

The Fate of Retained Gallstones Following Laparoscopic Cholecystectomy in a Prairie Dog Model

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ABSTRACT

Background and Objectives: Reported complications of retained gallstones following laparoscopic cholecystectomy (LC) are increasing. This study was undertaken to evaluate the effects of retained gallstones following LC in a prairie dog model.

Methods: Twenty-seven prairie dogs with diet-induced gallstones were divided into three groups of nine. Group I (control) had LC with removal of stones. Group II had LC followed by return of native stones intra-abdominally. Group III had LC followed by return of infected stones (stones dipped in *Escherichia coli*) intra-abdominally. Animals were euthanized at two months and the character and extent of intra-abdominal adhesions were scored.

Results: Adhesions were present in 56% of animals in Group I, 89% in Group II, and 100% in Group III. The character and extent of adhesions in groups II & III were significantly greater than the control group ($p < 0.03$). Group III exhibited the highest degree of adhesions when compared to control ($p < 0.007$). Histopathology revealed evidence of micro-abscess formation, foreign body giant cell reaction, and fat necrosis adjacent to retained stones.

Conclusion: Retained intra-abdominal gallstones, especially if infected, are associated with increased adhesions and inflammatory response in this LC model. Further investigation into the long-term consequences of this entity is warranted.

Key Words: Laparoscopic cholecystectomy, Retained gallstones, Complications, Prairie dogs.

INTRODUCTION

Cholecystectomy is one of the most common surgical procedures performed in the United States. Recently, major changes in the operative techniques for removing the gallbladder have occurred with laparoscopic cholecystectomy (LC) quickly becoming the surgical procedure of choice. Although laparoscopic cholecystectomy carries a low morbidity and mortality,¹⁻⁶ one complication that occurs more frequent with the laparoscopic procedure is perforation of the gallbladder followed by the subsequent release of bile and gallstones into the peritoneal cavity. In early series this complication was reported to occur in up to one-third of cases.^{7,8} Removal of spilled stones can often be difficult and time consuming, especially when stones are lost between loops of bowel or folds of the omentum.

Scattered reports of sequela from spilled gallstones have begun to appear in the literature. These reports include development of intra-abdominal abscesses,^{9,10} erosion of these stones into the sigmoid colon¹¹ or hepatic ducts,¹² and the formation of abdominal wall abscesses.^{11,13} The need for prolonging the operation to retrieve these stones laparoscopically or converting to an open procedure in order to reclaim these stones is a controversial topic in the literature. It is clear that an improved understanding of the fate of these retained intra-abdominal gallstones is necessary to better define this issue. Therefore, the purpose of this study is to evaluate the effects of retained intra-abdominal gallstones following LC in a prairie dog model.

Prairie dogs have been used as an animal model of human gallstones for many years, by a number of different groups.¹⁴ Prairie dogs have proven to be an excellent model for the study of gallstones, since they reliably form gallstones when fed a diet high in cholesterol. In fact, prairie dogs will form cholesterol gallstones nearly 100% of the time when fed an appropriate diet for 8 weeks.¹⁴

MATERIALS AND METHODS

Following approval by our Institutional Animal Care and Use Committee (IACUC), 27 male prairie dogs (*Cynomys ludovicianus*) were fed a diet of ground corn and alfalfa meal with 0.4% cholesterol added (Dyets Inc., Bethlehem,

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PA). After two months, three randomly selected prairie dogs received an ultrasound examination of their abdomen which confirmed the presence of gallstones in each animal. At the time of surgery, each prairie dog weighed between 1000 - 1300 g and was deprived of food overnight, but allowed free access to water. Following the induction of general anesthesia, a laparoscopic cholecystectomy was performed using a novel technique that we have developed. All animals in the study had gallstones found at the time of cholecystectomy. Group I (control, N = 9) had a LC performed with removal of the gallbladder. In Group II (N = 9), following LC, the gallstones were extracted from the gallbladder and then placed in the right lower abdomen via an existing port. Group III animals (N = 9) also had a LC performed; however, the extracted gallstones were dipped in a *Escherichia coli* suspension containing 10⁹ colony forming units/ml prior to being replaced in the animal's right lower quadrant. This group was added to simulate the spillage of infected stones that might occur at the time of LC for acute cholecystitis. The animals were allowed food and water ad libitum during recovery. All prairie dogs were given Cefotetan IM preoperatively (25 mg/kg) and Q 12 hours for 72 hours following the LC. Each prairie dog was sacrificed under anesthesia by a lethal intra-cardiac injection of Pentobarbital (86 mg/kg) two months after surgery, followed by immediate necropsy. The abdomen was opened via a mid-line incision from sternum to the pubis and careful assessment of gross pathologic changes asso-

