Research Article

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Diagnostic accuracy of Opisthorchis viverrini antigen methods for human opisthorchiasis: Systematic review and Meta-analysis

Kritpisut Maitongngam [1], Mathuros Tipayamongkholgul [2], Varakorn Kosaisavee[3]

[1] Master of Science (Public Health Infectious Diseases and Epidemiology), Faculty of Graduate Studies, Mahidol University, THAILAND

[2] Department of Epidemiology, Faculty of Public Health, Mahidol University, THAILAND

[3] Department of Parasitology and Entomology, Faculty of Public Health, Mahidol University, THAILAND

Correspondence: Varakorn Kosaisavee, Department of Parasitology and Entomology, Faculty of Public Health, Mahidol University, 420/1 Rajavithi RD., Rachathewi, Bangkok 10400. THAILAND, E-mail: varakorn.kos@mahidol.ac.th

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Abstract

Opisthorchiasis relates to cholangiocarcinoma occurrences. Regular screening for *Opisthorchis viverrini* can prevent loss of human and economy; however, optimal screening techniques have not been identified. There are various methods to detect *O. viverrini* infections, and each method has its strengths and limitations. A common detection method, conventional fecal examinations are cheap but has high false-negative and cannot differentiate between *O. viverrini* and Minute intestinal fluke (MIF) eggs. Antigen detection can result in cross-reactivity with other helminths; nevertheless, it can detect *O. viverrini* at early stage of infection and uses non-invasive human samples. This systematic review and meta-analysis evaluated the diagnostic accuracy of antigen detection for *O. viverrini*. This research searched various databases: MEDLINE, EMBASE, Google scholar, PubMed, Scopus, Science direct, Cochrane, AMED, IPA, CINAHL, and Thai Thesis Database. Study selection and data extraction were done by two researchers independently. Of 142 published articles, 4 articles met the inclusion criteria. Quality assessment was done by QUADAS-2 and found a low risk of bias. Pooled sensitivity is 91 % (95% CI = 82% - 96%) and pooled specificity is 68% (95% CI = 65% - 72%). The high sensitivity of antigen detection for *O. viverrini* suggested its potential to be an optimal tool for early detection and treatment. However, the moderate specificity reflects lower effectiveness to apply the antigen test for surveillance in low endemic areas. The study's findings provide evidence of the precision

of *O. viverrini* antigen detection. Public health decision-makers can employ antigen detection of *O. viverrini* as cost-effective screening tools in an epidemic area.

Keywords: Antigen detection, *Opisthorchis viverrini*, Opisthorchiasis, Systematic review, Meta-analysis

Introduction

Opisthorchiasis is a parasitic infection caused by ingesting raw or undercooked fish contaminated by the *Opisthorchis viverrini* (*O. viverrini*). It is endemic in several Southeast Asian countries, including Thailand, Lao PDR, Vietnam, and Cambodia[1]. It is estimated that over 10 million people from these countries are infected with *O.viverrini* [2]. Although some infected individuals are asymptomatic[3], prolonged infections can lead to severe symptoms and potentially life-threatening illnesses, particularly hepatobiliary diseases[4]. The World Health Organization's International Agency on Research in Cancer has recognized *O. viverrini* as a biological carcinogen for cholangiocarcinoma (CCA). CCA treatment is expensive, costing approximately 1.96 billion baht per year in Thailand[5]. Opisthorchiasis is one of the significant public health concerns which leads to socio-economic issues.

Epidemiological surveys in Northeast Thailand in 2017 showed a prevalence of over 10% for human opisthorchiasis[6,7]. According to the national health development plan of Thailand, the prevalence of opisthorchiasis should be lower than 1% by 2025[8]. Nevertheless, this target remains to be achieved due to several factors. The most critical factor is the tradition of consuming raw fish food. Moreover, there is still lack of proper sanitary system and sewage treatment system in the areas[8]. Furthermore, *O. viverrini* detection methods are the important assistance to take patients in the healthcare system.

