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Calot`s Triangle – and Hepatocystic triangle – Like Areas in Domestic Swine

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Due to the similarities to human anatomy the domestic swine is one of the most preferred species for experiments especially in developing new surgical techniques in the cholecystectomy in humans. The aim of the study was to establish the existence of both the Calot`s triangle and the hepatocystic triangle and the length of their borders in the domestic pigs. This research work was conducted on livers from 30 male pigs separated to different age groups. The results showed that Calot`s triangle – and the hepatocystic triangle-like areas were present. The borders of the triangles were identified, measured and statistically analyzed in immature and mature pigs and age dependent differences were identified. The similarities and differences in borders of triangles between pigs and humans were discussed. This study revealed that, like in humans, the Calot`s triangle and the hepatocystic triangle are presented and have important clinical significance.

Key words: Calot`s triangle, hepatocystic triangle, anatomy, morphometry, pigs

Introduction

The Calot`s triangle and hepatocystic triangle in humans are studied in detail because of the cholecystectomy surgery [18]. In order to develop new surgical techniques for gallbladder removing, the domestic swine is widely used as one of the most suitable experimental models for cholecystectomy [18]. Misinterpretation or lack of knowledge of this information contributes to intraoperative complications such as biliary injuries, which can cause serious morbidity and occasionally mortality [9]. Information about the presence of such triangles in animals was not found. The similarity in the anatomy of extrahepatic biliary tracts in both humans and pigs [10, 11] is well known. For example, both species possess a common hepatic duct, a common bile duct terminating in the duodenum by a papilla duodeni major and a cystic duct [10, 17]. Moreover, the cystic artery is a main vessel that supplies the gallbladder and has similar topography in both species.

Laparoscopic cholecystectomy became the preferred modern method for the treatment of symptomatic cholelithiasis [8]. Laparoscopic cholecystectomy has many

advantages over the standard open cholecystectomy: minimal trauma, decreased pain, shorter hospital stay, satisfactory cosmetic outcome, quick recovery, and return to work. However, numerous studies have shown that laparoscopic cholecystectomy is associated with a higher frequency of complications compared to the standard open cholecystectomy including lesions to the common bile duct, injury to the vascular and visceral structures [4]. Major complications (biliary and vascular) are life threatening and increase mortality rate, therefore creating the need for conversion to open surgical approach in order to treat them. The frequency of complications associated with laparoscopic cholecystectomy varies from 0.5 to 6% [4]. The most serious complications are associated with high mortality rate: injury of common bile duct with an incidence of 0.1-0.6% [12], injuries of large blood vessels 0.04-1.22% depending on the study [11]. They are more common in older age patients, male gender. During the cholecystectomy, Calot area was surgically opened, the vessel and duct of gallbladder were cut and clips applied, gallbladder was surgically removed from its adjoining area of liver.

The knowledge of anatomy of the borders and structures of Calot`s triangle can be very useful in preventing the intraoperative and postoperative complications during laparoscopic cholecystectomy in a treatment of cholelithiasis [4]. In 1891, the French surgeon Jean F. Calot described a triangular area formed by the cystic artery, common hepatic duct and the cystic duct. The borders of this triangle are as follows: the superior and inferior borders represented by the cystic artery and cystic duct, respectively which are equal and a little longer medial border, represented by the part of the hepatic duct, near the terminal part of the cystic duct. Later, Calot`s space was renamed as to hepatobiliary, hepatocystic or cystohepatic triangle; with a superior border given by the visceral surface of the liver, medial border – by the common hepatic duct and inferior border – by the cystic duct draining the gallbladder [6]. Therefore, the modern triangle appears to provide the surgeon with a more constant triangle boundary, one that would otherwise be variable, given the occasionally inconsistent pattern of the cystic artery [9]. The contents of cystohepatic triangle include the right hepatic arteria, cystic artery, lymph node of gallbladder, lymphatics and fibro-fatty connective tissue area. In patients without structural variations, laparoscopic removal of gallbladder stones is a routine technic for surgeons.

