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CagA-positive *Helicobacter pylori* may be associated with current infection of clonorchiasis

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This study aims to explore the relationship between *Helicobacter pylori (H. pylori)* infection, particularly CagA-positive strains, and Clonorchiasis. A total of 309 civil servants who underwent health check-ups and 73 hospitalized patients with Clonorchiasis from May 2019 to April 2023 were included. A control group of 100 healthy individuals matched by age, gender, and residence was included. *H. pylori* and Clonorchiasis antibodies in civil servants were detected using ELISA. Western blotting was used to identify *H. pylori* strains in hospitalized patients. Among civil servants, *H. pylori* and Clonorchiasis antibody positivity rates were 43.7% and 23.9%, respectively. *H. pylori* antibody positivity was comparable between those with (45.9%) and without Clonorchiasis (43.0%) infection. *H. pylori* infection among Clonorchiasis patients (61.6%) was slightly higher than in healthy controls (44.0%). Multifactorial analysis identified consuming raw fish (61.145; 22.263–167.93; 0.000) and CagA + *H. pylori* infection (3.7; 1.233–11.103; 0.020) as independent risk factors for Clonorchiasis. The rate of CagA + *H. pylori* infection is significantly higher in patients with Clonorchiasis than in healthy individuals. CagA + *H. pylori* infection and a history of consuming raw fish are independent risk factors for current Clonorchiasis.

Keywords CagA-positive *Helicobacter pylori*, Clonorchiasis, Current infection, Immunity

Clonorchiasis is a zoonotic disease characterized primarily by damage to the hepatobiliary system, caused by adult *Clonorchis sinensis* (*C. sinensis*) parasitizing the bile ducts. Taking *C. sinensis* as an example, this strain has been endemic in China for over 2000 years, with more than 15 million people in countries such as China, Japan, North Korea, and Vietnam currently at risk of infection[1,2]. The most severe complication of Clonorchiasis infection is cholangiocarcinoma, which the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has classified as a Group I carcinogen for cholangiocarcinoma. Due to its complex life cycle and wide range of definitive hosts, it poses a significant public health burden[3]. Differences in dietary habits among humans determine the heterogeneity of Clonorchiasis infection within populations, while climatic conditions, sanitation, economic status, and educational level are closely related to the prevalence and reinfection rates. Moreover, patients with high infection intensity before treatment are particularly prone to reinfection[4–7].

Different species of Clonorchiasis do not overlap in geographic distribution and are located in different climatic zones, but their life cycles and pathogenicity are similar[8]. After Clonorchiasis infects the human body, it induces a complex gene-protein-metabolism regulatory network in the host's liver, causing a series of immune pathological damages to the biliary tract through mechanical injury and the direct or indirect effects of excretory-secretory products (ESPs)[9]. ESPs mediate intercellular communication between biliary epithelial cells and hepatic stellate cells, inducing epithelial-mesenchymal transition (EMT) and biliary fibrosis by affecting cytokine secretion and protein expression levels, which promotes malignant transformation of cells in the biliary

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microenvironment and late-stage hepatobiliary abnormalities[10]. The role of host immune cells varies during different stages of infection, with CD4 + T cell subsets playing a dominant role[11]. Liver damage characterized by collagen deposition and inflammatory cell infiltration can occur within 24 h after Clonorchiasis infection[12], with early-stage responses primarily driven by macrophage-induced innate immunity and Th1-mediated cellular immunity. As the duration of infection increases, the levels of inflammatory cytokines (especially IL-6 and IL-1β) decrease, while anti-inflammatory cytokines, particularly IL-4, increase, indicating that a Th2-type immune response is triggered during the adult stage of Clonorchiasis to control excessive inflammation and promote tissue repair[13]. Additionally, the imbalance between regulatory T cells (Treg) and Th17 cells can also facilitate the fibrotic process[14]. IL-10 plays a crucial role throughout the infection process by inhibiting Th1 cell secretion of IFN-γ and IL-2, inducing Th2 cells to secrete IL-4 and IL-5, and protecting the host liver from damage by suppressing Th17 cell responses[15]. Therefore, the immune pathological damage of Clonorchiasis is the result of the combined actions of multiple cytokines.

