Review Article

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The Past and Present Situation of *Opisthorchis viverrini* Infection in Thailand

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ABSTRACT

Opisthorchis viverrini, a major foodborne trematode, remains a major public health concern in Thailand, particularly in the northern and northeastern provinces. *O. viverrini* can be transferred to people and reservoir hosts through the consumption of uncooked cyprinid fish with metacercariae. Chronic infection with *O. viverrini* can lead to a severe condition known as cholangiocarcinoma (CCA). According to the Nationwide Hospital Admission Database, Thailand has experienced the highest incidence of CCA globally in recent years. Particularly, between 2009 and 2013, there was an incidence rate of 14.6 cases per 100,000 individuals within the population and a corresponding mortality rate (MR) of 14%. In addition, high incidence rates of CCA were reported in Khon Kaen Province from 1989 to 2018, with a rate of 36.1 per 100,000 person-years in men and 14.4 per 100,000 person-years in women. Despite the reduction in the overall prevalence of *O. viverrini* at the national level, several studies have indicated that the infection remains highly prevalent in some communities in the northeastern and northern regions. Some communities have demonstrated a prevalence of *O. viverrini* exceeding 20%, and rates reaching as high as 45.7%. Therefore, in populations with a high prevalence, programs to control this parasite should be implemented, including monitoring eating habits. This review article provides a comprehensive overview of the current distribution of *O. viverrini* in Thailand, outlining its pathophysiology, background, and preventive and control strategies. In addition, this review suggests that individuals in high-risk communities should act to eradicate liver fluke, as this is crucial for the overall health of the community.

KEYWORDS: cholangiocarcinoma, foodborne trematode, *Opisthorchis viverrini*, prevalence

INTRODUCTION

Human liver flukes, including *Opisthorchis viverrini, Clonorchis sinensis,* and *Opisthorchis felineus,* belong to the family Opisthorchiidae, class Trematode, subclass Digenea, and phylum Pathyhelminthis[1]. *O. viverrini* is highly prevalent in Southeast Asia, including Thailand, Lao People's Democratic Republic (Lao PDR), Cambodia, Myanmar, and southern Vietnam[2-3]. *C. sinensis* is mostly found in some regions of northern Vietnam, southern China, and Korea[2], whereas *O. felineus* is widely distributed over eastern Europe and many regions of Russia[4]. Early infections of *O. viverrini, C. sinensis*, and *O. felineus* (clonorchiasis and opisthorchiasis) are usually asymptomatic; however, some patients present symptoms of abdominal pain, nausea, vomiting, diarrhea, and obstructive jaundice[5-7].

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Additionally, early symptoms seem to occur more often in patients with *O. felineus* infections than in patients with *O. viverrini* and *C. sinensis* infections[8]. In chronic infection, the clinical manifestations are associated with obstructive jaundice, cholangitis, cholelithiasis, cholecystitis, periportal fibrosis, and cholangiocarcinoma (CCA)[6-7].

A recent study in 2018 indicated that in critically endemic countries in Southeast Asia, a total of 12.39 million individuals have been reported to have acquired *O. viverrini* infections. Specifically, the total number of documented cases in Thailand was 6.71 million, Lao PDR accounted for 2.45 million, Vietnam reported 2.07 million, and Cambodia documented 1.00 million cases[9]. In Myanmar, the first formal survey of *O. viverrini* infection in humans was performed on 364 fecal samples and reported at 9.3% in three regions of Lower Myanmar[10]. In Thailand, the risk factor for *O. viverrini* infection is the consumption frequency of Koi Pla, or chopped raw fish salad, a traditional dish consumed by people in northeastern and northern Thailand. Other risk factors for opisthorchiasis include age, gender, occupation, endemic area, and raw food consumption attitude. However, a recent research indicated that education was not considered a major risk factor[3]. Although infection with *O. viverrini* affects people of all ages, there is an increased risk among the older population, and men are at a higher risk than women due to their frequent ingestion of Koi Pla with alcohol. To date, there are many prevention campaigns can effectively prevent *O. viverrini* infection and CCA; however, current studies on the approaches for preventing liver fluke are of poor quality. Nevertheless, it is important to understand the overall positive impact of health education in preventing infection. Therefore, in order to plan prevention and control programs, it is crucial to understand the epidemiology and distribution of *O. viverrini*, as they directly affect parasite populations, host dynamics, and human behaviors.

