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Inflammatory responses to *Opisthorchis viverrini* infection in animal models: A comparison between susceptible and non-susceptible hosts in different anatomical locations

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Abstract

Background: Inflammation caused by *Opisthorchis viverrini* infection increases the risk of cholangitis, cholecystitis, and leads to bile duct cancer (cholangiocarcinoma or CCA). However, only certain infected individuals are susceptible to CCA, suggesting the involvement of host factors in cancer development. In addition, there are reports indicating differences in the locations of CCA.

Aim: This study aims to investigate cellular inflammatory responses in the common bile duct (CB), intrahepatic bile duct (IHB), and gallbladder (GB) in susceptible and non-susceptible hosts following *O. viverrini* infection.

Methods: Thirty Syrian golden hamsters (a susceptible host) and 30 BALB/c mice (a non-susceptible host) infected with *O. viverrini* were studied at six time points (five animals per group). Histopathological evaluations were conducted on samples from the IHB, CB, and GB. Inflammatory cell infiltration was quantitatively assessed and compared between groups and time points. Statistical analysis was performed using one-way ANOVA, with a significance level of $p < 0.05$.

Results: Inflammation was significantly more pronounced in the IHB compared to the other two biliary locations. In comparison between susceptible and non-susceptible hosts, the intensity of inflammation was higher in the OV+H group than in the OV+M group ($p < 0.05$).

Conclusion: This study highlights the association between host response to inflammation, tissue location, and host susceptibility, with the IHB showing particular susceptibility to inflammation and pathological changes. These findings contribute to our understanding of the increased risk of CCA in susceptible hosts.

Keywords: *Opisthorchis viverrini*, Susceptible host, Non-susceptible host, Locations, Intrahepatic bile duct.

Introduction

Opisthorchis viverrini (*O. viverrini*), a foodborne trematode, remains a significant public health issue in the Greater Mekong Subregion, affecting approximately 12 million individuals (Sripa *et al*., 2021). Infection occurs through the consumption of raw or undercooked cyprinid fish containing the infective stage of the fluke, known as the metacercaria (Nair *et al*., 2011). Upon ingestion, the flukes migrate through the common bile duct (CB), primarily inhabiting the biliary tree, which includes both intra- and extrahepatic ducts as well as the gall bladder and pancreatic duct (PD) (Kaewkes and Sripa, 2004; Sithithaworn *et al*., 2014). Inflammation resulting from *O. viverrini* infection can lead to various biliary tract abnormalities, with the severity often associated with the specific location. Chronic inflammation of the gallbladder (GB) typically presents as cholecystitis, GB abscess, and cholelithiasis (Bhamarapravati *et al*., 1978; Harinasuta *et al*., 1984; Riganti *et al*., 1989), while lesions in the biliary tree can cause cholangitis, periductal fibrosis, and potentially progress to bile duct cancer (Sripa *et al*., 2018). Notably, the development of pathological

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lesions appears to be influenced by the location of the biliary tract.

Chronic *O. viverrini* infection is believed to contribute to the development of bile duct cancer, specifically cholangiocarcinoma (Campbell *et al*., 2017), through a «perfect storm» of carcinogenic stimuli (Sripa and Pairojkul, 2008; Sripa *et al*., 2012). However, less than 5% of individuals with opisthorchiasis develop malignancy (Sripa and Pairojkul, 2008; Parkin *et al*., 2010; Ghouri *et al*., 2015), indicating that susceptible individuals with a specific phenotype are more likely to develop cancer in response to persistent inflammation caused by the infection (Sripa *et al*., 2012). The Syrian golden hamster has been identified as a susceptible host for liver fluke infection in the liver fluke model, while mice naturally reject and expel the worms from their bodies (Choi *et al*., 2003; Chung *et al*., 2004; Boonmars *et al*., 2009). Furthermore, the Syrian golden hamster is a preferred model for cancer research, particularly in the study of CCA (Bhamarapravati *et al*., 1978; Sudsarn *et al*., 2014; Hanpanich *et al*., 2017; Loeuillard *et al*., 2019). These findings suggest that the host's susceptibility plays a crucial role in the development of CCA.