Early detection of *O. viverrini* can decrease the incidence of opisthorchis-induced cholangiocarcinoma. To date, there have been various *O. viverrini* detection methods in humans, for example, the conventional diagnostic method, molecular diagnosis, serological antibody test, and antigen detection[9]. Nevertheless, each detection technique has its limitations. Parasitological methods include the formalin ethyl acetate concentration technique (FECT, a simple smear, and Kato's thick smear. Light *O. viverrini* infection can lead to underdiagnosis of as much as 20%[10]. When patients encounter chronic infections, it can lead to biliary obstruction that hampers the passing of eggs into the stool[9,11]. There is difficulty in differentiating *O. viverrini*,

and other minute intestinal flukes (MIF) which are similar to *O. viverrini* [9,1,13]. As a result, it can yield lower specificity than expected. Molecular diagnosis has high specificity but varied sensitivity depends on the number of eggs found in feces. Moreover, a high amount of PCR inhibitors in feces might cause incorrect interpretation. This method is expensive and requires great expertise[9]. Immunological detection including antibody and antigen could be the optional detection method. However, the antibody detection method has varied sensitivity and specificity depending on the specimen used. Moreover, antibody detection cannot differentiate between active infection and past infection. Additionally, the detection of specific antibodies against *O. viverrini* antigens in serum or plasma has cross-reactivity with other helminth species[14].

While FECT is the gold standard for detecting *O. viverrini*, it can detect only at the egg stage, which takes four weeks to present in stool[3]. Antigen detection can detect the infections earlier, and initiate treatment in a timely manner, which prevents the patients from developing more severe conditions. Antigen detection yields high sensitivity and specificity; moreover, this technique yields optimal examination performance for non-invasive samples (i.e., urine and feces). However, antigen detection of *O. viverrini* has different degrees of cross-reactivity with other helminths from 3.57% with *S. stercoralis* to 10% in hookworm[15]. An antigen detection method facilitates screening and surveillance in epidemic areas.

Evidence-based information on the accuracy of antigen detection of *O. viverrini* in humans is still lacking. Furthermore, control policies in Thailand such as mass drug administration and health education on avoiding raw fish consumption have led to a shift from heavy to light *O. viverrini* infection[15]. The parasitological methods that are mainly used in Thailand have several limitations, including false positive results due to the misinterpretation of minute intestinal fluke eggs, or false negative results in cases of light infections and biliary duct blockage where fecal egg detection is not possible[16]. FECT is effective in detecting *O. viverrini* in medium to heavy infections[15]. Moreover, due to the limited sample size used, the accuracy of *O. viverrini* antigen detection varies and remains uncertain. This study therefore aimed to evaluate the diagnostic accuracy (sensitivity and specificity) of *O. viverrini* antigen detection in humans. The results from this study may serve as a reference for policy development and disease surveillance for earlier detection and medical intervention.

Materials and Methods

The systematic review and meta-analysis were conducted in accordance with the guideline of the Preferred Reporting Items for Systemic review and Meta-Analysis for Diagnostic Test Accuracy (PRISMA-DTA). The systematic review protocol was registered in International Platform of Registered Systematic Review and Meta-analysis Protocols (INPLASY), (the registration number: INPLASY202250123).

Eligible studies

Research articles on the antigen detection methods of *Opisthorchis viverrini* in humans that were published in databases between the years 2000 and 2021 were searched. We excluded the research articles that Formalin Ethyl acetate Concentration Technique (FECT) was not presented as the reference standard. All antigen detection techniques were accepted as the index test. The antigen detection of *O. viverrini* in animals' studies were also excluded. There were no considerations for study design, time, or language limitations. Consideration was given to studies that used reference standards and index tests on all individuals.

Searching strategy and Databases

This study was conducted in systematic review and meta-analysis by searching various databases. The searches have been updated from October 2021 – June 2022. The international published literatures were searched via databases: MEDLINE, EMBASE, Google scholar, PubMed, Scopus, ScienceDirect, Cochrane, AMED, IPA, CINAHL. Additionally, Thai-Journal Citation Index Centre, Online Computer Library Center, Inc. (www.worldcat.org) for theses and dissertations that including Thai Thesis Database were included as the databases for searching. Using existing knowledge and a review of the literature, the PICO statement was sought as follows: P: ("Opisthorchiasis" or "*Opisthorchis viverrini*"), I: ("Antigen detection" or "ELISA" or "monoclonal antibody" or "enzyme-linked immunosorbent assay" or "excretory-secretory antigen" or "crude antigen" or "somatic antigen" or "tegument antigen" or "egg antigen" or "sperm antigen"), C: ("FECT" or "Formalin ethyl acetate") and O: ("Sensitivity" or "Specificity"). During the screening, duplicates were manually deleted.