GEOGRAPHIC DISTRIBUTION OF *O. VIVERRINI* IN THAILAND

O. viverrini is a common species of human liver flukes that is highly prevalent in Thailand. In 1955, the initial report documented the significant prevalence of *O. viverrini* infection in Thailand, indicating that certain areas in the northern region of the country had a prevalence approaching 100%[11]. After three decades, a study of *O. viverrini* infection targeting 1,651, 1,585, and 1,447 individuals in 1980, 1981, and 1982 was conducted in several villages in Khon Kaen Province. The total prevalence of *O. viverrini* infection was reported to be 89.5% in 1980, 92.5% in 1981, and 88.6% in 1982, indicating Khon Kaen Province is the main endemic region for this parasite[12]. The first nationwide survey of *O. viverrini* infection was done from 1980-1981 and reported the prevalence at 14% (figure 1),

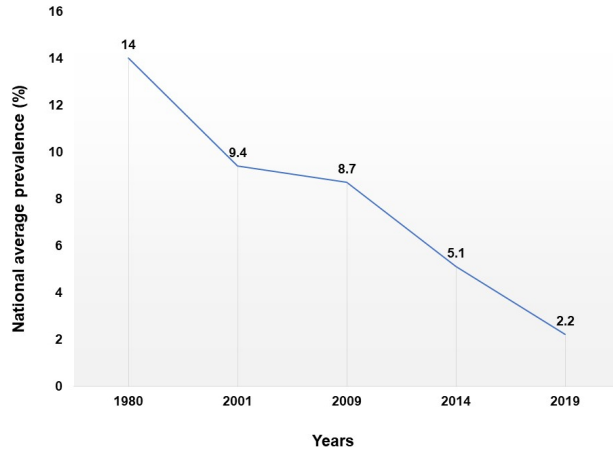

Figure 1 The average national prevalence of *Opisthorchis viverrini* infection in Thailand from 1980 to 2019

including 34.6% in the northeastern, 6.3% in the central, 5.6% in the northern, and 0.01% in the southern regions of the country[13]. In the average national survey of *O. viverrini* infection, the prevalence had decreased to 9.4% in 2001 due to continued and intensive control programs and public health campaigns. Notably, the prevalence of *O. viverrini* infection varied regionally, with rates of 0% observed in the South, 19.3% in the North, 15.7% in the Northeast, and 3.8% in the Central[13]. In 2009, the average national infection rate of *O. viverrini* was reduced to 8.7% from a survey including 15,555 people, of whom 1,351 cases were positive[14]. Furthermore, the average national prevalence of *O. viverrini* infection demonstrated a decline to 5.1% by the year 2014, exhibiting prevalence rates of 9.2% in the Northeast, 5.2% in the North, 0.9% in the Central, and 0% in the South. Following numerous preventative campaigns, the prevalence of *O. viverrini* has markedly reduced to 2.2% on a national level, as reported by the most recent National Helminthiasis Surveillance data in 2019 (figure 1), exhibiting a prevalence of 9.2% in the Northeast, 5.2% in the North, 0.9% in the Central, and 0% in the South[15]. The five national average surveys conducted between 1980 and 2019 provided evidence that Thailand has achieved significant progress in controlling opisthorchiasis over the last four decades. Consequently, the following aims are to eradicate *O. viverrini* and decrease the occurrence of CCA in Thailand.

Moreover, based on fecal screening for *O. viverrini* infection conducted in Khon Kaen Province between 1990 and 2001, the overall prevalence of *O. viverrini* infection was recorded at 24.5%, with variations observed across different districts ranging from 2.1% to 70.8%. Additionally, infection rate was higher among males (27.6%) than among women (21.4%)[16]. In 2010 and 2015, the results of surveillance that included 17 community-based surveys demonstrated a high prevalence of > 20%, and the highest prevalence (45.7%) was observed in a community in the northeastern region[17]. In 2023, the prevalence of *O. viverrini* in Chaiyaphum Province, northeastern Thailand, was 7.15%, which was higher than that in the national surveillance conducted in 2019, which reported a prevalence of only 2.2%[18]. Despite a decline in the overall prevalence at the national level, many studies have indicated that the infection rate of *O. viverrini* remains high in several communities within the northeastern areas[17-18].