Bile duct cancer manifests along the biliary system, and has been categorized into three types based on their anatomical site of origin: intrahepatic (iCCA), perihilar (pCCA), and distal CCA (dCCA) types (Sarcognato *et al*., 2021). However, not all biliary locations have an equal propensity for CCA development; certain areas, particularly the intrahepatic bile duct (IHB), are major sites for cancer establishment (Rizvi *et al*., 2018). A strong association has been established between *O. viverrini* infestation and the development of CCA (Cardinale *et al*., 2018). The inflammatory response is believed to be involved in cancer susceptibility, as individuals with a severe inflammatory response are considered more prone to developing CCA based on the concept of «helminth infection-induced malignancy» (Brindley and Loukas, 2017; Fried *et al*., 2011). Consequently, we formulate a hypothesis that the establishment phase of infection may be linked to the host's inflammatory response.

However, the association between inflammatory responses at usual locations of the biliary system, including IHB, CB, and GB, and host susceptibility has not yet been established. To investigate the pattern and response of cellular inflammation in three different locations among both susceptible and non-susceptible hosts, a study was designed using hamster and BALB/c mouse models. The objective of this study was to confirm the role of the inflammatory cell response in each location during the early phase of *O. viverrini* infection.

Materials and Methods

Fish collection, O. viverrini metacercariae isolation, and identification

To isolate and select the infective stage (metacercariae) of *O. viverrini*, freshwater cyprinoid fish were procured from water reservoirs located within the endemic region of Northeast Thailand. The fish underwent homogenization using an electronic blender, along with a solution comprising 0.25% pepsin and 1.5% hydrochloric acid (HCl) sourced from Wako Pure Chemical Industries, Osaka, Japan. This concoction was subsequently subjected to an incubation process within a water bath at 37°C, lasting for 1 hour. Upon completion of digestion, the resultant solutions underwent filtration through sieves of varying mesh sizes (1,000, 300, 106, and 250 µm) to meticulously isolate the metacercariae. Following this, the metacercariae were precipitated within a 0.85% NaCl solution, following a previously documented procedure (Bhamarapravati *et al*., 1978), and were subsequently identified based on morphological characteristics observed under a stereomicroscope (Kaewkes and Sripa, 2004).

Animal models and sample collection

Six-week-old male Syrian golden hamsters *(Mesocricetus auratus)* obtained from the Laboratory Animal Unit, Faculty of Medicine, Khon Kaen University, and 6-week-old male BALB/c mice from Nomura Siam International were used as animal models. A total of 30 animals were allocated to each group, with Group I (OV+M) consisting of BALB/c mice infected with *O. viverrini* and Group II (OV+H) consisting of Syrian golden hamsters infected with *O. viverrini* (Table 1). The animals were given 50 metacercariae by orogastric intubation. At specific time points Day 1, 2, 3, 7, 14, 28, and 56 (Table 1), five animals from each group were sacrificed using an overdose of isoflurane inhalation. Their livers including the IHB, CB, and gall bladder (GB) were dissected and collected for subsequent histopathological studies (Fig. 1).

Quantitative study of inflammatory pattern on various O. viverrini-residing locations

To quantitatively study, the inflammatory cell infiltration of different locations, namely the IHB, CB, and GB, the tissues from the biliary system

Table 1. The experimental groups consisted of Syrian golden hamsters and BALB/c mice that were infected with *O. viverrini* (Ov). At each designated time point, five mice and five hamsters from each group were euthanized for subsequent analysis.

Groups	Animal	*O. viverrini* infected	Sacrificing number per time point					
			D1	D2	D7	D14	D28	D56
OV+M	BALB/c mice	+	5	5	5	5	5	5
OV+H	Syrian hamster	+	5	5	5	5	5	5