Laparoscopic surgery is a technique often chosen in case of gallbladder stones [9]. Vascular and ductal variations can disorientate the surgeon during performing of laparoscopic technic [1]. In this relation, the knowledge of Calot`s triangle anatomy is of significant importance for the operator especially when there are arterial and biliary anomalies [4].

The domestic swine as omnivorous, monogastric species is regarded as a suitable animal model for human diseases. There are a lot of similarities to humans in anatomy and functions of the immune system. Based on the facts that the porcine organs are anatomically comparable in size and the porcine immune system resembles human's in > 80%, in contrast to mice with only 10% [3], the pigs are currently thought to be the best candidates for organ donation in xenotransplantation. Moreover, the pigs are inexpensive and easy to maintain in pathogen-free facilities, have relatively short gestation periods, large litters, and are easy to breed making them readily available [7].

All mentioned studies provoked the question if these two triangles are present in domestic pigs.

Based on the facts above, we aimed to establish the existence of both the Calot`s triangle and the hepatocystic triangle as well as to define their borders in the domestic pigs.

Materials and Methods

Animals

This research work was conducted on male crossbred pigs (Landrace×Danube White). A set of fresh liver with gallbladder, stomach, spleen and cranial part of the duodenum was collected from 30 pigs divided in three age groups – 10 animals at the age of 2 months (24-32kg), 10 animals at the age of 6 months (91-115 кg) and 10 animals at the age of 3 years (250-310 кg), at a legal slaughterhouse. All measurements involved in the study were done at a slaughterhouse. All procedures were performed in accordance with the Bulgarian legislation regarding animal care (Ordinance 20 of 01.11.2012 on the minimum requirements for the protection and welfare of experimental animals and the requirements for the sites for use, breeding and/or delivery) and in accordance with Directive 2010/63 / EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Macromorphometric method

After removing the sets of organs from the porcine bodies, the connective tissue surrounding the extrahepatic bile ducts was dissected and removed in order to visualize and prepare them for a macromorphometric study. The studied macrometric parameters were the length (in millimeters) of the common hepatic duct (*ductus hepaticus commun*is), cystic duct (*ductus cysticus*), common bile duct (*ductus choledochus*) and of the right margin of quadrate lobe of liver as borders of Calot`s and hepatocystic triangles. The distance (in millimeters) between dorsal margin of the liver and the point of union of *ductus cysticus, ductus hepaticus communis* and *ductus choledochus* was estimated as well. The length measurements were performed using a digital electronic caliper (with accuracy 0.01 mm). The angles were measured by protractor (a half circle with marked degrees from 0 to 180).

Statistical analysis

Data were processed by GraphPad Prism 6 for Windows (GraphPad Software, Inc., USA) via one-way analysis of variance (one-way ANOVA) followed by Tukey-Kramer's posthoc test and were presented as mean ± standard deviation (SD). P-values lower than 0.05 were considered statistically significant.

The terminology was consistent with the Nomina Anatomica Veterinaria [10] and with Terminologia anatomica [17].

Results

In order to visualize the structures of Calot`s and hepatocystic triangles following steps are needed to be done for safe identification of the triangles` structures: the first step is the clearance of the HC triangle – the HC triangle should be cleared of all the adipose

tissue (**Fig. 1**). It allows further safe dissection and identification of the cystic duct arising from the gallbladder neck and of the cystic artery which is the second step. The cystic artery can pass cranially or caudally to *ductus hepaticus communis* and predominantly on the left side of the cystic duct. The third step is to find the union of cystic duct to common bile duct. This can be achieved by following the direction of cystic duct to the common bile duct. Once reached the point of union, the three extrahepatic bile ducts can be visualized: the ventral duct – *ductus cysticus,* the left duct – *ductus hepaticus communis* and the right duct – *ductus choledochus* which terminates into the wall of duodenum. These ducts together with cystic artery are the anatomical landmarks for identification of both triangle borders.