Helicobacter pylori (H. pylori), classified as a Group I carcinogen, is a Gram-negative spiral bacterium first discovered and cultured in gastric biopsy samples by Robin Warren and Barry Marshall[16]. It utilizes adhesins (such as blood group antigen-binding adhesin, BabA; sialic acid-binding adhesin, SabA), enzymes (such as urease, Ure), toxins (such as vacuolating cytotoxin gene, VacA), and effector proteins (such as cytotoxin-associated gene A, CagA) to participate in colonization, immune evasion, and disease induction[17]. After entering the stomach, *H. pylori* releases virulence factors CagA and VacA, interacting with the host's gastric microenvironment and altering host cell signaling pathways, which induce, control, and regulate inflammatory responses in the gastric mucosa, maintaining chronic inflammation via epigenetic pathways in gastric epithelial cells[18]. These virulence factors primarily promote disease development by inducing changes in host inflammatory cytokines such as interleukin (ILs), tumor necrosis factor-alpha (TNF-α), and gamma interferon (IFN-γ)[19]. Research has confirmed a synergistic pathogenic effect between *H. pylori* and pathogens such as EBV, HBV, HCV, Brucella, and Orientia tsutsugamushi, indicating that co-infection may influence disease progression[20–24]. Additionally, *H. pylori* plays a protective role in asthma and inflammatory bowel disease (IBD) by balancing the homeostasis of pro-inflammatory and anti-inflammatory microbes, with this immunomodulatory effect being more pronounced in CagA-positive *H. pylori* strains[25,26].

The relationship between parasitic infections and *H. pylori* has been a longstanding topic of discussion. Some studies have found that parasitic infections can improve the clinical outcomes of *H. pylori*-related diseases. It is reported that the potential impact of co-infection with *H. pylori* and helminths on the immune response to Mycobacterium tuberculosis, inducing a Th2-type immune response to *H. pylori*[27]. In sub-Saharan Africa, a "dichotomy" is observed, with a 2/3 *H. pylori* infection rate and an extremely low incidence of gastric adenocarcinoma (2–3%). Researchers agree that this may be related to the high burden of soil-transmitted helminth (STH) infections in the area, where gastrointestinal helminths reduce the risk of *H. pylori*-induced gastric adenocarcinoma by inducing immune tolerance[28]. However, other studies also found inconsistent results, suggesting that co-infection of intestinal parasites and *H. pylori* may not always be protective. In certain contexts, H. pylori infection has been proven to relate to the increased risk of hepatobiliary abnormalities and cholangiocarcinoma, and co-infection with helminths may exacerbate tissue injury through chronic inflammation [29,30]. These inconsistent findings underscore the complex mechanisms underlying the impact of *H. pylori* co-infection on clinical outcomes. Both Clonorchiasis and *H. pylori* mediate immune responses through the balance of CD4 + T cell subsets, leading us to hypothesize that there may be some cross-influences between these biological behaviors. Therefore, we designed this cross-sectional study to explore the synergistic effects of *H. pylori* infection on current Clonorchiasis infection.

Methods

Study design and participants

We conducted a cross-sectional study ranging from May 2019 to April 2023 in two hospitals, including the Health Examination Center of Affiliated Hospital of Youjiang Medical University for Nationalities and the Infectious Disease Department of Beihai People's Hospital. A total of 482 participants were enrolled in this study, including (1) 309 civil servants undergoing routine health check-ups, (2) 73 hospitalized patients with current C. sinensis infection, and this group was further divided into *H. pylori*-positive (n = 45) and *H. pylori*-negative (n = 28), and (3) 100 healthy individuals.