Between 2013 and 2019, the prevalence of *O. viverrini* in 20 provinces in the northeastern regions was increased and recently reported to be 32.4%[19]. The above information indicates that the recent prevalence of *O. viverrini* in the northeastern region is still higher than the total national prevalence, especially in rural communities. Moreover, co-infection between *O. viverrini* and minute intestinal flukes (MIFs) is commonly found in Thailand, because they share a second intermediate host. In particular, the rate of co-infection of both parasites is high in northern Thailand. In northern Thailand, most foodborne trematode species are MIFs, and co-infection occurs between *O. viverrini* and MIFs in the genus *Haplorchis* spp., such as *Haplorchis taichui* and *Haplorchis yokogawai*[20-21]. Recently, reports have indicated that the total distribution of co-infection of *O. viverrini* and *H. taichui* was 37.2% and the total distribution of *O. viverrini* in residents of northern Thailand was 47.7% in Chiang Mai Province[21]. Due to immigration and environmental changes, a high prevalence of *O. viverrini* species has recently been found in nonendemic areas such as central Thailand. For example, in Chachoengsao Province, the incidence of *O. viverrini* infection was 21.4/100 person-years, and the prevalence of *O. viverrini* was 9.6%[22]. Moreover, cross-sectional studies were carried out in non-endemic regions of Thailand, demonstrating *Opisthorchis*-like egg infection prevalence ranging from 8.4% to 16.8% in high-risk districts[23-24]. Data on the epidemiology of *O. viverrini* infection indicate that some areas in rural communities still have a high prevalence of this parasite. Therefore, to reduce transmission and infection of this parasite in these endemic areas, control programs must be applied continually.

LIFE CYCLE OF *O. VIVERRINI*

O. viverrini has a complex life cycle and requires two intermediate hosts (i.e., freshwater snails and freshwater fish) and one definitive host (humans). Beginning with humans as the definitive host, adult worms reside inside the bile ducts and release fully developed eggs through feces. The eggs must have fallen into the water and been consumed by their first intermediate host, a freshwater snail of the genus *Bithynia*. After being eaten by a suitable snail, miracidiae undergo several stages of transformation into sporocysts, rediae, and cercariae. The cercariae are produced via an asexual reproduction process that involves germinal cells within the cercariae. About 21 to 30 days after infection, the free-living cercariae emerge from the snail and then penetrate the skin of freshwater fish. After penetrating the fish skin, the cercariae undergo a waiting period of 21 days before they transform into infective metacercariae. In addition, cats and dogs can be reservoir hosts of this liver fluke (figure 2). Approximately 18 species of fish belonging to the *Cyprinidae* family can be the second intermediate host (figure 3). Consumption of raw or undercooked fish containing metacercariae can infect humans, cats, and dogs. The metacercariae excyst in the duodenum of the definitive or reservoir host and then migrate upward through the bile ducts, developing into adult worms in approximately 1 or 2 months. After 3 to 4 weeks, these adult worms begin the process of egg deposition (figure 2).

During a thorough surveillance of *O. viverrini* infection in cyprinid fish, a total of 640 fish from 32 different regions in Nakhon Ratchasima Province in northeastern Thailand were subjected to investigation for *O. viverrini* metacercariae infection between the years 2010 and 2011[25]. The study revealed an infection rate of 12.3% (79 out of 640 fish), with *Cyclocheilichthys armatus*,

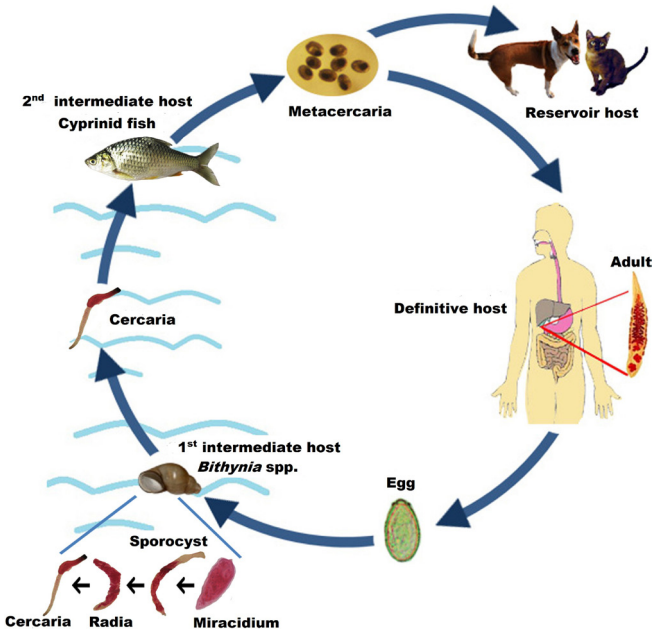

Figure 2 The life cycle of *Opisthorchis viverrini* requires a first intermediate host (*Bithynia* spp.) and a second intermediate host (freshwater fish of the *Crypinidae* family). Humans are the parasite's definitive hosts, whereas cats and dogs act as reservoir hosts.

Figure 3 The second intermediate host of *Opisthorchis viverrini* is freshwater fish in the family *Crypinidae*, also known as cryprinid fish.