Study selection

After keyword searching in the databases mentioned above, the researchers manually removed studies in each database according to the inclusion and exclusion criteria. Thereafter, screening from the abstract was done, and full texts assessed for eligibility were selected for the study. The disagreements from any issues of the systematic review were discussed with the third researcher as arbitrator.

Data extraction

When eligible studies were selected, the researchers extracted data from each study and filled in a self-created excel form. Data included study name, source of the database, published year, location of the studies, sample sizes, index tests, methods of testing used, reference standard, and duration of the studies. Moreover, the data on the diagnostic accuracy such as sensitivity, specificity, specimen used, true positive (tp), false positive (fp), false negative (fn), true negative (tn) were extracted by comparing the index tests and the reference showed in the selected studies. The data were ready for further analysis. True positive (tp) was considered as a diagnosis of opisthorchiasis confirmed by the reference standard method: FECT, false positive (fp) was considered a diagnosis of non-opisthorchiasis confirmed by the reference standard method: FECT, false negative (fn) was considered a diagnosis of non-opisthorchiasis by antigen method but confirmed to be opisthorchiasis by the reference standard method: FECT, true negative (tn) was considered a diagnosis of non-opisthorchiasis by the reference standard method: FECT.

Quality assessment

Two researchers independently evaluated the risk of bias by using a modified Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool which comprises four domains: 1) Patient selection 2) Index test 3) Reference standard 4) Flow and timing. We consulted previously documented guiding criteria to provide a quality rating (low, uncertain, high) to a specific area. The domain was evaluated uncertain/high risk of bias if research received one or more signaling questions with unclear or high results.

Data analysis

STATA BE 17 with the serial number: 301709029164 was the program used in this study. Sensitivity and specificity were the measurements of diagnostic accuracy of the antigen detection method of *Opisthorchis viverrini* in this study. The analyzed results are illustrated as the pooled estimate, forest plot graph showing sensitivity, specificity as average, 95% confidence interval-CI, and percentage of inconsistency index (I^2) measuring heterogeneity of each included study.

Analysis of I^2 is presented as a percentage; the higher the I^2 value, the more heterogeneity it is represented. The interpretation of I^2 is that I^2 equals or less than 25% meaning low heterogeneity, a value of 50%, meaning moderate heterogeneity, and a value of more than 75% (17), meaning high heterogeneity. Publication bias is analyzed by the result of Deek's test in the funnel plot[18]. When Deek's test result is more than 0.1, the publication bias does not exist.

Results

Study selection

According to electronic databases consisting of MEDLINE, EMBASE, Google scholar, PubMed, Scopus, Science direct, Cochrane, AMED, IPA, CINAHL, Thai Thesis Database, 142 related research articles were found. Of these, 108 duplicated articles were removed. As a result, 34 articles remained for abstract screening; thereafter all 34 articles were assessed. After assessment, 30 studies were excluded due to several reasons: 13 studies published before year 2000, 2 studies have no full text available, 10 studies are antibodies detection, 4 studies have no gold standard method as reference standard, 2 studies conducted on animals, and some studies were excluded for more than 1 reason (Figure 1). Once there was a conflict between 2 researchers during studies selection, there was a senior researcher to resolve the discrepancy. Two studies were cross-sectional studies, and the other two studies were experimental studies. The maximum sample size in the study was 1,043 samples, whereas the minimum sample size was 90 samples. All the studies were based in Thailand. Urine and fecal samples were used for the antigen detection method of *O. viverrini* infections.