The inclusion criteria were as follows: (1) individuals aged 18–65 years; (2) confirmed *H. pylori* or *C. sinensis* infection status by serological or parasitological methods; (3) written informed consent provided. The exclusion criteria were as follows: (1) history of liver or biliary tract diseases unrelated to *C. sinensis*; (2) immunodeficiency or use of immunosuppressive drugs; (3) incomplete clinical or laboratory data; (4) Patients who cannot cooperate.

General information

A total of 309 healthy individuals were recruited from the Health Examination Center of Affiliated Hospital of Youjiang Medical University for Nationalities, including 275 males and 34 females, with an average age of 44.34 ± 8.01 years. Seventy-three hospitalized patients with current Clonorchiasis were recruited from the Infectious Disease Department of Beihai People's Hospital, including 57 males and 16 females, with an average age of 42.15 ± 9.44 years. The control group comprised 100 healthy individuals matched for gender and age from Beihai People's Hospital during the same period, including 73 males and 27 females, with an average age of 43.29 ± 8.97 years. Detailed grouping of the study population and the technical approach is shown in Fig. 1. No significant difference was found in gender ($P = 0.978$) and age ($P = 0.553$).

Diagnosis of Clonorchiasis was based on the standards of the former Ministry of Health of the People's Republic of China WS309—2009, using a modified Kato thick-smear technique to detect *C. sinensis* eggs in the feces and duodenal fluid of the subjects as the basis for diagnosis. Enzyme-linked immunosorbent assay (ELISA)

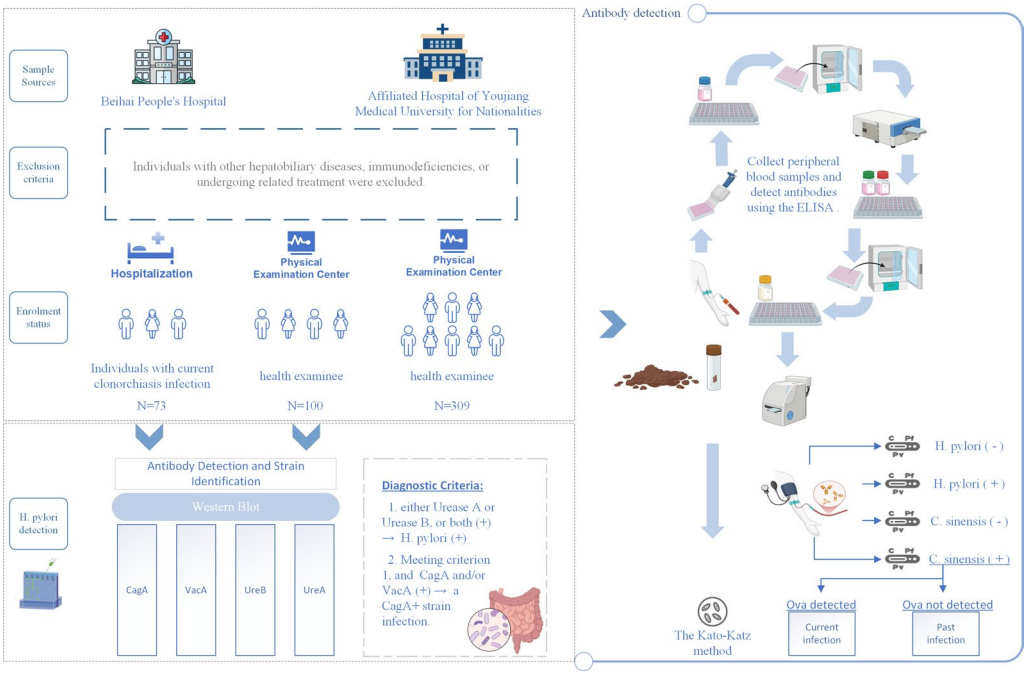

Fig. 1. Detailed grouping of the study population and the technical approach.

