

Laparoscopic Cholecystostomy in Pigs: Technique and Comparison with Traditional Open Cholecystostomy for Surgical Stress

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ABSTRACT

This article describes a simple technique of laparoscopic cholecystostomy (LC) and compares the LC technique with open cholecystostomy (OC) for clinical outcome and surgical stress response. LC was performed under general anesthesia using three portals. The gallbladder was fixed to the anterior abdominal wall using two sets of simple interrupted sutures placed between the anterior abdominal wall and gallbladder, rather than the traditional single set. A cauterized incision was made at the gallbladder fundus such that it lay at the center of a purse-string suture; an 18-F Malecot catheter was introduced into the gallbladder lumen through this incision. The mean duration required for completing LC was 38±12 min, which was decreased by 16 min with increasing experience. In a matched group of pigs, OC was performed using the conventional approach. All animals underwent tube cholecystocholangiography and follow-up laparoscopy at 15 days and 1 month after the surgery, respectively. The investigations showed that the gallbladder was viable with no bile leakage or abscess formation and that it remained adherent to the abdominal wall. In addition, the serum concentrations of cortisol, interleukin-6, and C-reactive protein showed that the surgical stress response was lower for LC than OC. Thus, in terms of surgical stress, and possibly operative time, laparoscopic placement of the Malecot catheter using a three-portal approach and two suture anchors may be more beneficial than OC for the creation of a short-term access route into the gallbladder and bile ducts in pigs, under both interventional and experimental settings.

Keywords: Laparoscopy; Tube Cholecystostomy; Technique; Response Stress; Miniature Pig.

INTRODUCTION

Percutaneous cholecystostomy (PC) is a safe alternative to cholecystectomy and surgical exploration of the bile ducts in the management of acute cholecystitis (1-5) and inspissated bile syndrome (6) in humans. PC is also performed to facilitate diagnostic T-tube cholangiography in humans (7). In veterinary medicine, experiments related to procedural development and *in vivo* evaluation of techniques and/or devices, as well as professional training protocols for biliary interventional procedures, require the establishment of a safe and easy access route into the gallbladder and the bile

ducts (8-10) and such purposes a porcine model is frequently sought. Previous studies on large animal models involved either open surgery with anchoring of the gallbladder and subsequent placement of large-bore external tubes or placement of a permanent catheter that was inserted percutaneously under radiological or ultrasonic guidance and retained until the formation of a mature tract (11-16).

In our experience, the laparoscopic approach enables good visualization of the abdominal cavity while maintaining good hemostatic control, enabling fast recovery, and affording an efficacy level that was comparable to that afforded by open

surgery. However, a previous study has not shown differences between the two surgical approaches in regard to surgical stress response (17). To achieve minimally invasive treatment of benign and malignant diseases of the biliary tract and to facilitate interventional procedures and experimental studies related to the biliary system, it is necessary to establish an easy and reliable route to access the gallbladder and the bile ducts.

In this study, we employed a simple method for performing LC in pigs using two suture anchors and inserting the Malecot catheter into the gallbladder. In addition, we compared LC and OC in terms of the clinical outcome and surgical stress response.

MATERIALS AND METHODS

Study design

The study was carried out on Chinese experimental miniature pigs that were equally divided into 2 groups: the LC group, in which LC was performed and the OC group, in which open surgery was performed using the conventional approach. The experimental protocol was approved by the ethics committee of Northeast Agricultural University (NAUEC2014-0205).

Animals

The animals used in this study were 14 Chinese experimental miniature pigs (3 males and 4 females in each group) of ages 3–6 months and weight 15.5–25.8 kg. Seven animals each were enrolled in the 2 treatment groups. The pigs were housed individually and fed a standard piglet diet with tap water *ad libitum*. The animals were allowed a 14-day period to acclimatize to the laboratory conditions before the start of the study. Care and handling of the animals were in accordance with regulations for the administration of affairs concerning experimental animals (Approved by the State Council on October 31, 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on November 14, 1988).