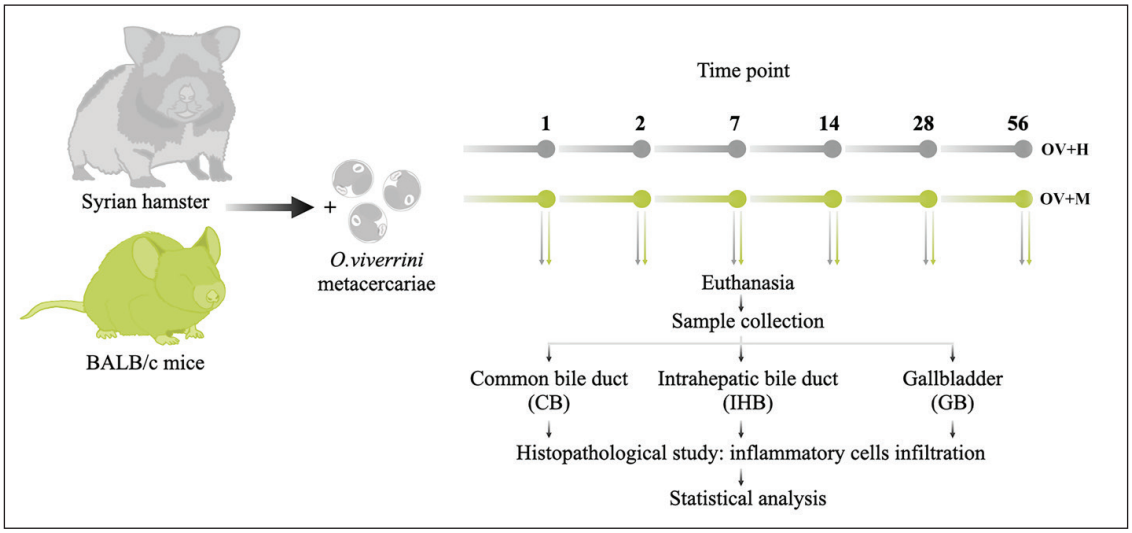

Fig. 1. In the location study, the experimental design encompassed the infection of two different animal species. Subsequently, these animals underwent sacrifice, and sample collection was conducted at specific time intervals: 1, 2, 7, 14, 28, and 56 days. The experimental groups were designated as follows: OV+M (BALB/c mice with *O. viverrini* infection), and OV+H (Syrian golden hamsters subjected to *O. viverrini* infection).

were processed using routine histology techniques. The tissues were embedded in paraffin blocks, and 4 µm-thick sections were cut using a microtome and placed on coated glass slides. Subsequently, the tissue sections were deparaffinized and stained with routine hematoxylin and eosin. Inflammatory cell infiltration was identified following the method described by Dulaimi *et al*. (2018). Infiltrating leukocytes were then quantitatively evaluated by counting the cells from ten non-overlapping high-power fields under a light microscope (Suyapoh *et al*., 2021a).

Statistical analysis

Statistical analysis in this study was conducted using SPSS version 23.0. To compare the mean values between different groups, a One-way ANOVA with Fisher's Least significant difference post hoc test was employed. A *p*-value of less than 0.05 was considered statistically significant.

Ethical approval

The study protocol was subjected to review and approved by the Animal Ethics Committee of Khon Kaen University approved the study protocol (IACUC-KKU 78/2561).

Results

Infiltration pattern of inflammatory cells across distinct biliary in susceptible and non-susceptible hosts

To investigate the pattern of inflammatory responses in the biliary tract system of hamsters and mice infected with *O. viverrini*, we assessed the infiltration of inflammatory cells in three relevant tissues: the IHB, CB, and GB. Quantitative cell counts were performed through histopathology at six different time points after infection (Table 2).

Overall, the pattern of inflammatory cell infiltration differed between the early and chronic phases but remained similar across all biliary and GB tissues. Initially, a small number of neutrophils, eosinophils, and mononuclear cells were observed in the lamina propria and submucosa of IHB, CB, and GB. These cells were particularly detected at the portal area where *O. viverrini* resided. As the infection progressed beyond the acute phase, a greater infiltration of mononuclear cells was observed, with some of these cells penetrating the intra-epithelium of the mucosa, particularly in the IHB and GB tissues. In addition, a high number of eosinophils were detected during this period, primarily located beyond the mucosal area infected by the worm. The extent of inflammatory cell infiltration varied but was most extensive in IHB, followed by CB and GB, respectively (Fig. 2).

In the hamster model, the pattern of inflammatory cell infiltration in the CB tissue exhibited low levels during the acute phase, specifically at 1–2 days post-infection (d.p.i.). Subsequently, the inflammation increased significantly during 7–14 d.p.i. and reached its maximum level at 28 d.p.i. The inflammation remained stable thereafter until 56 d.p.i. On the other hand, the IHB tissue showed a similar trend, with inflammation increasing during the early phase but declining at 56 d.p.i. On the contrary, the levels of leukocyte infiltration in the GB tissue remained unchanged throughout the infection period. In the mice model, the inflammation levels at CB and IHB slightly increased during the 1–7 d.p.i., reached a significant peak at the 14 d.p.i., and then declined at 28–56 d.p.i. Meanwhile, the infiltration pattern at the GB tissue showed slight detection at the early period of infection, dropped during 7–14 d.p.i,

Table 2. Quantitative inflammatory cell infiltration in the CB, IHB, and GB in hamsters and mice at different time periods is presented below. *O. viverrini* infected hamster (OV+H), *O. viverrini* infected BALB/c mice (OV+M). The data is reported as mean with standard deviation (SD).