Characteristics	Current Clonorchiasis infection (n = 73)		χ^2/*t-value*	*P-value*
	***H. pylori* positive(n = 45)**	***H. pylori* negative(n = 28)**		
Male, n (%)	35 (77.8)	22 (78.6)	0.001	0.978
Age, (mean ± standard deviation) yr	41.27 ± 8.59	42.62 ± 10.60	1.994	0.553
Residing in rural areas, n (%)	15 (33.3)	7 (25.0)	0.311	0.577
Accompanied by clinical symptoms, n (%)	35 (77.8)	6 (21.4)	1.589	0.207
History of consuming raw fish, n (%)	40 (88.9)	24 (85.7)	2.244	0.134

Table 1. Comparison of general characteristics between *H. pylori* positive and *H. pylori* negative patients with current *C. sinensis* infection.

was used to detect *C. sinensis* antibodies in the patients' blood, supplementing the pathogenic diagnosis[31], along with epidemiological history and clinical manifestations for confirmation.

Based on the presence of clinical symptoms, the collected cases of Clonorchiasis infection were divided into two categories: (1) current infection, defined by the presence of *C. sinensis* eggs in feces and/or typical clinical symptoms, with or without positive *C. sinensis* antibodies; (2) past infection, defined by positive *C. sinensis* antibodies alone, with no egg detection in feces and no clinical manifestations. one being current infection, characterized by positive C. sinensis antibodies, detection of eggs in feces, and/or characteristic clinical symptoms; the other being past infection, indicated solely by positive C. sinensis antibodies, negative fecal egg detection, and/or absence of clinical manifestations. Exclusion criteria included individuals with other liver and biliary diseases, immunodeficiency, or those undergoing related treatments. All case data collection was approved by the ethics review committees of the two hospitals. All patients were informed of the risks and benefits of participating in the study before it began, and they voluntarily participated in the research and signed informed consent forms. Based on *H. pylori* detection results, the 73 patients with current Clonorchiasis infection were divided into *H. pylori* positive (45 cases) and negative (28 cases), with a comparison of general information between the two groups detailed in Table 1.

Methods

Peripheral blood samples were collected from 309 healthy individuals, and ELISA was used to simultaneously detect overall infection rates of *H. pylori* antibodies and *C. sinensis* antibodies in the general population. Based on the positivity of *C. sinensis* antibodies, the *H. pylori* infection status of the subjects was analyzed. In addition, the 100 healthy individuals recruited from Beihai People's Hospital were matched controls used specifically for comparative analysis with 73 hospitalized clonorchiasis patients. Peripheral blood samples were collected from 73 hospitalized patients with current *C. sinensis* infection and 100 healthy individuals, and *H. pylori* antibodies were detected using the Western blot method (products from Shenzhen Blot Biological Company). The reagents

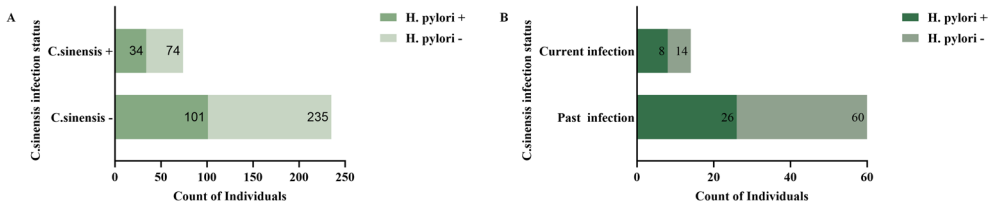

Fig. 2. Survey of *H. pylori* and *C. sinensis* infection status among civil servants using ELISA.