Preoperative protocol

Before commencement of the experiments, food and water were withheld for 12 and 6 hours, respectively, to reduce the gastrointestinal content and minimize the risk of regurgitation during surgery. To induce anesthesia, each pig was administered intramuscular (IM) injections of atropine

(Harbin Pharma, China), tiletamine/zolazepam (Huaen Pharma, China), xylazine (Libang Pharma, China), and tramadol (Jiupai Pharma, China) at doses of 0.02 mg/kg, 3.0 mg/kg, 1.2 mg/kg and 1.6 mg/kg, respectively (18). The core body temperature (measured using a rectal probe) was maintained between 36°C and 38°C by warming the operating table and using an infrared heating lamp. Throughout the procedure, physiologic saline (0.9% NaCl, Kelun Pharma, China) solution was continuously infused at a rate of 3 ml/kg/h via the marginal ear vein of the pig and the vital signs, blood gas levels, and respiratory parameters of the animal were monitored.

Technique of the cholecystostomy

The 7 animals in the LC group were placed in the supine position, and the ventral aspect of the abdomen (from the xiphoid to the pubis and the inguinal folds) was shaved, aseptically prepared, and draped for laparoscopy. A Veress needle (Olympus Corporation, Tokyo, Japan) was then inserted into the peritoneal cavity to establish a carbon dioxide pneumoperitoneum of pressure 12 mm Hg. A 3-portal approach was adopted for LC (Figure 1).

The laparoscope provided good visualization of the peritoneal cavity. The gallbladder was located adjacent to the right medial liver lobe and showed a grayish discoloration in several cases. The site of the planned cholecystostomy on the anterior abdominal wall was identified and digitally palpated under laparoscopic visualization. To decompress the gallbladder, a 22-gauge, 6-inch spinal needle was inserted into the gallbladder through the abdominal wall, after which the stylet was removed and the gallbladder contents aspirated as much as possible. A single traction suture was placed with an intracorporeal laparoscopic suture at a distance of approximately 1 cm from the gallbladder fundus (19). A purse-string suture (2-0 polyglycolic acid; Pudong Jinhuan Medical Products Co., Ltd., Shanghai, China) was then placed in the wall of the gallbladder fundus approximately 1 cm caudal to the first fixation point of the gallbladder. A cauterized incision extending up to the gallbladder lumen was made at the center of the purse-string suture by using a laparoscopic monopolar hook electrode (Tonglu Medical Instruments Co., Ltd., Hangzhou, China) introduced through portal B (Figure 2). A sterilized, modified 18-F Malecot catheter (Zhanjiang Star Enterprise Co., Ltd., Zhanjiang, China) was advanced through the cannula at portal A. The gallbladder was then

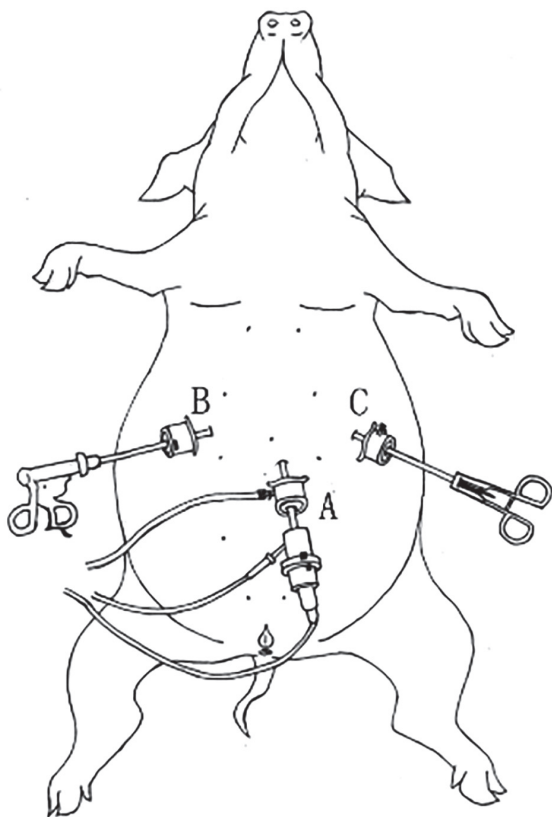


Figure 1: The locations of the portals A, B, and C. Portal A (laparoscope) was located 5–8 cm below the umbilicus along the ventral midline (in male pigs, portal A was shifted 2–3 cm to the left). Portals B and C were 3–5 cm cranial to portal A and 8–10 cm to the left and right of the ventral midline, respectively; they were used for introducing laparoscopic surgical instruments.