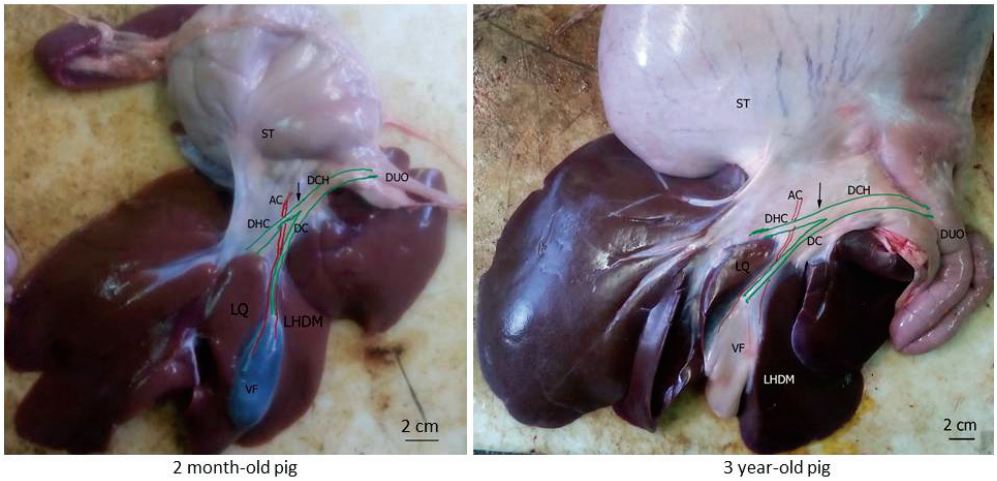

Fig. 1. Relevant anatomical structure before dissection – *(DC) ductus cysticus; (DHC) ductus hepaticus communis, (AC) a. cystica,(DCH) ductus choledochus, (LQ)lobus hepatis quadratus, (ST) stomach, (VF) vesica fellea; (DUO) duodenum; (arrow)* indicates the distance between the dorsal margin of the liver and the union of the bile ducts.

In this study, the borders of Calot`s triangle were visualized and their length was measured in the three aged groups (**Fig. 2, Table 1**):

1. The right border was represented by the cystic duct which length increased with age and a statistical significant difference was detected.

2. The left border was given by cystic artery which length increased with age without any statistical significant difference.

3. The dorsal border was represented by common hepatic duct which length increased with age without any statistical significant difference.

Calot`s triangle is referred to a scalene because none of the sides of the triangle have equal lengths. Its side represented by *ductus cysticus* was the longest, followed by that represented by *a. cystica* and *ductus hepaticus communis*, respectively. The Lymph node in the Calot`s triangle was not present, so that the main content was the adipose tissue.