Cyclocheilichthys repasson, *Hampala dispar*, *Puntioplites proctzysron*, and *Hampala macrolepitota* being the predominant infected species[25]. Subsequently, from 2011 to 2012, a study was conducted to assess the distribution of *O. viverrini* in cyprinid fish across 20 provinces in northeastern Thailand, encompassing various aquatic environments such as rivers, dams, ponds, and lakes. During this period, six species were identified as harboring *O. viverrini* metacercariae: *C. armatus*, *P. proctozysron*, *P. orphoides*, *H. dispar*, *Henicorhynchus siamensis*, and *Osteochilus hasselti*, with an infection rate of 24.3%[26]. In addition, during the surveillance period from 2019 to 2021, metacercariae of *O. viverrini* were detected in freshwater fish across 20 districts in the upper regions of northeastern Thailand, and infection rates of *O. viverrini* in fish ranged between 3.9% and 21.1% with the average prevalence of 7.1%[27]. Moreover, *O. viverrini* metacercariae were detected in five different fish species, with the following prevalence rates: 13.7% in *H. siamensis*, 12.7% in *Cyclocheilichtys* spp., 8.1% in *Hampala* spp., 6.9% in *Systomus* spp., and 5% in *Barbonymus goniatus*[27]. From 2018 to 2019, a total of 2,149 freshwater fish, consisting of 20 different species, were examined for the prevalence of *O. viverrini* metacercariae across five districts in Nakhon Phanom Province, northeastern Thailand. The species most frequently found infected with *O. viverrini* metacercariae were *H. dispar* and *Anematichthys repasson*, with an average of 0.23 metacercaria per fish. Furthermore, the prevalence displayed district-level variation, with rates ranging from 0.07 to 0.52[28].

The geographical distribution of *Bithynia* spp., the first intermediate host of *O. viverrini*, varies by region, including *Bithynia funiculata* in the north, *Bithynia siamensis goniomphalos* in the northeast, and *Bithynia siamensis siamensis* in central Thailand[29-30]. A previous study demonstrated that the prevalence of *O. viverrini* in *Bithynia* spp. was generally < 1% which was significantly lower than that observed in cyprinid fish[31-34]. For example, a study performed between 2008 and 2011 in 48 locations in northeastern Thailand and Lao PDR indicated that the average prevalence of *O. viverriini* in *B. siamensis goniomphalos* was 0.73% (42/5,790) in northeastern Thailand and 1.08% (63/5,848) in Lao PDR[32]. In addition, seasonal prevalence of *O. viverrini* in *B. siamensis goniomphalos* in northeastern Thailand showed significant variations from 2012 to 2013, with rates of 0.31%, 1.05%, and 0.37% during rainy, cold, and hot seasons, respectively[33]. Another study in Bangkok's canal network from 2018 to 2019 found a total prevalence of *O. viverrini* infection of 0.05% (4/7473) in *B. siamensis*[34].

For a study on the prevalence of *O. viverrini* infection in reservoir hosts, Aunpromma et al. conducted a study in Khon Kaen Province from 2007 to 2009, revealing a high prevalence of *O. viverrini* infection in cats (35.51%, or 76/214), compared to dogs (0.37%, or 3/821). In addition, cats with severe infections showed gall bladder wall thickening and hyperechoic liver parenchyma on ultrasound, indicating cats act as the main reservoir for human opisthorchiasis in this endemic area[35]. Another study in 2016 around Ubolratana Dam found higher infection rates in cats (30.92%, or 77/249) than dogs (0.20%, or 2/1,018), especially in raw fish-eating cats, were more likely to develop risk factors as potential disease transmitters[36]. Therefore, the epidemiology of *O. viverrini* in secondary hosts should be considered for improved control and education programs. Interestingly, the study of *O. viverrini* infection in fish-eating animals in northeastern Thailand, including monkeys, rodents, small domestic mammals, and avian species, revealed the absence of *O. viverrini* infection, suggesting these animals are unlikely to act as reservoir hosts for *O. viverrini* in these endemic areas[37].

CLINICAL MANIFESTATIONS AND PATHOGENESIS OF *O. VIVERRINI* INFECTION

The primary symptoms of opisthorchiasis and arise from inflammation and damage to the bile ducts. Symptoms may not be evident during