Groups	Time point (Day)					
	1	2	7	14	28	56
CB						
OV+H	77.60 (63.23)	72.20 (31.41)	147.40 (30.46)	243.40 (59.70)	856.00 (109.05)^{D,H,K,M}	842.00 (464.76)^{E,I,L,N}
OV+M	61.80 (46.93)	83.60 (55.59)	158.60 (113.90)	255.80 (71.56)^{C,G}	218.00 (52.25)^{D,H}	180.80 (153.22)^{E}
IHB						
OV+H	161.60 (46.48)	160.50 (29.20)	230.80 (85.20)	1139.20 (124.51)^{C,G,J}	2026.50 (610.13)^{D,H,K,M}	1184.90 (54.09)^{E,I,L,O}
OV+M	96.90 (13.68)	215.00 (77.98)	839.40 (197.30)^{B,F,K,L}	1036.50 (245.55)^{C,G,M,N}	371.60 (344.19)^{D}	120.70 (42.26)
GB						
OV+H	128.80 (49.94)	97.40 (123.86)	80.80 (33.28)	121.20 (144.65)	84.60 (52.48)	223.00 (113.92)^{I,L,O}
OV+M	43.80 (17.28)	14.60 (14.31)	12.80 (11.39)	0.00 (0.00)^{M}	66.60 (90.54)	132.80 (64.30)^{E,I,L,N,O}

Superscript clarified significance between groups at $p < 0.05$—i.e., A = Infection day 1–2, B = Infection day 1–7, C = Infection day 1–14, D = Infection day 1–28, E = Infection day 1–56, F = Infection day 2–7, G = Infection day 2–14, H = Infection day 2–28, I = Infection day 2–56, J = Infection day 7–14, K = Infection day 7–28, L = Infection day 7–56, M = Infection day 14–28, N = Infection day 14–56, O = Infection day 28–56 in the same group of infection.

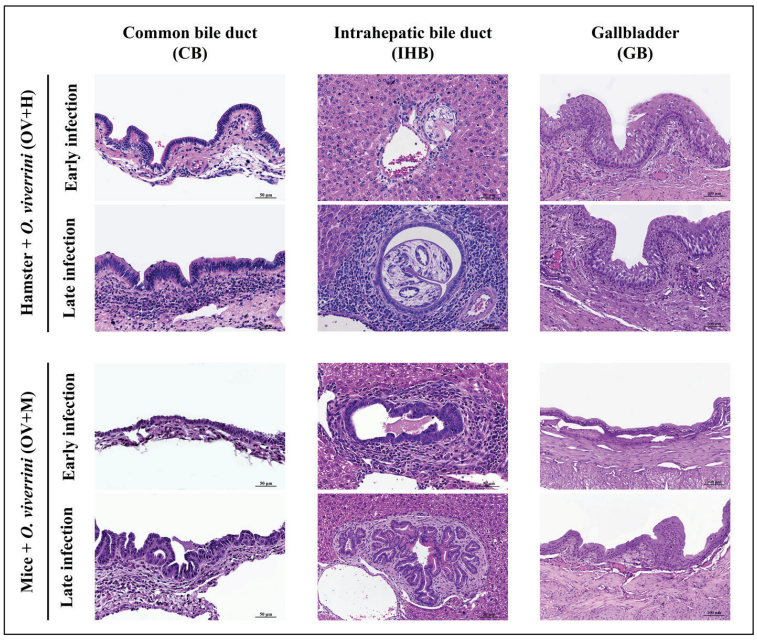

Fig. 2. Histopathological features of inflammatory cell infiltration in the biliary tract of *O. viverrini* infected hamsters and mice. Notably, there are distinct patterns observed during the early and late infection periods. In both the IHB and CB, the inflammation is more pronounced in the early stage of infection when compared to the later stages in both groups (left and middle column). Conversely, the GB exhibits a moderate and consistent level of leukocyte infiltration in the OV+H group, which contrasts with the OV+M group (right column) [H&E, original magnification = ×40, the scale bar represents 50 µm].

and then rose toward the end of the experiment. For more detailed information on the quantitative cell count of overall inflammation (Table 2).