ciated with the surgery and retained intra-peritoneal stones was performed. The extent and characteristics of the intra-abdominal adhesions were noted and categorized. The location of retained gallstones was also noted. Representative areas of tissue containing adhesions and sites of retained stones were sampled and prepared for hematoxylin and eosin staining. Histopathologic analysis to determine the degree of inflammatory response and other sequela of stone retention was performed. Similar areas of tissue were harvested from the control animals and studied in the same manner to serve as the histopathologic control.

Intra-abdominal adhesions were assessed for both extent (**Table 1**) and characteristics (**Table 2**) and assigned a score for each category (**Table 3**) as well as a combined Total Adhesion Score (TAS) (**Table 4**). The Kruskal-Wallis and Mann-Whitney statistical tests were used to compare groups for extent and characteristics of the adhesions, with the alpha value set at $p < 0.05$.

RESULTS

Twenty-seven prairie dogs underwent laparoscopic cholecystectomy in this protocol. Two months following their surgeries each animal was euthanized and a necropsy immediately performed. The necropsy of the control animals (Group I, N = 9) revealed intra-abdominal adhesions in 5/9 (56%) with only 2/9 (22%) being characterized as

Table 1.

Extent of intra-abdominal adhesions found at necropsy two months following laparoscopic cholecystectomy.

	None	Localized to Gallbladder Fossa	Extending to Other Areas of Liver	Extending to Other Organs (Diaphragm, Abdominal Wall, Small Bowel)	Group Comparisons
Group I (Control)	4/9 (44%)	2/9 (22%)	1/9 (11%)	2/9 (22%)	Group I vs III P = 0.08
Group II (Stones Only)	1/9 (11%)	2/9 (22%)	2/9 (22%)	4/9 (44%)	Group I vs II P = 0.12
Group III (Infected Stones)	0/9 (0%)	1/9 (11%)	3/9 (33%)	5/9 (44%)	Group II vs III P = 1.00

Table 2.

Characteristics of intra-abdominal adhesions found at necropsy two months following laparoscopic cholecystectomy.

	None	Thin/Avascular	Filmy/Vascular	Dense Adhesions	Total Adhesions	Group Comparisons
Group I (Control)	4/9 (44%)	1/9 (11%)	2/9 (22%)	2/9 (22%)	5/9 (56%)	Group I vs II p = 1.0
Group II (Stones Only)	1/9 (11%)	2/9 (22%)	3/9 (33%)	3/9 (33%)	8/9 (89%)	Group I vs III p = 0.15
Group III (Infected Stones)	0/9 (0%)	1/9 (11%)	2/9 (22%)	6/9 (67%)	9/9 (100%)	Group II vs III p = 0.20

dense adhesions. Group II (native stones replaced) revealed intra-abdominal adhesions in 8/9 (89%) with 3/9 (33%) being characterized as dense adhesions. Group III (infected stones replaced) exhibited adhesions in 9/9 (100%) with 6/9 (67%) characterized as dense adhesions (**Table 1 and Table 2**). When combined, Groups II and III were associated with greater adhesions in both character and extent, as compared to Group I ($p < 0.03$).

A Total Adhesion Score (TAS) was determined for each experimental group. The TAS was calculated by adding the extent of adhesions score to the characteristics of adhesions score for each group. The mean TAS for the infected stone group (Group III) was 5.00 ± 1.3 , which was significantly greater than the control group (Group I) whose mean TAS was 2.33 ± 2.4 ($p < 0.007$). Group II (native stones) had a mean TAS of 3.89 ± 1.83 which did not differ statistically from either Group I or Group III.