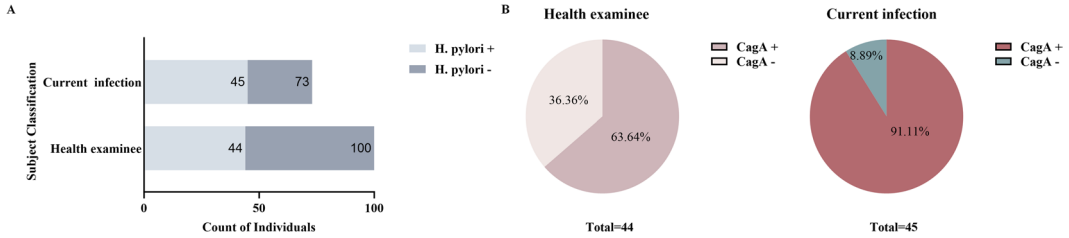

Fig. 3. Infection status of different *H. pylori* strains in patients with current C.sinensis infection using the Western blot method.

were purchased from Shenzhen Blot Biological Company (batch number: 161216), and the experimental procedures were strictly followed according to the instructions. The Western blot method can detect antibodies against *H. pylori* cytotoxin CagA (bands at 128 kD and 116 kD), vacuolating cytotoxin-associated protein VacA (bands at 95 kD and 91 kD), and urease ureB (66 kD) and urease ureA (30 kD). Diagnosis criteria: (1) A positive result for either urease A or urease B, or both, indicates *H. pylori* strain infection; (2) If condition (1) is met, and CagA and/or VacA are positive, it indicates infection with CagA + strains. Declaration: All experimental methods in this study were strictly carried out in accordance with relevant guidelines and regulations, to ensure the scientific nature and ethical compliance of the research.

Statistical methods

A database was established using SPSS 27.0 statistical software. Continuous variables were expressed as mean ± standard deviation ($x \pm s$), and the categorical variables were presented as frequency and percentage (n, %). The comparison of *H. pylori* infection status between the two groups was conducted using the chi-square test. Univariate analysis of risk factors for current *C. sinensis* infection was performed using the chi-square test, and variables with statistically significant factors were included in the logistic regression model for multivariate analysis, with a significance level of $\alpha = 0.05$, using two-sided tests.

Results

Survey of *H. pylori* and *C. sinensis* infection status among civil servants

A total of 482 participants were included in this study, the baseline characteristics of *C. sinensis* infection patients stratified by *H. pylori* infection are listed in Table 1. By the ELISA method, antibodies against *H. pylori* and *C. sinensis* were tested in 309 civil servants. The results showed that the *H. pylori* antibody positivity rate was 43.7% (135/309) (95% CI: 38.13–49.25%), and the *C. sinensis* antibody positivity rate was 23.9% (74/309) (95% CI: 19.16–28.73%). Further analysis of *H. pylori* infection status between *C. sinensis*-infected and non-infected individuals revealed that the *H. pylori* antibody positivity rate among *C. sinensis*-infected individuals was 45.9% (34/74), which was similar to the 43.0% (101/235) in non-infected individuals, with no statistical difference ($P = 0.654$). A further analysis of *H. pylori* infection status in individuals with current infection, past infection, the results showed that the *H. pylori* antibody positivity rate was 57.1% in the current *C. sinensis* infection group, and 43.0% in the healthy control group. Although a slightly higher positive percentage of current infection than the healthy control group, the difference was not statistically significant ($P = 0.299$). In contrast, patients with past *C. sinensis* infection showed no significant increase in *H. pylori* antibody positivity compared with non-infected individuals. See Fig. 2 for details.

Infection status of different *H. pylori* strains in patients with current c.sinensis infection

The Western blot method was used to detect *H. pylori* antibodies in 73 patients with current *C. sinensis* infection and 100 healthy controls, followed by strain identification. The results showed that the *H. pylori* infection rate in patients with current *C. sinensis* infection was 61.6%, slightly higher than the rate of 44.0% in healthy individuals, but this difference was not statistically significant ($P = 0.249$). However, the infection rate of CagA + strains in patients with current *C. sinensis* infection was 56.2%, significantly higher than the 28.0% in healthy individuals ($\chi^2 = 13.960$, $P < 0.001$, indicating a significant difference. The infection rate of CagA strain in healthy infected individuals was 16.0%, significantly higher than that in current C. sinensis infected individuals ($P = 0.033$). See Fig. 3 for details.