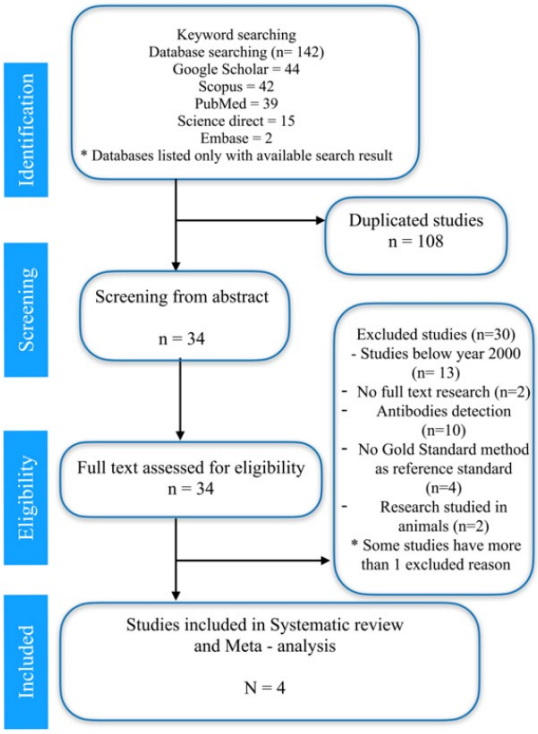

Figure 1 Flow chart of the process in article selections

Study characteristics

The characteristics of the selected studies were shown in Table 1. Considering participants in each study, all samples were selected from Khon Kaen province, Thailand (High endemic area for liver fluke); however, one study expanded the research to 2 more provinces: Sakon Nakhon and Chaiyaphum which are endemic areas as well. Average age of included participants was between 47.7 and 63.7 years. Nevertheless, there is one study (19) used samples stored in sample bank so there was no explicit clarification on age and gender of participant characteristics.

Table 1 Study characteristics

Study	Study design	Sample (n)	Location	Study Period	Specimen	Index test	Antigen
(Teimoori *et al.*, 2017)	Laboratory analysis	90	Thailand	2012 - 2014	Feces	Sandwich ELISA	Somatic antigen
(Watwiengkam *et al.*, 2013)	Laboratory analysis	141	Thailand	NA	Feces	Mab-ELISA	OV excretory-secretory (ES)
(Worasith *et al.*, 2015)	Cross sectional	470	Thailand	2013 - 2014	Urine	Indirect sandwich ELISA	OV excretory-secretory (ES) Crude OV
(Worasith *et al.*, 2019)	Cross sectional	1043	Thailand	2015 - 2016	Urine and feces	Mab-ELISA	OV excretory-secretory (ES)

OV: Opisthorchis viverrni

Quality assessment

QUADAS-2 was used as a tool for quality assessment. To interpret QUADAS-2, when an answer for all signaling questions is "yes" the risk of bias is considered "low". However, when all answers are "no" there is possibility for having risk of bias [20]. For this study, after the quality assessment was done, the result demonstrated a low risk of bias in the majority of studies. However, the patient selection in one study was not clear, as it did not elaborate explicitly on the methods of patient recruitment for the study. Another unclear point was the reference standard in the same study that not mention how FECT was used. Additionally, it did not mention any specific in diagnostic clearly (Figure2).

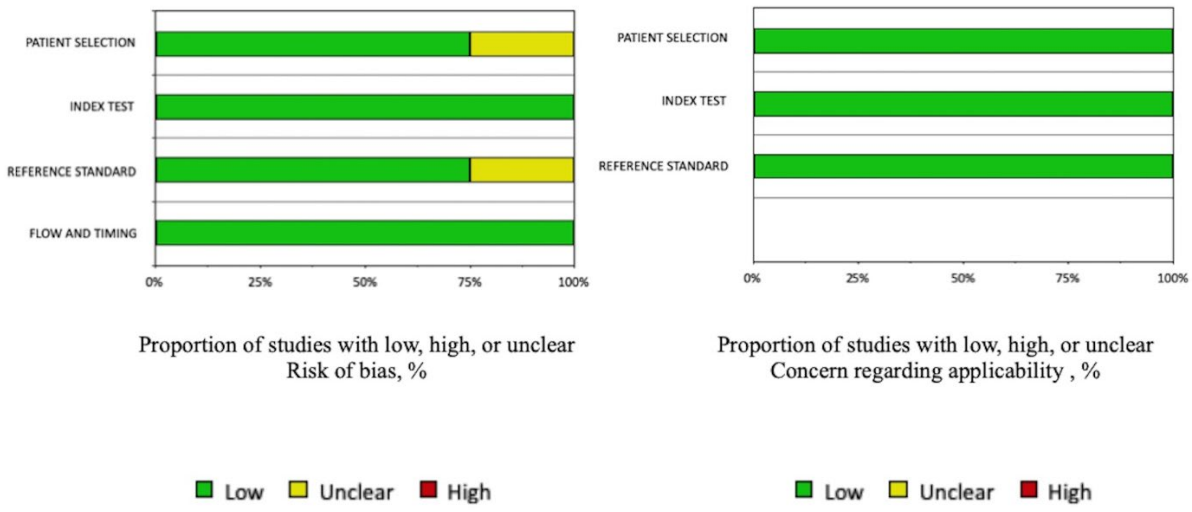

Figure 2 Risk of bias and applicability-concerns: A summary of authors' judgement about each domain presented as percentages across the included studies.