secured with an atraumatic grasping forceps (Tonglu Medical Instruments Co., Ltd., Hangzhou, China). Under direct laparoscopic visualization, the Malecot catheter was advanced into the gallbladder lumen through the cauterized incision mentioned above, by using a laparoscopic left-curved preparation forceps (Tonglu Medical Instruments Co., Ltd., Hangzhou, China). The catheter was then withdrawn slightly to allow the winged end of the catheter to expand and anchor itself in the gallbladder tissue; the purse-string suture was then pulled tightly. The catheter was subjected to mild tension and infused with 10 mL of sterile saline (0.9% NaCl) solution to check for leakage around the site of entry into the gallbladder. Another set of simple interrupted sutures was placed opposite to the first fixation point in a manner similar to that described above for the first set of sutures (Figure 3). The two sets of sutures were used to attach the gallbladder to the anterior abdominal wall at the point where the catheter exited the peritoneal cavity.

The intra-abdominal pressure was then reduced from 12 mm Hg to 6 mm Hg, and the gallbladder fundus was approximated to the parietal peritoneum by retracting the catheter, to prevent biliary leakage. A hemostatic forceps was then advanced into the peritoneal cavity through a stab incision made in the ventral body wall parallel to and approximately 1–2 cm from the ventral midline. The forceps was used to clamp the catheter outside the abdominal cavity. The abdomen was then completely deflated; the sutures were

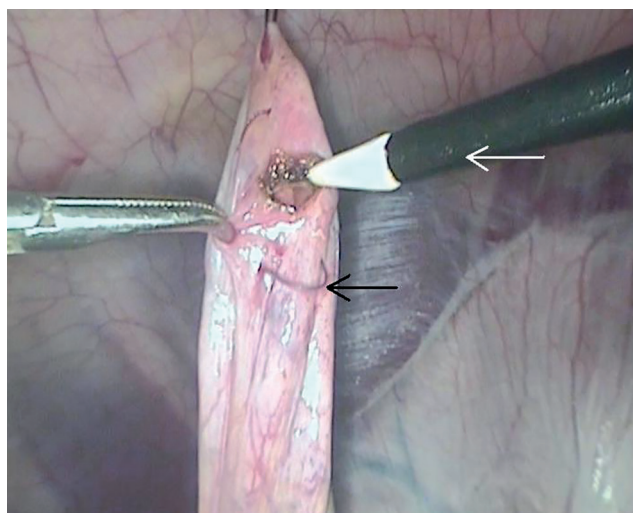


Figure 2: Intraoperative view showing a cauterized incision made by the laparoscopic monopolar hook electrode (white arrow) in the middle of the purse-string suture (black arrow).

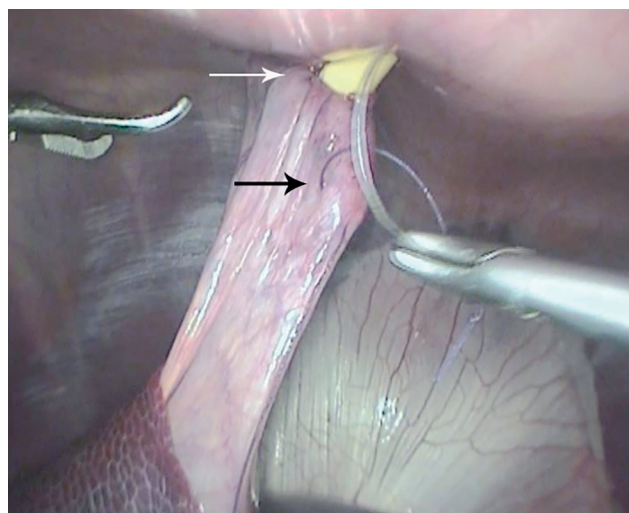


Figure 3: Intraoperative view showing the second set of simple interrupted sutures (black arrow) placed opposite to the first fixation point (white arrow).

tightened and knotted extracorporeally; and the knots were buried in the subcutaneous tissues (20, 21). Immediately afterwards, a tube cholecystocholangiogram was obtained using 60% diatrizoate sodium (Hansen Pharma Co., Ltd., Changsha, China).