Comparison of inflammatory pattern between susceptible and non-susceptible hosts

The quantitative comparison of biliary tract inflammation between OV+H and OV+M was assessed based on histopathological sections during the early and late phases (Fig. 3). In general, the inflammation observed in the OV+H was more intense compared to the OV+M across all locations. Specifically, in the CB, the OV+M group exhibited slightly higher inflammatory cell infiltration during the early phase (2–7 d.p.i.) than in the late phase, and there was no significant difference between the 2 groups (Fig. 3a). However, in the OV+H group, *O. viverrini* infection significantly enhanced leukocyte infiltration during the late period, particularly at 28 and 56 d.p.i. with

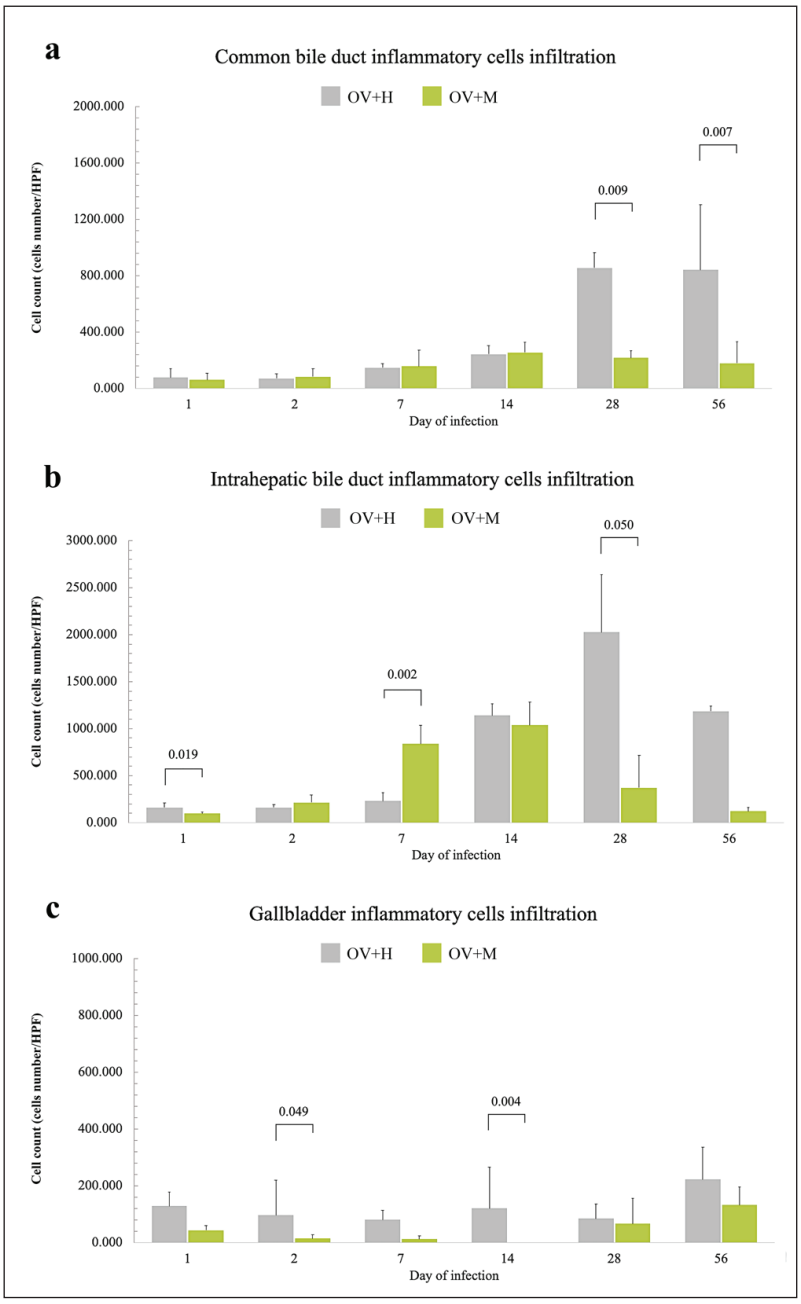

Fig. 3. A comparison of inflammatory cell infiltration at the biliary and GB area of the hamsters and mice infected with *O. viverrini* from 1 to 56 days. (a) CB. (b) IHB. (c) GB. The comparison data was illustrated with a significant difference, $p < 0.05$.

p = 0.009 and 0.007, respectively (Fig. 3a). These findings suggest that the inflammatory response in the biliary tract, specifically in the CB, differs between the two animal models. While the OV+M group shows a relatively stable level of inflammation, the OV+H group exhibits a significant increase in leukocyte infiltration during the late phase of infection.