early-stage infections. However, long-term infections can cause liver and bile duct inflammation, formation of gallstones, periductal fibrosis, and CCA, subsequently damaging the liver and bile ducts[5,38]. At 2 to 4 weeks after infection, initial pathological alterations involve a rapid inflammatory response in the first- and second-order bile ducts and the connective tissue surrounding the portal. In addition, localized coagulation necrosis is observed in the liver lobules and more cells in the bile ducts[39]. At 12 weeks after infection, adult worms cause chronic pathological changes, including hyperplasia, adenomatous changes, granuloma formation, and thickening fibrous tissue around the bile ducts, resulting in the obstruction and gradual enlargement of the bile ducts[39-40]. In addition, in a hamster model, the biliary epithelium demonstrated inflammation and fibrosis along its entire length, leading to tumorigenesis and development of CCA[39,41]. Periductal fibrosis observed in animals with persistent infection was similar to that observed in humans, as evaluated by ultrasonography[42]. In addition, in patients with chronic opisthorchiasis, a strong association was detected between the severity of *O. viverrini* infection and the increased occurrence of advanced periductal fibrosis, which is also associated with a higher incidence of CCA[6]. Cholangiocarcinogenesis associated with *O. viverrini* infection is a complicated process that is triggered by several factors. Initially, the sucking activity during feeding and the movement of adult worms result in mechanical damage to the ductal epithelial cells. In addition, *O. viverrini* produces proteases, including cathepsin F and cathepsin B1, which contribute to tissue damage via the breakdown of extracellular proteins[38]. In addition, the excretory secretory products of *O. viverrini* stimulate proinflammatory responses, resulting in an increase in Toll-like receptors and interleukins 6 and 8, which activate downstream chemokines and promote CCA development[43]. In addition, *O. viverrini* induces persistent damage to the bile duct tissue by the actions of reactive oxygen species (ROS). produced by phagocytic cells, leading to DNA damage and mutagenesis, which increases the risk of CCA. Moreover, cell proliferation during development of CCA is promoted by the activation of mitogenic and antiapoptotic factors[44]. Similarly, by increasing cell proliferation, stimulation of mitogenic and antiapoptotic proteins enhances the formation of CCA[45]. Finally, the degradation of tissue by cathepsin F and cathepsin B1 can change the surrounding extracellular matrix environment, leading to the collapse of the basement membrane and the invasion and spread of CCA, which is characterized by high invasiveness, rapid development, high spreading potential, and a very poor prognosis[5,7]. To date, the chronic infection of *O. viverrini* can cause CCA; *C. sinensis* has also been strongly reported to be associated with CCA; and both liver flukes are classified as Type 1 carcinogens[6,8,46]. For *O. felineus* infection, there are a few studies related to CCA. For example, one epidemiological study from Russia suggested an increased incidence of CCA in areas endemic to *O. felineus*[47]. Additionally, some animal models revealed that CCA is a sequalae of *O. felineus* infection[48]. However, there is less research on *O. felineus* associated with CCA than on *C. sinensis* and *O. viverinni*; therefore, it is difficult to explain the association between *O. felineus* infection and CCA, despite the fact that such a correlation seems to exist.

INCIDENCE OF CCA IN THAILAND

CCA is a rare cancer that develops and arises from bile duct epithelium cells. However, CCA is more commonly observed in Thailand, where its incidence is relatively high. In addition, high incidences of CCA have been reported in Thailand's northern and northeastern regions, which are geographically associated with widespread *O. viverrini* infection[6,13]. As from many researches, *O. viverrini* has been categorized as a group 1 carcinogenic agent, which is a major risk factor for CCA[46]. Moreover, the top 10 provinces in Thailand with the highest CAA MR

were published in 2005. The highest CCA MR was observed in Sakol Nakhon Province, northeastern Thailand, at 61.4 per 100,000 people, followed by Prae Province in northern Thailand, at 55.8 per 100,000 population. In addition, the remaining provinces with high MRs were found in the northeastern region of Thailand, particularly in Roi-Et Province (MR = 54.8), Nongbua Lamphoo Province (MR = 54.1), Kalasin Province (MR = 50.9), Amnat Charoen Province (MR = 47.8), Mahasarakham Province (MR = 44.9), Udon Thani Province (MR = 44.3), Nakhon Phanom Province (MR = 40.3), and Yasothorn Province (MR = 39.9) (figure 4)[7]. Between 1988 and 2012, the age-standardized rate of liver cancer and bile duct cancer in Thailand ranged from 40.5 to 33.9 per 100,000 in males and 16.3 to 12.9 per 100,000 in females. According to Green et al., the prevalence of CCA in Khon Kaen Province in 1988 was 135.4 per 100,000 for males and 43.0 per 100,000 for females[49]. For individuals older than 35 years, there was a threefold increase in prevalence of 317.6 cases per 100,000 person-years. Furthermore, as reported by Banales et al.[50]. There was a significant decrease from 1989 to 2018 in the incidence of CCA among males and females in Khon Kaen Province. The reduction in CCA incidence can be attributed to the low birth rate in this population[51]. According to the predicted data, the age-standardized rate for men in 2028 is estimated to be 7.6 per 100,000 individuals, whereas it is projected to be 3.6 per 100,000 for females. Since the incidence rates of CCA decreased in Khon Kaen Province between 1989 and 2018 and will continue to decline until 2028, the five-year relative survival analysis for CCA was only 10.9%, indicating the survival of the patients remains low[51]. Therefore, the survival rate of patients with CCA is a major obstacle in achieving positive patient treatment outcomes.