The external portion of the Malecot catheter was sutured to the skin using the finger-trap method (20) and clipped at about 4–5 cm from the point of its exit from the peritoneal cavity to prevent its dislodgement. Sutures of 2-0 polyglycolic acid (Shanghai Medical Instruments Co., Ltd., China) were used to close portal A in 2 layers and portals B and C, in a single layer. All the skin wounds were closed with a simple interrupted 3-0 nylon suture (Shanghai Medical Instruments Co., Ltd., China).

The 7 pigs in the OC group underwent OC performed with the conventional technique (14). All the procedures were performed by the same team comprising a surgeon and 2 assistants.

Postoperative care

After the surgery, the temperature, heart rate, respiratory rate, and arterial blood pressure of the pigs were monitored until the values returned to the preoperative levels and the animals regained consciousness; additionally, clinical examination and blood tests for the complete blood count were conducted daily. For analgesia, fentanyl patches (5 mg; Durogesic, Janssen Pharma, Xian, China) were applied on the median line of the back after shaving every 3 days for 6 days, and for antibiotic coverage, ampicillin (20 mg/kg IM; Lukang Pharma, Jining, China) was administered every 8 hours for 3 days. The pigs were housed individually in dry and clean barns to avoid damage to the catheters and contamination by feces. The extracorporeal portion of the catheter was washed every day. In both groups, the Malecot catheter was removed 15 days after the surgery and laparoscopic examination was conducted one month after the surgery to assess the status of the abdominal cavity and its viscera.

Measurements of serum cortisol, interleukin-6, and C-reactive protein concentrations and white blood cell count

For all pigs in both groups, blood samples (2.5 mL) from the precaval vein were collected at the following time points: 1 h before surgery; immediately (0 h) after the surgery; and 4 h and 1, 2, 3, 5, and 7 days after the surgery. The samples were

used to measure the serum concentrations of cortisol (COR), interleukin-6 (IL-6), and C-reactive protein (CRP) and the white blood cell (WBC) count. The serum IL-6, CRP and COR concentrations were measured using enzyme-linked immunosorbent assay kits (Yapu Biological Technology Co., Ltd., Shanghai, China) for the measurement of the porcine parameters (sensitivity, 0.1 µg/mL, 0.1 mg/L, and 1.0 ng/mL, respectively). The WBC count was determined using an automatic blood cell analyzer (Mindray Bio-medical Electronics Co., Ltd., Shenzhen, China).

Statistical analysis

For the LC group, the operating time was defined as the duration from the initial stab incision to the closure of the last portal. For the OC group, this value was defined as the time from the start of the skin incision to its closure. The data were expressed as mean ± standard error of the mean. Inter- and intragroup differences in values were determined by one-way analysis of variance, which was performed using the SPSS software (version, 17.0; SPSS Institute, Cary, NC, USA). The significance of differences was set at a *P* value of <0.05.

RESULTS

Surgical procedure

LC was successfully performed for all the pigs, without any major intraoperative, surgery-related complications. Minimal hemorrhage and biliary leakage were noted in a few cases during the puncturing of the gallbladder with the needle and the monopolar hook electrode; nevertheless, intraoperative bleeding was negligible for LC. The total length of the skin incisions, including those for all the 3 portals, was 2 cm for the LC procedure; this value was significantly less than that required for the OC procedure (10 ± 2 cm) (*P* < 0.01). The mean operating time was 38 ± 12 min and 33 ± 10 min in the LC group and OC group, respectively. Further, the LC operative time was reduced by 16 minutes with increasing experience.