In the IHB, the inflammatory pattern in both the short and long term exhibited a similar trend to that observed in the CB. On the first day of infection, a significantly high number of infiltrating leukocytes was detected in the OV+H group (p = 0.019), but this number declined by day 2 post-infection. At day 7 post-infection, the average number of infiltrating leukocytes in the OV+M group was significantly higher than in the OV+H group (p = 0.022). However, at later infection periods (14, 28, and 56 d.p.i.), a higher number of inflammatory cells were detected in the OV+H group. The maximum number of leukocyte infiltrations was significantly found at day 28 (p = 0.050) (Fig. 3b). Regarding the GB, minimal levels of inflammation were observed in both the OV+H and OV+M groups. However, a higher infiltration of inflammatory cells was observed in infected hamsters compared to mice. The significantly highest inflammation was found on days 2 and 14 in the OV+H group (p = 0.049 and 0.004, respectively) (Fig. 3c). These results indicate that the inflammatory response in the IHB and GB differs between the OV+H and OV+M groups. While the OV+H group exhibits a higher number of infiltrating leukocytes in the IHB and GB during the later stages of infection, the OV+M group shows a relatively higher number of inflammatory cells in the IHB during the early phase of infection.

Discussion

Opisthorchis viverrini is classified by the International Agency for Research on Cancer [28] as a Group I biological carcinogen to humans, specifically causing bile duct lesions and cholangiocarcinoma (Group 1) (IARC, 1994). Chronic inflammation resulting from *O. viverrini* infection is believed to be a risk factor for severe biliary abnormalities (Aksorn *et al*., 2018; Sripa *et al*., 2009). This phenomenon typically appears in different locations along the biliary tree (Pungpak *et al*., 1985; Riganti *et al*., 1989; Sripa and Kaewkes, 2002). Unfortunately, some individuals affected by *O. viverrini* infection may ultimately develop cholangiocarcinoma (Ghouri *et al*., 2015; Parkin *et al*., 2010; Sripa and Pairojkul, 2008). This bile duct cancer can be classified into 3 subtypes based on anatomical locations: intrahepatic type (iCCA) which develops from either large or small IHBs; perihilar (pCCA), located at the hilar region, and distal CCA (dCCA) arising from the extrahepatic bile duct (EHB) (Bragazzi *et al*., 2018; Sarcognato *et al*., 2021). The severity of lesions and symptoms appears to be associated with individual susceptibility, both in human and animal models (Boonmars *et al*., 2009; Choi *et al*., 2003; Chung *et al*., 2004; Khuntikeo *et al*., 2018). In this study, a systematic investigation of the inflammatory response in different biliary locations and susceptible/non-susceptible animal models was conducted, revealing that *O. viverrini* infection leads to more severe inflammation in the susceptible hamster model compared to the non-susceptible mouse model. Among the different biliary locations, the inflammation in the IHB was found to be particularly intense. These results suggest that hosts susceptible to *O. viverrini* infection experience more severe inflammation, especially in the IHB area.

Inflammatory cell infiltration including neutrophils, eosinophils, macrophages, lymphocytes, and mast cells, is commonly observed in areas infected by *O. viverrini* (Bhamarapravati *et al*., 1978; Lvova *et al*., 2012; Sripa *et al*., 2018; Suyapoh *et al*., 2021a). Studies have reported minimum reactions with neutrophils, macrophages, eosinophils, and lymphocytes in hamsters, occurring in the EHB including CB and GB on days 1–7 of infection (Sripa and Kaewkes, 2002). This suggests that the inflammation may be a response to the migration of juvenile liver fluke [3, 37]; and the secretory antigens by immature parasites [38], the first-line defense mechanisms such as mucus hypersecretion (Kaewkes and Sripa, 2004; Nithikathkul *et al*., 2007), and the mucosal immune response in bile fluid, specifically involving IgA and IgE (Wongratanacheewin *et al*., 1988).

During migration, immature fluke reaches the IHB and induces a slightly inflammatory response with neutrophils and eosinophils (Bhamarapravati *et al*., 1978; Sripa and Kaewkes, 2000). As the flukes develop into adult worms, they are commonly detected in the larger ducts of the intrahepatic biliary tree (Pungpak *et al*., 1985; Pungpak *et al*., 1989). Mature *O. viverrini* worms induce strongly cell-mediated immune responses in the periductal area (Bhamarapravati *et al*., 1978; Sripa *et al*., 2018). Various animal studies have described patterns of leukocyte infiltration, where chronic inflammation with intense mononuclear cells, a moderate number of eosinophils, and mast cells are detected after a week post-infection (Bhamarapravati *et al*., 1978; Sripa and Haswell, 2021; Sripa and Kaewkes, 2000). In heavy infection with *O. viverrini*, the worms can be found in other locations, including EHB, PD, GB, and CB (Pungpak *et al*., 1985; Pungpak *et al*., 1989). The adult helminths are more frequently detected in GB than in the CB, likely due to the larger space available in the GB. Gross findings may reveal thickening, opacity, and dilatation of the GB (Boonmars *et al*., 2009; Wonkchalee *et al*., 2011). Microscopically, mild-to-moderate infiltrations of eosinophils and mononuclear cell infiltration can be seen 7–14 d.p.i. (Sripa and Kaewkes, 2002). The severity of inflammation depends on the number of flukes in close contact with the mucosal surface of those organs (Sripa and Kaewkes, 2002).

