstones in male sheep (Petruzzi et al., 1988).

In this study, most of the gallstones found in the goats were pigment stones. This is in agreement with the results reported by Petruzzi et al. ,1988, Cavallini et al., 1991; Khaki, 2005. All gallstones that they found in sheep and cattle were pigment type. These findings indicate that pigment cholelithiasis is more common in ruminants. In most reports of cholelithiasis in horses the chemical analysis has shown that choleliths have a mix composition, containing bilirubin, bile pigments, cholesterol esters, esters of cholic and carboxylic acid, calcium phosphate, and sodium taurodeoxycholate (Gerros, 2009). In one study, 80% of the choleliths in horses contained less than 10% cholesterol (Johnston et al., 1989).

Bacteriologic analysis of the bile in 3 of the 5 goats with gallstones revealed bacteria (*Escherichia coli* and *Salmonella* spp.). Isolation of bacteria from bile is common. In one study, bacteriologic analysis of the bile in 10 sheep with gallstones and 10 controls (without gallstones) revealed bacteria in 50% of the first group and 75% of the second group (Cavallini et al., 1991). Several pathogenic bacteria (*Salmonella* spp., *Escherichia coli*, *Aeromonas* spp., *Citrobacter* spp., group D *Streptococcus* spp., *Clostridium perfringens*) have been cultured from the bile ducts of horses and cows with cholelithiasis. Whether these bacteria were the cause or the result of the stone formation remains unclear (Gerros, 2009).

Microscopic examination of gallbladders revealed cystic glands, necrosis and atrophy of mucosal layer, edema, focal and diffuse infiltration of lymphocytes in submucosal layer, and hypertrophy of smooth muscles. These findings indicate chronic cholecystitis which is most likely to be due to the mechanical irritation of the gallbladder by biliary calculi. Cholecystitis may occur as a result of bacterial

infections such as salmonellosis. Other bacteria, either derived from the blood or ascended from the intestine, can cause acute or chronic cholecystitis. Chronic cholecystitis typically accompanies prolonged bacterial infection of the biliary tree or ongoing irritation from choleliths or parasites of the gallbladder (Cullen, 2007).

Based on the results of this study, the prevalence of both types of gallstones in goats are low. Although cholelithiasis can cause chronic inflammation of the gallbladder, it is not likely to become clinically significant.

## Acknowledgements

The authors wish to thank Dr. A. Khodabakhsh Sarbandi, Dr. H. Shojaei, Dr. A. Badakhsh and Dr. S. Baradaran for their contributions and support.

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مجله طب دامی ایران، ۱۳۹۱، دوره ۶، شماره ۲، ۷۷-۷۳

# بررسی میزان شیوع و یافته‌های آسیب شناسی سنگ‌های صفراوی در بز

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(دریافت مقاله: ۲۱ دی ماه ۱۳۹۰ ، پذیرش نهایی: ۲۹ خرداد ماه ۱۳۹۱)

## چکیده

**زمینه مطالعه:** سنگ‌های صفراوی توده‌های سفتی هستند که در داخل صفرا شکل می‌گیرند. به نظر می‌رسد دو نوع اصلی سنگ‌های صفراوی (رنگدان‌های وکلسترولی) توسط مکانیسم‌های کاملاً متفاوتی از نظرآسیب‌شناسی بروز می‌یابند. **هدف:** این مطالعه به منظور تعیین میزان شیوع، بررسی ترکیب شیمیایی و یافته‌های باکتری شناسی وآسیب شناسی سنگ‌های صفراوی در بز انجام گرفت. **روش کار:** در این تحقیق تعداد ۴۱۱ راس بز درکشتارگاه جونقان در استان چهارمحال وبختیاری مورد بررسی قرار گرفتند. **نتایج:** در ۵ راس (۱/۲٪) از بزهای مورد بررسی، سنگ‌های کیسه صفرا یافت شدند. موارد بروز سنگ‌های صفراوی در دام‌های بالغ به طور معنی‌داری ($p<0/05$) بیشتر بود. تفاوت معنی‌داری بین دام‌های نر و ماده از نظر حضور سنگ‌های صفراوی دیده نشد. تجزیه شیمیایی سنگ‌ها نشان داد که در ۴ راس از بزهای مبتلا سنگ‌ها از نوع رنگدان‌های (بیلی-روبین) و در ۱ راس از نوع کلسترولی می‌باشد. ترکیب شیمیایی صفرا در بزهای مبتلا بررسی شد. ازکشت باکتریایی صفرا در ۳ راس از بزهای مبتلا باکتری‌های اشرشیاکلی و سالمونلا جدا شد. در مطالعه میکروسکوپی کیسه‌های صفرای حاوی سنگ، غددکیستی، آتروفی ونکروز لایه مخاطی، ادم، ترشح کانونی ومنتشر لنفوسیت‌ها در لایه زیرمخاطی وهیپرتروفی عضلات صاف دیده شد. **نتیجه‌گیری نهایی:** این تحقیق نشان داد که میزان شیوع هر دو نوع سنگ صفراوی در بز پایین است و با این که سنگ‌های کیسه صفرا می‌توانند باعث آماس مزمن کیسه صفرا شوند ولی بعید است که از نظر بالینی اهمیت چندانی داشته باشند.

**واژه‌های کلیدی:** بز، سنگ‌های صفراوی، شیوع، باکتری‌شناسی، آسیب‌شناسی.

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