Clinical outcome

The catheter was left in place for 15 days and washed once a day under sterile conditions, before feeding; the clamp was released once a day to drain the gallbladder under sterile conditions. Intraperitoneal bile leakage or dislodgement of or



Figure 4: Follow-up cholecystocholangiogram performed 15 d after surgery shows absence of bile extravasation and catheter occlusion.

damage to the catheter did not occur in any of the pigs while the catheter was left *in situ*. In the case of one pig, the stoma continued to leak small amounts of bile even after the drain was removed; however, this was resolved within 5 days after the formation of healthy granulation tissue. Furthermore, two pigs developed irritation at the site of the stoma. Since bacterial culture of the bile samples was not performed, we are unable to comment on the status of postoperative infections of the gallbladder or bile tract. However, it appears that there may have been no clinical infection since the total blood counts were within the reference range for all the pigs at 5 postoperative days and none of the pigs showed any evidence of digestive complications during the 1-month postoperative follow-up period.

Follow-up cholecystocholangiography was successfully performed 15 days after the surgery confirming the absence of bile extravasation and catheter occlusion in the LC group (Figure 4). The drainage tube allowed easy access to the gallbladder for performing repeat cholangiography. Repeat laparoscopy was performed 1 month after the surgery in both groups. The findings of

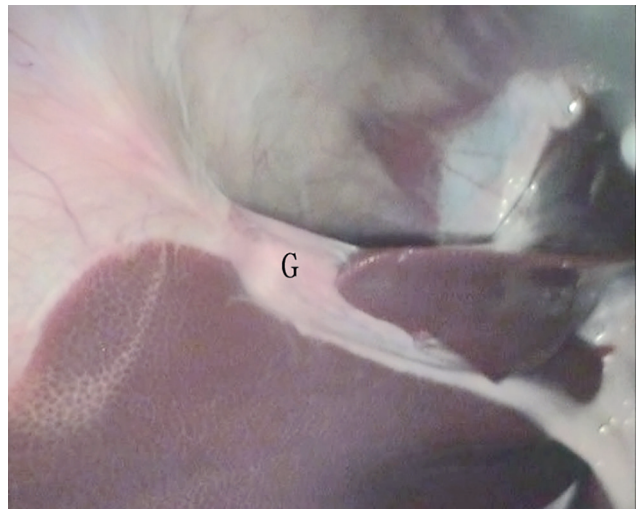


Figure 5: Repeat laparoscopy performed 1 month after the surgery shows close attachment and adherence of the gallbladder (G) to the anterolateral abdominal wall.

repeated laparoscopy in the animals of the LC group were as follows: a viable gallbladder; the absence of bile leakage and abscess formation; close attachment and adherence of the gallbladder to the anterolateral abdominal wall (Figure 5); absence of omental attachment to the suture anchors; and presence of white or reddish, oval, and raised structures at the trocar sites, indicating advanced peritoneal healing. On the other hand, the OC group showed omental attachment to the site of the suture anchor in two pigs and to the abdominal incision in one pig. No other complications were observed in either group within 3 months of the surgery until the animals were euthanized with administration of pentothal sodium (4–8 mg/kg) and potassium chloride injection (2 g, 20 ml) via the marginal ear vein successively.