DIAGNOSIS OF *O. VIVERRINI*

MICROSCOPIC EXAMINATION

Conventional techniques for diagnosing *O. viverrini* infection consist of the direct, simple smear technique; the Kato–Katz technique; and the formalin–ethyl acetate concentration technique (FECT) for detecting parasite eggs in feces[52-53]. In addition, the Mini Parasep® stool kit was recently developed to identify parasite eggs in feces, including liver fluke eggs. However, the use of only microscopic examination for detecting *O. viverrini* will result in false positives with MIF eggs, because these eggs are very similar and difficult to distinguish under a light microscope.

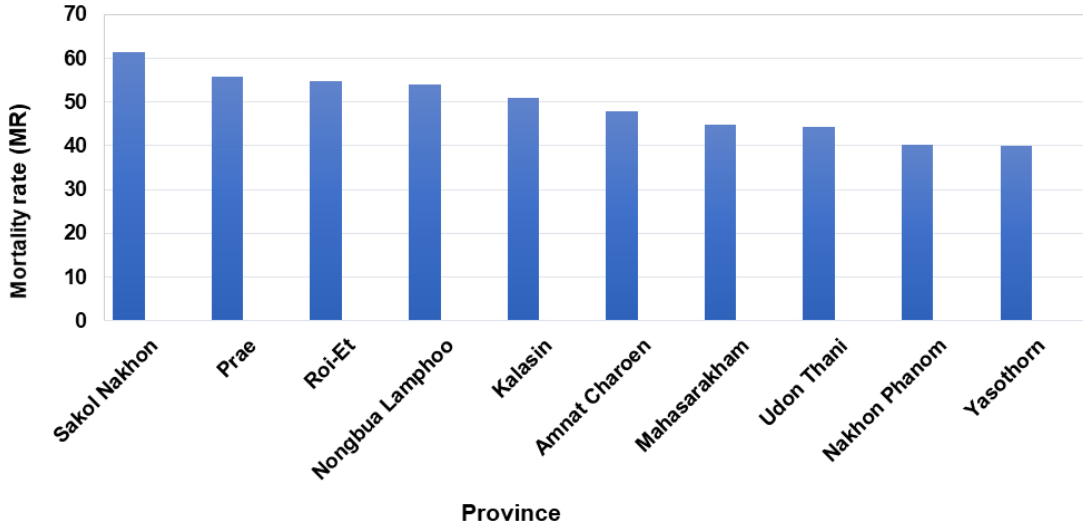

Figure 4 The top province with mortality rates of cholangiocarcinoma in Thailand. The mortality rate is per 100,00 people[7].

Furthermore, it has been shown that when there are fewer than 20 adult worms of *O. viverrini*, no eggs could be found in the feces. As compared with the sensitivity of diagnostic techniques, the direct, simple smear; Kato–Katz; FECT; and stool kits (Mini Parasep Kit) had sensitivity values of 12.4%, 68.3%, 91.0%, and 32.4%, respectively[52]. Moreover, one study demonstrated that FECT, Kato–Katz, and stool kits (Mini Parasep Kit) had diagnostic sensitivity values of 75.5%, 66.0%, and 67.3%, respectively, for detecting *O. viverrini* eggs[53]. Therefore, FECT provides higher sensitivity for detecting *O. viverrini* in feces than other diagnostic methods[53].

SEROLOGICAL DIAGNOSIS

Antibody detection assays are preferred for the detection of *O. viverrini*. However, there were limitations due to the cross-reactivity of antigens, and positive results may not always indicate active parasite infection. For example, crude antigens (17-18 kDa) of *O. viverrini* were employed in ELISA, exhibiting sensitivities of 91% -92% across diverse subject groups, including opisthorchiasis patients, those with mixed infections of *O. viverrini*, individuals with other parasitic infections, and healthy individuals[54]. However, specificity ranged from 70% to 80%, with noted cross-reactions in individuals infected with parasites such as *Paragonimus heterotremus*, *Schistosoma* spp., *Ascaris lumbricoides*, and others[54]. In 2001, Wongsaroj et al. successfully extracted the oval antigen of *O. viverrini* using monoclonal antibodies, achieving 100% sensitivity, specificity, and accuracy in detecting *O. viverrini* infection via dot-ELISA[55]. This method is ideal for mass screening, especially in newly endemic regions. In 2007, an ELISA study using crude *O. viverrini* antigen found sensitivities ranging from 23.1% to 99.2% and specificities from 29.6% to 93.0%. This study revealed a correlation between the *O. viverrini* egg count in feces and specific IgG and IgG_4 levels in serum and urine[56]. A lateral flow immunochromatographic (ICT) kit, using soluble *O. viverrini* worm extract and colloidal-gold-labeled IgG antibody conjugates, was developed and tested on 347 simulated whole-blood samples[57]. The ICT kit exhibited diagnostic outcomes of a sensitivity of 95.5%, a specificity of 87.0%, a positive predictive value of 80.5%, a negative predictive value of 97.2%, and an overall accuracy of 90.1% in diagnosing opisthorchiasis.