Result of Meta-analysis

The funnel plot was implemented to see whether publication bias is presented. The Deek's test revealed symmetrical data distribution with P = 0.56 (>0.1) (Figure3). Therefore, publication bias was not presented in this study. Random effects model was used and found pooled sensitivity of 91% (95% CI = 82% - 96%) and pooled specificity of 68% (95% CI = 65% - 72%) (Figure 4). The sensitivity and specificity in each selected study were summarized in table 2. The I^2 of sensitivity and specificity were 74.63% and 19.82%, respectively. If I^2 is less than 25%, it is considered low heterogeneity. When I^2 is less than 75%, it is moderate heterogeneity. This means that heterogeneity of sensitivity and specificity are low and moderate, respectively based on the interpretation provided in the material and method section.

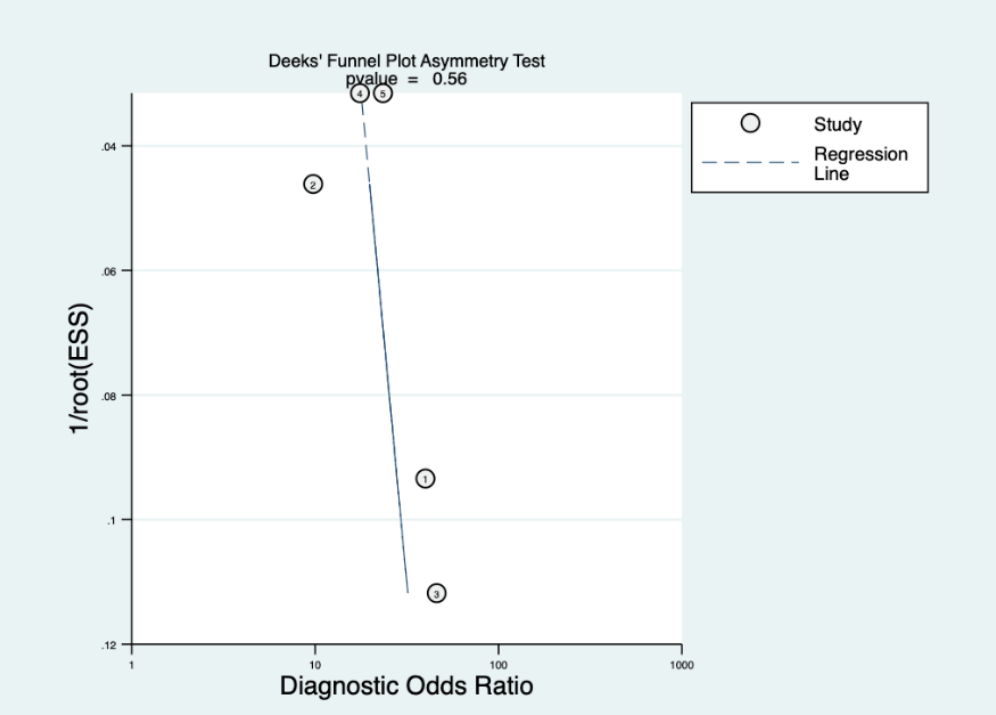

Figure 3 Funnel Plot

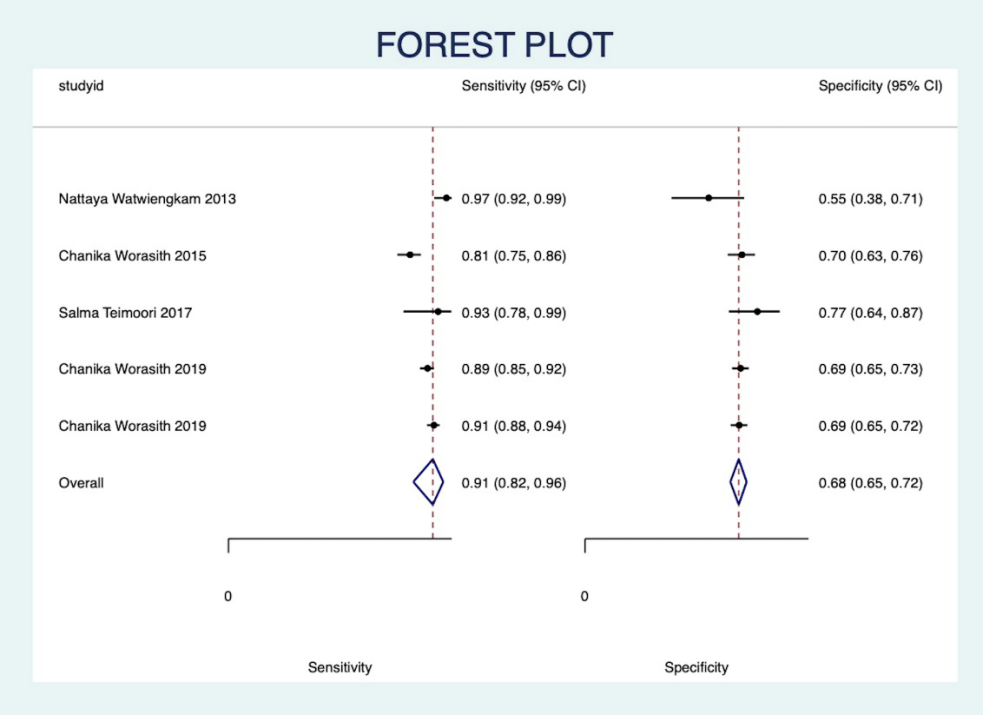

Figure 4 Forest plot

Discussion

The systematic review and meta-analysis revealed pooled sensitivity of 91 % (95% CI = 82% - 96%) and pooled specificity of 68% (95% CI = 65% - 72%). Sensitivity in all four articles was in the high percentage from 81 % to 97.9%. Specificity in three studies was between 70% - 76.7%. However, one study (21) reported moderate specificity at 54.2% even though sensitivity was high at 97.9%. The lower specificity compared to other studies was probably due to the cross reactivity with other helminths[25], leading to higher false positive.

There are many detection methods for *O. viverrini*, but each has limitations. The accuracy of the parasitological methods varies according to sensitivity and specificity. The sensitivity of the simple smear, Kato's thick smear, and FECT for detecting *O. viverrini* are 12.5% - 31.7%[22], 62.3% - 72%[23,24] and 49.3% - 91%[23,24], respectively. The simple smear involves the