Histopathological examination of necropsy specimens revealed a wide range of pathology. At two months, one animal in Group II had developed a macro-abscess (sterile) adjacent to the gallbladder fossa. In general, fat necrosis, foreign body giant cell reactions, acute and chronic inflammation, and micro-abscess formation typified the findings in Group II animals. Group III animals were similar histopathologically to Group II except for more pronounced acute inflammatory reactions throughout the sampled tissues. Group I animals showed significantly less inflammatory changes and fat necrosis on histopathology when compared with Group II and Group

III. Adhesions with cholesterol stones were clearly evident on H&E staining. Cholesterol clefts (gallstone remnants) are shown adherent to the omentum (**Figure 1**), and to the abdominal wall (**Figure 2**). **Figure 3** demonstrates a micro-abscess between the liver and small bowel present in a Group II prairie dog.

DISCUSSION

We have developed a model for laparoscopic cholecystectomy in the prairie dog which incorporates standard laparoscopic techniques commonly used in the field of



Figure 1. Cholesterol clefts or gallstone remnants (arrow) are shown adherent to the omentum.

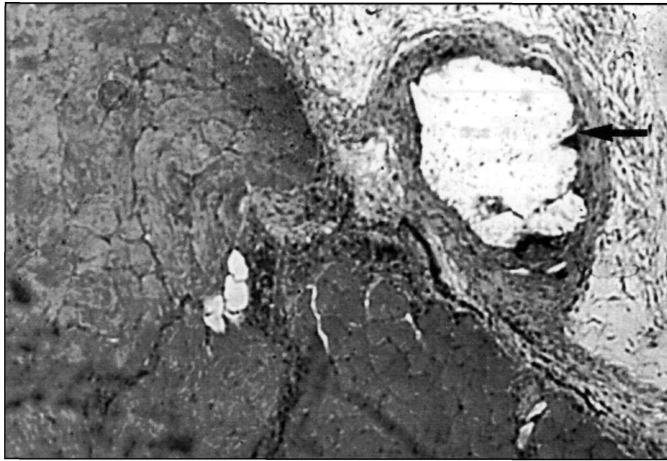


Figure 2. Gallstone (arrow) adherent to abdominal wall.

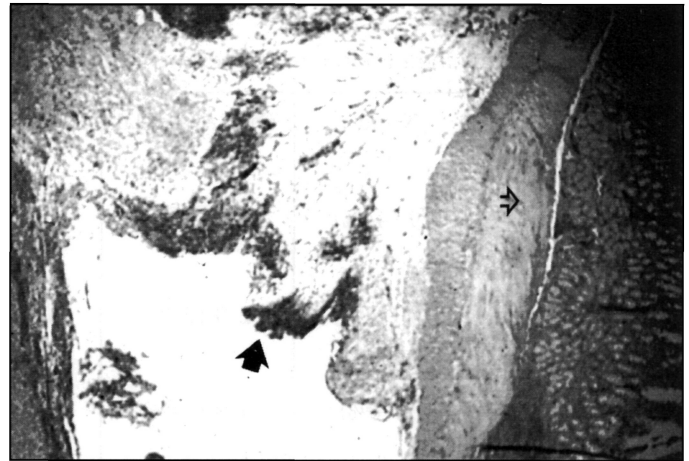


Figure 3. Micro-abscess formation (closed arrow) between liver on left and small bowel (open arrow) on right.

surgery today. Prairie dogs have proven to be an excellent model for laparoscopic cholecystectomy since nearly 100% of these animals begin to form gallstones after only two months of being fed a diet high in cholesterol.¹⁴ In addition, the biliary anatomy in the prairie dog is similar to that of the human. One complication related to LC is the intra-abdominal spillage of bile and gallstones which has been reported to occur in up to one-third of cases.^{7,8} Spillage of gallstones generally occurs due to traumatic graspers, during dissection of the gallbladder from the gallbladder fossa with electrocautery, or during the vigorous extraction of the gallbladder through the umbilical port. Sequela related to these retained stones is now being reported with more frequency. Complications recorded include intra-abdominal^{9,10} and abdominal wall abscesses,^{11,13} and erosion of stones into adjacent structures such as the sigmoid colon¹¹ or hepatic ducts.¹² Other reported complications include bowel obstructions,¹⁰ trocar site abscesses¹⁵ and development of draining cutaneous sinus tracts.¹⁶ There is also one report of gallstones found in an abscessed ovarian dermoid cyst.¹⁷ Patients with cholelithiasis have been shown to have bile colonized by bacteria in 20 - 40 percent of cases. The organisms most commonly cultured from bile include *Escherichia coli*, *Klebsiella pneumoniae*, and enterococci, although *Enterobacter* and *Pseudomonas* species are now becoming common organisms in the infected biliary tract.¹⁸ In order to simulate infected stones, we subjected harvested gallstones to a suspension of *Escherichia coli* prior to returning the stones back into the abdomen of

Group III animals. *Escherichia coli* was chosen as the organism since it was felt to be less pathogenic than the other common organisms in this survival study.