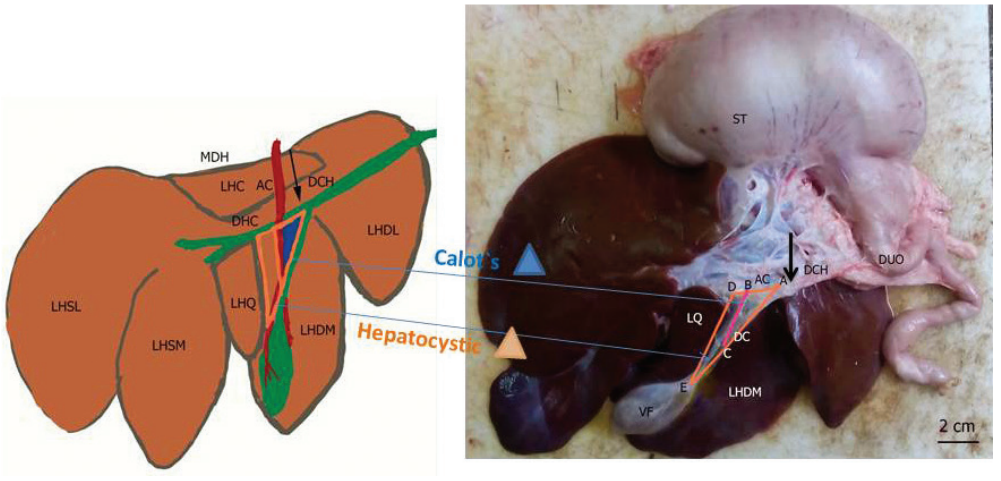

Fig. 2. Anatomy of hepatocystic and Calot's triangles in 6 month-old pig.
(Original figure, I. Stefanov) **Left:** Hepatocystic triangle (orange outline); **Right:** Calot's triangle (in blue); *(DC) ductus cysticus; (DHC) ductus hepaticus communis, (AC) a. cystica, (DCH) ductus choledochus, (LQ, LHQ) lobus hepatis quadratus, (LHSL, LHSM, LHDL, LHDM, LHC) lobus hepatis sinister lateralis, lobus hepatis sinister medialis, lobus hepatis dexter lateralis, lobus hepatis dexter medialis, lobus hepatis caudatus, espectively, (MDH) margo dorsalis hepatis;(ST) stomach, (VF) vesica fellea; (DUO) duodenum; (arrow)* indicates the distance between the dorsal margin of the liver and the union of the bile ducts.

The borders of the hepatocystic triangle also were visualized and their length was measured in the three aged groups (**Table 1**):

Table 1. The length (mm) of the borders of Calot`s triangle and hepatocystic triangle as well as the distance (mm) (MDH-UEBD) between *margo dorsalis hepatis* (MDH) and the union of the extrahepatic bile ducts (UEBD).

Calot`s triangle borders	**2 month-old pigs Length (mean±SD)**	**6 month-old pigs Length (mean±SD)**	**3 year-old pigs Length (mean±SD)**
ductus hepaticus communis Min-max	14.49±9.97**a1** 3.76-29.38	19.95±12.74 **a4, b1** 5.38-43.33	29.32±9.56 **a4** 15.23-44.98
ductus cysticus Min-max	35.71 ±4.16 **A2, B4** 29.71-40.28	60.71±13.26 **c2** 43.22-89.12	71.49 ±11.94 **c3** 52.67-92.12
arteria cystica Min-max	25.73±12.92 9.22-40.29	38.38±17.87 10.48-61.15	42.95±17.78 13.22-64.33
Hepatocystic triangle borders	**2 month-old pigs Length (mm) (mean±SD)**	**6 month-old pigs Length (mm) (mean±SD)**	**3 year-old pigs Length (mm) (mean±SD)**
ductus hepaticus communis Min-max	21.78±10.99 **a1** 6.28-32.19	35.35 ±13.87 **a4** 16.37-55.01	36.14±14.39 **a4** 16.92-57.22

Table 1.

ductus cysticus *Min-max*	39.07 ±4.11 **A3, B4** 31.28-44.15	65.28± 12.40 **d2** 50.18- 92.19	76.28±11.03 **d4** 58.73-94.28
lobus quadratus hepatis Min-max	32.98±14.48 **A1, B3** 13.27-49.92	50.00±14.40 19.77-69.87	51.87±15.88 21.22-71.88
Distance MDH-UEBD	23.92±3.77 **A4, B4** 16.11-27.72	36.84±5.44 28.43-44.38	36.71±5.55 29.88-43.28

Legend:
1,2,3,4 ($P < 0.05;0.01; 0.001;0.0001$, respectively)
A – Statistical significant difference against the age of 6 months
B – Statistical significant difference against against the age of 3 years
a – Statistical significant difference against the *ductus cysticus*
b – Statistical significant difference against the *a. cystica*
c – Statistical significant difference against the *a. cystica*
d – Statistical significant difference against the *lobus quadratus hepatis*

1. The right border was represented by the cystic duct which length increased with age and a statistical significant difference was detected.

2. The left border was given by the right margin of *lobus hepatis quadratus* which length increased with age and a statistical significant difference was detected.

3. The dorsal border was represented by common hepatic duct which length increased with age without any statistical significant difference.

The side of hepatocistic triangle represented by *ductus cysticus* was the longest, followed by that represented by *lobus hepatis quadratus* and *ductus hepaticus communis*, respectively.

Obviously, the area of hepatocystic triangle is bigger than that of the Calot`s triangle. So that the Calot`s triangle was formed as a part of hepatocystic triangle.

The main content of hepatocystic triangle is the large amount of adipose tissue and *arteria cystica* (**Figs 1, 2**).