application of a small number of fecal materials onto microscopic slides and examination under a light microscope; therefore, when infection is light, helminth eggs might not present in feces, leading to low sensitivity. False negatives may present in the case of fecal collection when few or no *O. viverrini* eggs are found. The high false negative of FECT can lead to misdiagnosis, causing such severe consequences as cholangiocarcinoma. FECT can reliably detect *O. viverrini* when infection is moderate or heavy[15].

The antigen detection method is more expensive than FECT technique, but it can help detect patients with even light infections. The pattern of *O. viverrini* infection in Thailand has changed from heavy to light infection due to the implementation of control policies such as mass drug administration and health education on avoiding raw fish consumption[15,16]. Moreover, antigen detection method allows early detection of *O. viverrini* at the young adult stage, but FECT can only detect it in the egg stage, which is 4 weeks behind. With antigen detection, patients with the infection can receive treatment in time and prevent the development of more severe conditions. In one included study[16] utilized FECT to test samples and found 40 negative results. The same samples were then tested with Mab-ELISA, which identified 15 positive samples using an antigen detection method. These positive results were confirmed by the third method, DNA detection to prevent false positive. This could mean that about 37.5% negative result of FECT yields false negatives. A similar result was observed in another included study[15] which used 63 negative samples detected by FECT to retest with Mab-ELISA, antigen detection

yielded 28 positive samples, meaning 44.4% false negative by FECT. The reason behind these occurrences might be the limited ability of FECT at detecting light infections.