Human gallstones can generally be divided into several groups based on their respective composition. Generally, there is a very low incidence of infected bile found with either cholesterol stones or the black (pure pigment) stones. In contrast, the incidence of bacteria present in other types of stones such as bile pigment calcium stones and combination stones is relatively high.¹⁹ To date there has been no reported study linking the type of retained gallstones to their intra-abdominal adhesion potential. In an animal study by O'Leary et al., bacterial contamination

Table 3.
Adhesion scoring scale.

Extent of Adhesions	Points
None	0
Localized to Gallbladder Fossa	1
Extending to Other Areas of Liver	2
Extending to Other Organs	3
Characteristics of Adhesions	Points
None	0
Thin / Avascular	1
Filmy / Vascular	2
Thick / Dense	3

Table 4.
Total adhesion score (TAS) for each group along with group comparisons.

	Mean TAS	Group Comparisons
Group I (Control)	2.33 ± 2.4	Group I vs III p < 0.007
Group II (Stones Only)	3.89 ± 1.8	Group I vs II p > 0.05
Group III (Infected Stones)	5.00 ± 1.3	Group II vs III p > 0.05

proved to be a potent cause of postoperative peritoneal adhesions (8/9, p = 0.002) compared with uninfected controls (3/10).²⁰ Although all groups in this study developed adhesions, there was no statistical significance noted between Groups I and II. However, the extent and density of the adhesions was statistically more significant in Group III, which had the infected stones replaced. Clearly, decreased adhesion formation is one of the accepted advantages of laparoscopic surgery. Our laparoscopic control group showed adhesions in 56% (5/9) which is higher than 0 - 15% reported in human LC. Yet only 22% (2/9) had significant adhesions extending to areas other than the gallbladder fossa or nearby liver capsule.

The fate of retained gallstones has been studied by Welch et al. at Creighton University where aseptically collected human bile and cholesterol gallstones were implanted into the peritoneal cavity of New Zealand white rabbits.²¹ After two months, localized fibrosis and fatty necrosis were typical, with no evidence of residual inflammation. Additionally, there was no evidence of either macro or micro-abscess formation. A similar study was performed by Johnston et al. where sterile saline, human bile and/or gallstones were injected into 90 rats.²² They concluded that while small amounts of bile may be harmless, bile in combination with gallstones was associated with a significant risk of postoperative adhesions and intra-abdominal abscess formation. One limitation of these two studies was their use of human gallstones that were placed via open surgical incision. Our study differs in that we have developed an experimental animal model of gallstone

spillage during LC that uses native stones, which simulates the events that occur in humans more directly.

Jones et al. performed follow-up surveys (mean, 41 mos.) of 220 patients following LC in which 73 (33%) sustained an intraoperative gallbladder perforation.²³ Their survey revealed no late wound complications, and no patient required intervention for intra-abdominal sepsis or bowel obstruction.

CONCLUSIONS

This study demonstrates that retained gallstones, especially when infected, are associated with increased intra-abdominal adhesions and inflammatory response in a prairie dog LC model. Although no significant intra-abdominal complications occurred during this study, controversies still exist as whether or not to convert a LC to an open procedure when multiple stones are spilled. At this time the consensus in the literature does not recommend conversion to an open procedure, since the reported complication rate of retained stones is relatively low. The use of postoperative antibiotics in patients with spillage of bile and retained stones is not clear-cut. Our model showed that retained stones, even if infected, did not cause any clinical evidence of infection. Further, since the reported infectious complications resulting from retained stones is relatively small, we would not recommend continued use of antibiotics postoperatively. Our results in this short-term model (2 months) support the view that every attempt should be made to remove as many stones as possible laparoscopically, as retained gallstones appear to cause a significant inflammatory response as well as adhesions, especially when infected. Nonetheless, the ultimate incidence of morbidity associated with retained gallstones remains to be determined. Further investigation into the long-term consequences of this commonly occurring entity is warranted.

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