The mechanisms of hepatobiliary inflammation initiated by adult flukes of *O viverrini* are multi-factorial and involve various factors, including mechanical damages, bacterial colonization, and secreted proteins (Brindley and Loukas, 2017; Gouveia *et al*., 2017; Sripa *et al*., 2012; Sripa *et al*., 2017). The mechanical action of sucking, facilitated by the oral and ventral suckers of the fluke, contributes to *O. viverrini*-mediated inflammation (Sripa *et al*., 2012). In addition, the liver fluke plays a significant role as a carrier of carcinogenic bacteria, *Helicobacter pylori* (Deenonpoe *et al*., 2015). The presence of *O. viverrini* enhances the migration and colonization of *H. pylori*, leading to severe inflammation and pathological changes in the biliary system (Suyapoh *et al*., 2021a; Suyapoh *et al*., 2021b). Furthermore, there is evidence that *O. viverrini* can modify the intestinal microbiome. Plieskatt *et al*. (2013) reported that *O. viverrini* infection results in alterations in the composition of the intestinal flora the evidence of liver fluke-modification of intestinal microbiome. This dysbiosis of the intestinal microbiota can promote the translocation of microorganisms toward the liver, leading to an immunological response known as the «Leaky-Gut Hypothesis» (Giordano *et al*., 2018).

The «leaky-gut hypothesis» suggests that impaired intestinal barrier function can result in increased permeability, allowing the translocation of gut bacteria and their products into the liver. This translocation can trigger an inflammatory response (Giordano *et al*., 2018). In the case of *O. viverrini* infection, it has been proposed that the parasite may disrupt the intestinal microbiome, leading to dysbiosis and increased intestinal permeability (Harris and Loke, 2017; Plieskatt *et al*., 2013). This, in turn, may facilitate the translocation of bacteria and their products to the liver and biliary system. The chronic inflammation caused by *O. viverrini* infection is believed to be a key factor in the development of cholangiocarcinoma (Oh *et al*., 2014). *O. viverrini* secretes excretory/secretory products (OvES) that have been implicated in the pathogenesis of liver fluke-induced bile duct cancer (Jittimanee *et al*., 2007; Nair *et al*., 2011). OvES may promote the proliferation of bile duct epithelial cells and contribute to the development of IHB cancer (Nair *et al*., 2011). Liver fluke infection is also associated with alterations in the host's immune response. It can modulate cytokine production and antigen presentation, which may contribute to the chronic inflammation and carcinogenesis observed in infected individuals (Chen *et al*., 2012).

Indeed, *O. viverrini* infection can have multiple effects on the liver and biliary system that contribute to chronic inflammation and the development of cholangiocarcinoma. The alteration of the liver microbiome and the promotion of *H. pylori* growth by the parasitic infection can enhance inflammation and potentially increase the risk of malignancy [50, 53]. Various studies have reported the production of excretory/secretory products (OvES) by *O. viverrini* (Oh *et al*., 2014; Rim, 2005). The production of OVES by the fluke, which increases as the parasite matures, has been associated with heavy leukocyte infiltration and proliferation of bile duct epithelium (Nair *et al*., 2011; Sripa and Kaewkes, 2000). This proliferation of the bile duct epithelium, triggered by the endocytosis of OvES by cholangiocytes, is thought to contribute to the higher incidence of IHB cancer compared to other types of cholangiocarcinoma. The increased production of OvES with the maturation of the parasite corresponds to the heavy infiltration of leukocytes, indicating a possible role in the inflammatory response. Studies have demonstrated that OvES can promote the growth of cholangiocarcinoma cells both *in vitro* and *in vivo*, indicating a potential role in the development of cholangiocarcinoma (Chen *et al*., 2012; Harris and Loke, 2017). Epidemiological studies have extensively documented the association between *O. viverrini* infection and CCA, further supporting the idea that chronic inflammation induced by the parasite plays a crucial role in the development of this cancer (Chen *et al*., 2012; Sripa *et al*., 2018).