In order to define more easily the union of the three extrahepatic bile ducts the distance between the dorsal margin (*margo dorsalis*) of the liver and the level of union was evaluated (**Table 1**). This distance in 6 month- and 3 year-old pigs was larger than in immature ones.

The angles of Calot`s triangle and of hepatocystic triangle were estimated (**Table 2**).

Table 2. Values (degrees ^{0}) of the angles (∠CAB, ABC, ACB, ADE and AED, see **Figs**. **1, 2**) of Calot`s triangle and of hepatocystic triangle in pigs from the three age groups as well as between the *ductus cysticus* and the *ductus choledochus* (∠DC-DCH), between the *ductus cysticus* and the neck of gallbladder (∠DC-GB) to the long axis (LA) of the gallbladder.

Angles	**2 month-old pigs (mean±SD)**	**6 month-old pigs (mean±SD)**	**3 year-old pigs (mean±SD)**
∠CAB	50.4 ±21 **A4, B4**	48.7±1.3	48.9 ±1.1
∠ABC	101.7±2.6 **A4, B4, a4**	109.4±1.3 C2,a4	112.0±1.7 a4
∠ACB	24.0±1.0	20.10±0.9	20.00±0.8

∠ADE	110.3±0.8**A4, B4**	114.9± 1.7 C4	116.4±1.4
∠AED	30.0±1.2	28.5±0.5	28.0±0.7
∠DC-DCH	125.5±9.84 110.0-140.0	130.3±17.33 110.0-162.0	133.0±20.17 110.0-170.0
∠DC-GB	126.5±12.70 115.0-153.0	126.0±23.07 90.00-166.0	135.5±23.62 100.0-173.0

Legend:
∠CAB – the angle between *ductus cysticus* and *ductus hepaticus communis*
∠ABC – the angle between *ductus hepaticus communis* and *a. cystica*
∠ACB – the angle between *ductus cysticus* and *a. cystica*
∠ADE – the angle between *ductus hepaticus communis* and *right margin of lobus hepatis quadratus*
∠AED – the angle between *ductus cysticus* and right margin *of lobus hepatis quadratus*
2, 4 ($P < 0.01$; 0.0001, respectively*)*
A – Statistical significant difference against the age of 6 months
B – Statistical significant difference against the age of 3 years
C – Statistical significant difference against the age of 3 years
a – Statistical significant difference between ∠ABC and ∠ADE

The angles between *ductus cysticus* and *ductus hepaticus communis*, between *ductus cysticus* and *a. cystica,* between *ductus cysticus* and right margin of *lobus hepatis quadrates* decreased with age*,* while the angles between *ductus hepaticus communis* and *a. cystic* as well as the angle between *ductus hepaticus communis* and right margin of *lobus hepatis quadrates* increased with age (**Figs. 1, 2, Table 2**).

In addition, the angles between cystic duct and common bile duct as well as between the cystic duct and the neck of gallbladder were evaluated (**Table 2**). The both angles showed similar values and were the largest in all age groups.

Discussion

In this study, for the first time, the borders of Calot`s triangle and of hepatocistic triangle were identified and their length was measured in the three aged groups of pigs. The similarities and differences between human and porcine triangles were discussed as well. It was found that the borders of Calot`s triangle in porcine liver are identical to that of Calot`s triangle in humans. In both pigs and humans the area of hepatocystic triangle is bigger than that of the Calot`s triangle. So that the Calot`s triangle is formed as a part of hepatocystic triangle. However, due to the different anatomical position of the body in both species the different terms are used in the definition of sides of the triangles in pigs and in humans. The right side of porcine Calot`s triangle was represented by the cystic duct, the left side was given by cystic artery, the dorsal side was represented by common hepatic and none of the sides of the triangle have equal lengths. In humans the superior and inferior borders of Calot`s triangle, represented by the cystic artery and cystic duct, respectively are equal and a little longer medial border, represented by the part of the hepatic duct, near the terminal part of cystic duct [6]. Therefore the Calot`s triangle in pigs is referred to as scalene but in humans it's referred to as isosceles.