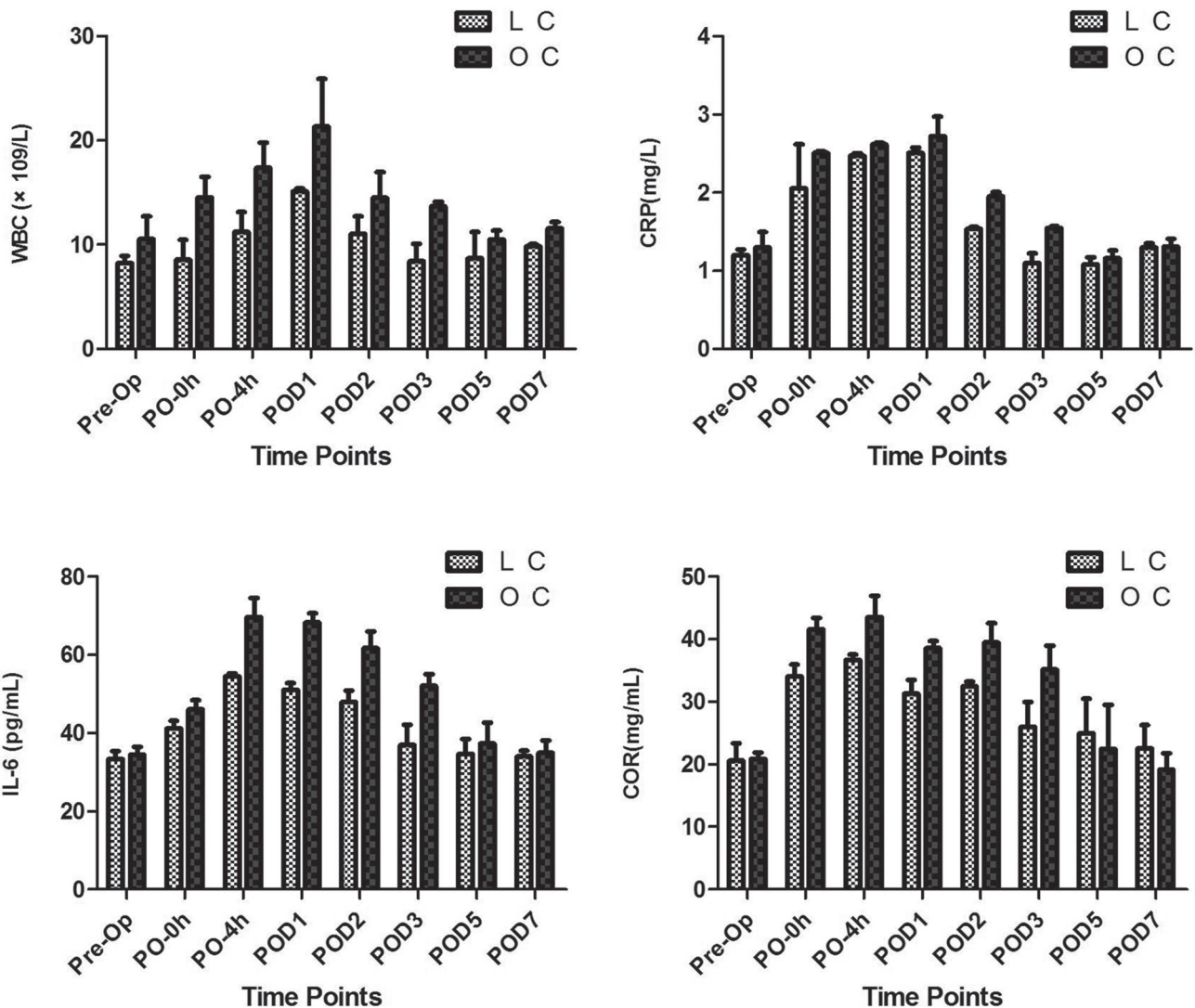
White blood cell count

At 24 hours after the surgery, the mean WBC count increased significantly from preoperative levels of $8.34 \pm 0.61 \times 10^9/L$ and $9.43 \pm 2.19 \times 10^9/L$ to $15.21 \pm 0.30 \times 10^9/L$ and $21.22 \pm 4.60 \times 10^9/L$ in the LC and OC groups, respectively ($P < 0.01$, for all comparisons). However, in both groups, the values did not exceed the upper limit of the reference interval ($11\text{--}22 \times 10^9/L$) in this study. The increased WBC levels in the LC and OC groups returned to the preoperative levels on days 3 and 5, respectively ($8.56 \pm 1.52 \times 10^9/L$ and $10.39 \pm 0.78 \times 10^9/L$, respectively) (Table 1).