Biomolecular technique has high specificity but there is a limitation in sensitivity depending on the number of eggs found in feces. For example, when there are more than 1,000 helminth eggs per 1 gram of feces, it has 100% sensitivity, while it has 68.2% sensitivity when there are between 200 - 1,000 eggs per 1 gram of feces and 50% sensitivity when there are fewer than 200 eggs per gram[9,15]. In addition, a large amount of PCR inhibitors are present in feces, which might cause incorrect interpretation[15]. Besides, it incurs high costs and requires high expertise. The antigen detection method, by comparison, is relatively inexpensive. Antibody detection methods are diagnostic tests that can use three types of specimens: blood, saliva and urine. Sensitivity and specificity vary depending on the specimen used. Antibody detection showed high sensitivity in blood specimens but low sensitivity in urine samples; blood and saliva showed low specificity[14]. Moreover, the interpretations of the results of antibody detection methods are limited. If patients are detected as positive, it cannot be concluded that they still have symptoms at that point or previously had symptoms; they still present with an Ab level in the blood. Antigen detection, unlike antibody detection, can differentiate active infection from past infection.

This study shows that antigen detection has high accuracy. The usage of trichloroacetic acid (TCA) can enhance detection performance and increase sensitivity of antigen detection methods[15,21]. There are several antigens used for diagnostic; for example, excretory-secretory antigen, sperm antigen, tegument antigen, adult worm antigen[25], the included research used excretory-secretory antigen and copro-antigen as target antigen. Another advantage of antigen detection is that it produces low cross reactivity with other helminths. Antigen detection is a noninvasive test because of the ease of collecting urine or feces for examination, and it is more convenient to perform because there is a strip test that can be used in different endemic areas. Antigen detection can be used for surveillance and disease prevention in epidemic areas.

From the final included four articles, two studies were conducted in the cross-sectional design, and the other two were laboratory analyses. In cross-sectional studies, all suspected cases were tested using both index test and reference standard test. Cross-sectional designs tend to reflect justifiable estimate diagnostic accuracy[26]. None of the included research papers used case-control designs. The case-control design may possibly lead to exaggeration of

diagnostic accuracy and is not ideal for evaluating a test's accuracy[26]. The over diagnostic accuracy from including case-control design to the study is because enrolled patients are previously known as people with disease: case group and people without disease: control group. Moreover, in general, mild cases that were difficult to examine were excluded from case-control study resulting in overestimated of sensitivity and specificity[27].

All four papers included samples from endemic areas of Khon Kaen Province. Moreover, one study[16] extended to two more endemic areas: Sakon Nakhon province and Chaiyaphum province. Taking samples from endemic area to perform antigen detection test is consistent with the result found from this study that antigen detection should be done in endemic area to obtain a more accurate result avoid high false positive cases.

The limitation found in the study was that published literatures are only in English language. There are no Thai language research on antigen detection methods. Moreover, all articles conducted in Thailand and the low numbers of publication studies. However, articles searching was done in reliable databases such as MEDLINE, EMBASE, Google scholar, PubMed, Scopus, Science direct.

Diagnostic tests with high sensitivity but low specificity yield high false positives. Using this particular test in non-endemic areas leads to low positive predictive values and high false positive. As a result, financial resources, human resources, and time are used ineffectively. However, high sensitivity and low specificity tests can be effectively used in endemic areas. The included studies[15,21,16] had prevalence as detected by FECT as 53%, 66.2% and 41% respectively, when positive predictive value (PPV) by antigen detection were 75%, 80.7% and 66% respectively. The data illustrated that the higher prevalence, the more positive predictive value. When prevalence is 66.2%, it yields PPV as high as 80.7%. To apply these data, antigen detection of *O. viverrini* should be used in villages in Northeastern of Thailand which have high prevalence as much as 85.2%[5] since it will yield high positive predictive value; as a result, using this test will be efficient. However, it will be less effective to use the test in northern of Thailand, where the prevalence is 45.6%[5], since that would lead to high false positive cases.

Conclusion

Based on the systematic review and meta-analysis of this study, it was found that *Opisthorchis viverrini* antigen detection in human has average sensitivity of 91% (95% CI = 82%

- 96%) and average specificity of 68% (95% CI = 65% - 72%). The data is representative of diagnostic accuracy which can be used to apply in screening of early detection of *Opisthorchis viverrini* in endemic area promptly and also facilitate public health authorities to set appropriate policy effectively. Moreover, future study can use the idea from this study to develop or modify early detection methods of *Opisthorchis viverrini*, especially increasing the screening of opisthorchiasis in non-endemic areas.

Ethical Approval Statement

Not required.

Author Contributions

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Conflicts of Interest

All authors have no conflicts of interest to disclose.

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