Additionally, the assay demonstrated the ability to detect clonorchiasis, yielding a sensitivity of 85.7%, a specificity of 87.0%, a positive predictive value of 53.6%, a negative predictive value of 97.2%, and an overall accuracy of 86.8%[57]. Moreover, a rapid ICT was recently developed to detect anti-*O. viverrini* IgG and IgG4 subclass antibodies in the serum of CCA patients. Among the 36 CCA patients tested, 61.1% were positive for IgG antibodies and 41.6% for IgG_4 antibodies. In comparison, IgG ICT and IgG_4 ICT assays were positive in 25.5% and 27.7%, respectively, of patients with liver cirrhosis and other malignancies. The patients diagnosed with CCA exhibited a 6.53-fold higher positivity rate for anti-*O. viverrini* IgG antibody and a 3.27-fold increase in positivity for anti-*O. viverrini* IgG_4 antibody compared to the non-CCA group[58]. Therefore, this ICT shows potential for developing a diagnostic biomarker for predicting CCA risk linked to *O. viverrini* infection. A recently developed lateral flow ICT kit demonstrated high sensitivity and specificity for diagnosing opisthorchiasis and detecting co-infections with clonorchiasis, offering potential for field surveys in endemic areas[58]. Recently, a urine *O. viverrini* antigen-based rapid diagnostic test (OV-RDT) was developed in 2023 using an ICT technique. This OV-RDT revealed a high sensitivity of 94.2% and a high specificity of 93.2% in comparison to the commonly used FECT for detecting 423 urine specimens. Furthermore, the urinary OV-RDT had a low 2% of cross-reactions with other helminth infections, indicating that it might be beneficial for large-scale screening campaigns targeting opisthorchiasis in fields due to the simplicity of collecting urine samples[59].

MOLECULAR DIAGNOSIS

As compared with microscopic examination, the molecular-based polymerase chain reaction (PCR) technique has a higher specificity and sensitivity for detecting *O. viverrini* eggs in fecal samples. In 2001, Wongratanacheewin et al. developed a PCR assay to detect as little as 2×10^{-17} ng of *O. viverrini* genomic DNA in fecal samples. When the numbers of eggs per gram were > 1,000, > 200, and < 200, the sensitivity of the assay was 100%, 68.2%, and 50%, respectively[60]. In addition, multiplex PCR was used to identify *C. sinensis* and *O. viverrini* infections by targeting the mitochondrial NADH dehydrogenase subunit 2 (*nad2*) gene; this technique was able to detect one egg of *O. viverrini* and two eggs of *C. sinensis* in a fecal sample[61]. Because the egg morphologies of *O. viverrini* and MIFs are very similar, a PCR assay was developed to discriminate between these parasites using internal transcribed spacer (ITS)1 and ITS2 as targets with 76.2% sensitivity for ITS1 and 95.2% for ITS2[62]. Moreover, conventional PCR and real-time quantitative PCR were also developed to detect and discriminate between *O. viverrini* and *H. taichui* by amplification of mitochondrial cytochrome c oxidase subunit I (*cox1*) genes, demonstrating 100% sensitivity for the detection of *Opisthorchis*-like eggs[63]. Moreover, *O. viverrini* is considered to be a species complex consisting of at least two siblings, one in Thailand and the other in Lao PDR, using multilocus enzyme electrophoresis. Furthermore, to study the genetic structure and genetic variation of *O. viverrini*, mitochondrial DNA sequencing of subunit 1 of the NADH dehydrogenase gene (*nad1*) and the cytochrome oxidase gene (*cox1*) were used, which indicated the monophyletic group of this parasite[23,64-65]. In addition, random amplified polymorphic DNA was used to study *O. viverrini* from northeastern Thailand and Lao PDR, and the results suggested that this parasite is composed of different genotypes[66]. In a recent study, microsatellite DNA analysis was used to assess the genetic diversity and population structure of *O. viverrini* across various geographical regions. The findings revealed genetic variation in the population of *O. viverrini* at a localized level, with corresponding patterns observed at a broader scale[67]. Since 2007, the use of multilocus enzymes to study systematics and genetic variation in *O. viverrini* populations has revealed a species complex, termed "*O. viverrini* sensu lato," which includes cryptic species in Thailand and Lao PDR wetlands[68-69]. Subsequently, genetic variation in mitochondrial genes, specifically *cox1* and *nad1* nucleotide sequences, was observed in *O. viverrini* sensu lato across Thailand and Lao PDR, with variations ranging from 0% to 0.3% for *nad1* and 0% to 0.5% for *cox1* in *O. viverrini*[64]. Furthermore, a novel cryptic *O. viverrini* sensu lato population was identified in Pangkon district, Sakon Nakhon Province, exhibiting distinctive genetics compared to other isolates based on *nad1*, *cox1*, cathepsin F gene (CF-int6), paramyosin gene (Pm-int9), ITS2, and 28S rDNA sequence analysis[70]. Additionally, the random amplified polymorphic DNA analysis also showed genetic differences between *O. viverrini* populations in northeastern Thailand and Lao PDR[66]. At present, microsatellite DNA marker analysis conducted on four neighboring villages in Khon Kaen Province revealed genetic variation and distinct alleles, along with sub-structuring within the *O. viverrini* population across these areas in Thailand[67]. Additionally, Namsanor et al. (2020) identified a cryptic species of *O. viverrini* sensu lato in Sakon Nakhon Province, northeastern Thailand, using microsatellite DNA, nuclear DNA, and mitochondrial DNA markers[71]. Moreover, genetic studies revealed co-evolution between *O. viverrini* sensu lato and *Bithynia* spp., the first intermediate host, with at least three distinct genetic groups. Population genetics of *O. viverrini* indicated complex species across Thailand and Lao PDR[68,72-73]. As the distribution of *O. viverrini* infection continues to spread in new regions of Thailand, the evolutionary association between *O. viverrini*