The variability in the inflammatory response to *O. viverrini* infection between different host types is indeed interesting. In BALB/c mice, the inflammatory response in the CB and GB was generally low, with mild to moderate inflammation observed at 1–2 weeks post-infection, consistent with previous studies (Boonmars *et al*., 2009). Gross examination of the livers of infected mice did not reveal any notable abnormalities. The complexity of the inflammatory response to trematode infections, such as *Clonorchis sinensis* (*C. sinensis*), is influenced by various factors, including the parasite type, the host immune response, and the genetic background of the host. Notably, *C. sinensis*, similar to *O. viverrini*, is commonly found in the IHB (Rim, 2005; Oh *et al*., 2014). In *C. sinensis* infection, a peak of leukocyte infiltration was reported at 2–3 weeks post-infection, followed by a gradual decrease (Choi *et al*., 2003). This non-susceptibility phenomenon may be associated with an early significant increase in Th1 cytokines, such as IFN-γ, IL-2, and IL-10, at this time point (Choi *et al*., 2003; Wang *et al*., 2021). In addition, similar results were recently reported in another liver fluke, *Opisthorchis felineus* (Avgustinovich *et al*., 2021). In non-susceptible hosts, it is possible that the immune system effectively eliminates the parasite with the assistance of immune cell infiltration. Furthermore, in other opisthorchiasis models, the infiltration of immune cells appears to depend on the host's master coregulatory or MTA1, which plays a role in host-parasite interactions (Nair *et al*., 2011). One study found that intense bile duct inflammation was only detected in wild-type Mta1+/+ mice, whereas inflammation was less severe in Mta1-/- mice (Nair *et al*., 2011). This suggests that host factors, including coregulators

and genetic factors, can influence the inflammatory response to opisthorchiasis.

The extensive cellular infiltration observed in the biliary system of Syrian golden hamsters in our study is consistent with previous findings reported by Bhamarapravati *et al*. (1978) and Lvova *et al*. (2012). It has been suggested that the higher inflammation observed in hamsters is associated with the early expression of IL-12 in the liver in response to the parasite antigens, followed by a shift toward Th2 cytokine, particularly IL-10 (Jittimanee *et al*., 2007). The predominance of a Th2-type immune response and reduced Th1 response may contribute to immune homeostasis and allow the survival of the parasite (Chen *et al*., 2012; Harris and Loke, 2017). Our results further support the notion that hamsters exhibit a more intense cellular immune response compared to mice, especially during the late phase of infection (4 weeks or one month). This heightened immune response in susceptible hosts like those hamsters may contribute to the long-term survival of *O. viverrini*. However, it is important to acknowledge the differences in immune responses between species. The immune system of humans is more complex and may respond differently to *O. viverrini* infection compared to animal models. Further studies are required to fully understand the mechanisms underlying the pathogenesis of opisthorchiasis and the immune response to *O. viverrini* infection in humans. Nonetheless, the data obtained from animal models such as hamsters and mice are valuable in elucidating the fundamental mechanisms involved in host-parasite interaction and can provide insights into potential therapeutic targets for further exploration.

Conclusion

Indeed, the inflammatory responses to *O. viverrini* infection are multifaceted and influenced by a range of factors. The interplay between the host's immune system, the parasite's virulence factors, and the specific location of the infection all contribute to the observed inflammatory patterns. The intense inflammatory response observed in the IHB tissue of susceptible hosts suggests a potential link to the development of intraductal cholangiocarcinoma. On the other hand, the lower inflammatory reaction seen in non-susceptible hosts may indicate a reduced risk of developing this particular type of cancer. Gaining a comprehensive understanding of the mechanisms underlying these differences in susceptibility and immune response is crucial for developing effective treatments and prevention strategies for parasitic infections. Further research is needed to elucidate the specific immune pathways and molecular mechanisms involved in the host-parasite interaction during *O. viverrini* infection.

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Conflict of interest

The authors declare that there is no conflict of interest.

Author contribution

Conceptualization, ST; Methodology, TT, WDW, and ST; Validation, WS, ST; Formal Analysis, WS; Investigation, ST; Resources, TT, ST; KS, PS Writing—Original Draft Preparation, WS, ST; Writing—Review and Editing, WS, ST; Supervision and Editing, SS, ST, PT. All authors have read and agreed to the submitted version of the manuscript.

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Data availability

All data are provided in the manuscript.

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