Table 1: White blood counts and serum concentrations of C-reactive protein, interleukin-6, and cortisol

	WBC ($\times 10^9/L$)		CRP (mg/L)		IL-6 ($\mu\text{g/mL}$)		COR (ng/mL)	
	LC	OC	LC	OC	LC	OC	LC	OC
Pre-Op	8.34±0.61	9.43±2.19	1.19±0.06	1.29±0.22	34.39±1.86	33.49±1.99	20.57±2.79	21.11±1.01
POD-0h	8.68±1.79	14.43±1.88**▲▲	1.98±0.55**	2.49±0.03**▲	40.22±1.82**	45.98±2.53**	33.22±1.78**	40.59±1.90**▲
POD-4h	11.34±1.81*	17.24±2.58**	2.50±0.04**	2.63±0.02**	53.60±0.53**	68.69±4.68**▲	37.12±0.91**	43.66±3.38**▲
POD-1d	15.21±0.30**	21.22±4.60**▲	2.53±0.06**	2.69±0.26**	50.13±1.58**	67.28±2.24**▲	31.30±2.30**	38.75±1.20**▲
POD-2d	11.18±1.56*	14.39±2.51**▲	1.55±0.03*	1.99±0.07**▲	48.03±2.83**	60.68±.32**▲	32.74±0.67**	39.50±3.22**▲
POD-3d	8.56±1.52	13.57±0.34**▲▲	1.09±0.11	1.56±0.04**▲▲	37.12±5.30	51.99±2.79**▲	25.94±4.04	35.41±3.91**▲
POD-5d	8.81±2.41	10.39±0.78	1.10±0.08	1.18±0.09	35.67±3.79	36.89±5.73	25.01±5.56	22.44±7.13
POD-7d	9.86±0.19	11.44±0.53	1.20±0.04	1.29±0.11	33.90±1.48	35.03±3.82	23.10±3.77	19.19±2.77

Note: Pre-Op = pre-operation; POD = postoperative day; WBC = white blood cell; CRP = C-reactive protein; IL-6 = interleukin-6; COR = cortisol; LC = laparoscopic cholecystostomy; OC = open cholecystostomy; Compared with preoperative values: * 0.01 < P < 0.05; ** P < 0.01; Compared with the OC group: ▲ 0.01 < P < 0.05; ▲▲ P < 0.01; one-way ANOVA.



Serum concentrations of C-reactive protein, interleukin-6, and cortisol

In the LC group, the postoperative serum CRP concentrations were significantly higher than the preoperative values (1.19 ± 0.06 mg/L) at the following time points: immediately after surgery (1.98 ± 0.55 mg/L), at 4 hours after surgery (2.50 ± 0.04 mg/L), and 1 day after surgery (2.53 ± 0.06 mg/L) ($P < 0.01$, in all cases). The postoperative levels were the highest (2.53 mg/L) at 24 hours after the surgery. Compared to the OC group, the LC group showed a slower rise in the postoperative levels of serum CRP, but a more rapid decrease to the preoperative levels (Table 1).

The serum IL-6 concentration in the LC group showed an immediate increase after surgery compared to the preoperative level (34.39 ± 1.86 pg/mL) and was the highest (53.60 ± 0.53 pg/mL; $P < 0.01$) at 4 h after the surgery. As was the case with the serum CRP concentrations, the serum IL-6 levels showed a more rapid restoration to preoperative levels in the LC group than in the OC group. The postoperative rise in the serum IL-6 levels in the OC group was significantly higher than that in the LC group (Table 1).

The mean serum COR concentrations in both groups increased immediately after surgery, but the levels were lower in the LC group than in the OC group (33.22 ± 1.78 ng/mL and 40.59 ± 1.90 ng/mL, respectively). The serum COR concentrations did not show any statistically significant intergroup differences at any time point but they remained lower in the LC group than in the OC group at all time points (Table 1).

DISCUSSION

Several techniques have been proposed for performing PC in humans (2, 22). In this study, we performed cholecystostomy using a laparoscopic approach in swine models. We tested this method in the miniature pig since this is a useful model for research on laparoscopic techniques by virtue of their similarity with humans with respect to the hepatobiliary anatomic and functional aspects (19).

We successfully completed LC in all the 7 healthy pigs without any intraoperative or immediate postoperative complications. Further, the operative time was reduced by 16 min (74 min to 58 min) with increasing experience and further improvement may be expected with additional practice.