and *Bithynia* spp. remains unclear. Future studies should focus on evaluating genetic diversity and population dynamics in endemic areas, using microsatellite DNA markers. Additionally, it is essential to conduct thorough investigations into the genetic characterization of *O. viverrini* infections in cyprinid fish, which serve as the secondary intermediate host, in order to enhance our understanding of their life cycle and transmission dynamics.

PREVENTION AND CONTROL OF *O. VIVERRINI* INFECTION

Since 1987, national control strategies for *O. viverrini* have been initiated to eradicate *O. viverrini* infection[74]. First, the strategies were used to control liver fluke, including identifying infected individuals through stool examination and following treatment with praziquantel to eliminate *O. viverrini* in humans and reservoir hosts[75]. Second, as an important prevention step against infection, promoting health education programs emphasizing the consumption of cooked fish is crucial. This should be followed by attempts to enhance sanitary defecation habits by promoting hygiene behaviors for feces disposal, such as the use of latrines at the household level and rice paddies to effectively reduce environmental contamination with *O. viverrini* eggs. In addition, human consumption of raw or unprocessed freshwater fish is the main factor contributing to the persistence of liver flukes in rural areas. Therefore, health education is required to change the eating habits of people in endemic areas, which could also reduce the future prevalence and incidence of CCA. However, prevention and control programs have not been successful in some endemic areas. In Thailand, the major strategies for prevention and control are fecal examination to detect parasite infection and praziquantel treatment to eliminate adult worms in human hosts. However, some patients become reinfected with *O. viverrini* in some areas at a rate of approximately 10.9%[76]. Therefore, to reduce the transmission and distribution of *O. viverrini* infection in Thailand, an effective prevention and control program is still required.

CONCLUSION

O. viverrini remains an important trematode in Thailand, with a high prevalence in northeastern and northern Thailand. Although the total national prevalence is decreasing, the prevalence remains high in some areas. Moreover, *O. viverrini*–associated CCA results from by multiple factors, including parasite factors, host immunological responses to parasites, and exogenous or endogenous nitrosamine. All of these factors are derived from chronic infection with *O. viverrini*. In addition, CCA is a malignancy with a poor prognosis and a high MR; therefore, it is necessary to eradicate this parasite to reduce the incidence of CCA in Thailand. As an example, implement ongoing campaigns to discourage raw fish consumption and provide health education on *O. viverrini* infections at community and school levels to alter eating habits, particularly in high-prevalence endemic regions of northern and northeastern Thailand. Additionally, an effective control strategy involves reducing infection prevalence by screening individuals via fecal examination, treating infected humans, and treating reservoir animals (such as cats and dogs) to minimize transmission and act as primary prevention for CCA. Additionally, suspected CCA patients should undergo ultrasonography screening for periductal fibrosis to determine the need for CCA monitoring, then a diagnosis and suitable treatment.

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