The sides of hepatocystic triangle in humans and pigs are represented by similar structures. However, again due to the different anatomical position of the body in both species, the different terms are used in definition of the sides of triangles. In pigs, they are represented by the right margin of the visceral surface of *lobus hepatis quadratus* forming the left side, *ductus cysticus* forming the right side and *ductus hepaticus communis* forming the dorsal side. The cystohepatic triangle in humans has a superior (cranial) side given by the visceral surface of the liver, medial (right) side – by the common hepatic duct and inferior (caudal) border – by the cystic duct [6]. The right hepatic arteria, cystic artery, cystic lymph node, lymphatics and fibro-fatty connective tissue are the contents of cystohepatic triangle.

The borders and contents of the hepatobiliary triangle are the main landmarks used by surgeons when performing the laparoscopic technique. In humans, a detailed study regarding the anatomy of hepatobiliary triangle and its variations was carried out by Ahmad et al. [1] using a laparoscope. These authors found out that 63.6% of patients expressed cystic duct, cystic lymph nodes and cystic artery variations. Among them 12% depicted cystic duct variations, 32.2% of patients demonstrated cystic lymph nodes variations and 19.4% of the patients showed cystic artery variations. Fat deposition, fibrosis and adhesions were also observed in hepatobiliary areas of female patients. Ahmad et al. [1] revealed that 32.2% of the patients had cystic lymph node variations. Cystic lymph node was found posterior to cystic duct in 8.1%, anterolateral to cystic duct 8.1%, and outside hepatobiliary triangle in 8% of the patients. Normal cystic duct was documented in 83.85% of the patients. Percentages of cystic duct variations included broad cystic duct in 4%, long cystic duct in 3.67%, short cystic duct in 4.33%, absence of cystic duct in 0.33%, spiral cystic duct in 2.70%, double cystic duct in 0.33%, accessary cystic duct in 0.10%, adherent cystic duct in 0.33%, and parallel insertion of cystic duct to form common bile duct in its retroduodenal part in 0.15% cases. Bleeding and biliary injury force surgeon to do open abdominal operation especially when structural variations are encountered [15].

The cystic artery is the key structure clipped or ligated during laparoscopic or conventional cholecystectomy [5, 9]. Ahmad et al. [1] identified single cystic artery in hepatobiliary triangle in 76.02% of cases, double artery in hepatobiliary triangle – in 9.88%. In terms of syntopic relations of *a. cystica* to *ductus cysticus* in pigs, two variations were described by us in previous study [13]. The first type of variation (92% of cases) showed that a. *cystica* passed on the left of *d. cysticus* and caudally to v. *portae* and *d. hepaticus communis*. In this case *a. cystica* originated from *r. dexter medialis* and from *a. gastroduodenalis*. The second type of variation (8% of cases) represented the origin of *a. cystica* from the common trunk of *r. dexter lateralis* and *r. dexter medialis.* In this case*,* the beginning of *a. cystica* was located on the right of *ductus cysticus*. Then *a. cystic* directed ventrally passing cranially to *ductus choledochus* to the place of its division.

Anatomical variations increase the risk of structural injuries during the laparoscopic cholecystectomy can be prevented by precise operative technique, clear visualisation of anatomical landmarks, and careful dissection of tissues [11]. The risk is further increased when these variations are encountered during laparoscopic visualization rather than open surgery. Of all the structural injuries following a cholecystectomy, bile duct injury BDI is the most feared because it can result in high morbidity, long-term hospitalization, and may be life-threatening [2, 14]. The identification of the Calot's

triangle borders is very important in order to prevent bile duct injury (BDI) [2, 16]. The potential for BDI remains statistically greater with the laparoscopic approach [2, 8]. The incidence of BDI in open cholecystectomy is estimated at 0.1-0.25% whereas the figure is higher in the laparoscopic approach, at 0.5% [13, 14]. Other studies indicate that the cases of injuries to the common bile duct varies from 0.1 to 0.6% [12, 11].

Conclusion

This study revealed that, like in humans, the Calot`s triangle and the hepatocystic triangle with similar borders are presented. Full knowledge of the borders and contents of the Calot`s triangle as important anatomical landmarks can be very helpful for surgeons in developing new technics for safe execution of cholecystectomy and to avoid intraoperative and postoperative bleeding and biliary leakage.

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