The serum concentrations of CRP, IL-6, and COR are

considered useful in evaluating the extent of surgical trauma (21). In our study, the postoperative increase in serum levels of CRP and IL-6 were significantly greater for OC than for LC. Moreover, the serum cortisol concentrations were lower for the LC group than the OC group at all time points. This implies that the surgical stress and postoperative pain associated with LC are less than those associated with OC.

Unlike the case with surgery requiring celiotomy, LC precludes the need for the exposure of the abdominal viscera to air; allows for the completion of all surgical procedures within the abdomen; enables direct visualization of the gallbladder through the laparoscope; and entails minimal invasion, reduced bleeding, and a quick recovery. Moreover, direct laparoscopic visualization allows proper positioning of the aspiration needle; this avoids entry into the gallbladder through a liver lobe, which is sometimes unavoidable when cholecystostomy is performed under ultrasonographic, computed tomographic, or fluoroscopic guidance (22).

In the present study, a 3-portal approach was used to perform LC. The laparoscope was introduced through a portal located below the umbilicus and on the ventral midline; this position allowed for excellent visualization of the gallbladder and its associated structures. The two instrument portals were located 3–5 cm cranial to the laparoscope portal, one on each side of the midline (8–10 cm away). The 3 portals were positioned in a triangular formation that facilitated optimal manipulation of the gallbladder. This triangular positioning of the 3 portals, along with the infra-umbilical placement of the laparoscope portal, appears to be suitable for the manipulation of the gallbladder and catheter placement. Additionally, in our technique, we used 2 sets of sutures to attach the gallbladder to the abdominal wall. This was intended to prevent the malpositioning and damage to the Malecot catheter.

In the presence of excessive fullness or internal pressure in the gallbladder, penetration of the gallbladder wall with the needle may be challenging. A quick, forceful jabbing action, however, usually enables penetration with minimal leakage of the remaining contents. Bile leakage may also be controlled by placing a double purse-string suture; suture anchoring of the gallbladder with the parietal peritoneum is also useful in preventing cholecystic hemorrhage, inadvertent peritoneal migration of the catheter, and peritoneal leakage of the gallbladder contents (16, 23). Further, we suggest that the intra-abdominal pressure be decreased to 6 mm Hg be-

fore tying the fixed sutures since this will reduce the tension between the ventral body wall and the gallbladder.

Repeat laparoscopy performed 1 month after the respective surgeries in both groups showed the presence of focal fibrous adhesions between the gallbladder and the ventral body wall. These adhesions are useful in that they reduce the risk of bile leakage into the abdomen. Further, the adhesions should be strong so as to avoid kinking of the catheter.

The findings of this study should be considered in the light of a couple of limitations. Since cultures of bile samples were not performed, we could not conclusively assess the status of postoperative infection. However, since relevant clinical and laboratory signs were not observed, infection may be presumed to be absent. Postoperative infections of the gallbladder and biliary tract can be avoided by the employment of appropriate sterilization techniques and proper administration of intensive postoperative care. Another limitation of our study is the short follow-up duration. This may be overcome by further investigations on the long-term advantages of LC. Silicone-coated tubes may be useful only for short-term biliary drainage, i.e., for a period less than 2 weeks. Further investigations are necessary to determine whether tubes manufactured using other types of material, such as teflon, polyurethane, or polyvinyl chloride, are superior to silicone-coated ones for cholecystostomy (24).

In conclusion, our results indicate that LC using a three-portal approach with placement of 2 sets of sutures for the insertion of the Malecot catheter is better than OC for the establishment of a short-term access route to the gallbladder and bile ducts of swine, with a lower level of surgical stress response. LC appears to be a simpler and safer method compared to the conventional approach involving the surgical insertion of the percutaneous catheter in swine; our findings also show that with an increase in the surgeon's experience, it may be possible to further reduce the operative time for LC. Further studies are necessary to evaluate the efficacy of the laparoscopic approach for cholecystostomy in clinically affected pigs as well as over the long term.

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ETHICAL STANDARD

The experiments comply with the current laws of the People's Republic of China and Institutional Animal Care and Use Committee (Northeast Agricultural University, China).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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