Variable	β coefficient	Standard	Wald x^2	P-value	OR (95% CI)
Gender (Male/Female)	0.17	0.58	0.09	0.767	1.19 (0.38, 3.69)
Residency (Rural/Urban)	-0.63	0.55	1.31	0.252	0.53 (0.18, 1.57)
Raw Fish Consumption History (Yes/No)	4.11	0.52	63.67	<0.001	61.15 (22.26, 167.93)
H. pylori（CagA+）	1.31	0.56	5.45	0.020	3.70 (1.23, 11.10)

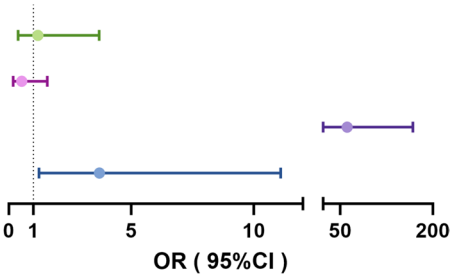

Fig. 4. Multivariate regression analysis of the relationship between CagA + *H. pylori* infection and current *C. sinensis* infection.

Multivariate regression analysis of the promoting effect of CagA + *H. pylori* infection on current *C. sinensis* infection

Factors such as patient gender, residence, history of consuming raw fish, and *H. pylori* CagA status ($CagA^+$/$CagA^-$) were incorporated into the multivariate regression model, and the results indicated that a history of consuming raw fish (61.145; 22.263–167.93; 0.000) and CagA + *H. pylori* infection (3.7; 1.233–11.103; 0.020) were independent risk factors for current *C. sinensis* infection. See Fig. 4.

Discussion

Currently, over 35 million people globally are affected by *C. sinensis* infection, but only about 1.5–2 million exhibit symptoms or complications. Among individuals with current infection, some patients present acute symptoms such as fever, jaundice, and liver function abnormalities, while others show more subtle symptoms, only testing positive for eggs in fecal examinations[32]. The clinical phenotypic differences of *C. sinensis* disease are the result of the combined effects of the host's immune status, genetic background, characteristics of the infecting strain, infection load, and the host's age, gender, nutritional status, and environmental factors. *H. pylori* infection primarily occurs in childhood, with its prevalence increasing with age, while Clonorchiasis predominantly affects adults. Poor dietary habits and unfavorable socioeconomic conditions are common risk factors for both *H. pylori* and Clonorchiasis infection. Additionally, both Clonorchiasis and *H. pylori* mediate immune responses through the balance of CD4 + T cell subsets, leading us to hypothesize potential cross-interactions between these two microorganisms. The classification of infections, particularly past infections, involves a combination of approaches. For instance, serological testing is commonly used, where IgM antibodies indicate recent infection and IgG antibodies reflect past exposure due to their prolonged presence. Moreover, advances in molecular diagnostics, including metagenomic sequencing, facilitate direct detection of pathogen nucleic acids, enhancing the identification of current or prior infections, especially in immunocompromised individuals. The integration of immunological evidence, clinical coding, symptom timing, and molecular methods thus provides a comprehensive framework for accurately categorizing infections in both clinical and research settings.

The clinical phenotypic differences of C. sinensis disease are the result of the combined effects of the host's immune status, genetic background, characteristics of the infecting strain, infection load, and the host's age, gender, nutritional status, and environmental factors. *H. pylori* infection primarily occurs in childhood, with its prevalence increasing with age, while Clonorchiasis predominantly affects adults. Poor dietary habits and unfavorable socioeconomic conditions are common risk factors for both *H. pylori* and Clonorchiasis infection. Additionally, both Clonorchiasis and *H. pylori* mediate immune responses through the balance of CD4 + T cell subsets, leading us to hypothesize potential cross-interactions between these two microorganisms.

In this study, we first employed the ELISA method to detect both *H. pylori* antibodies and *C. sinensis* antibodies in 309 civil servants undergoing health check-ups. The results indicated that the infection rate of *C. sinensis* among healthy individuals in this region was as high as 23.9%, likely related to local dietary habits that favor raw freshwater fish consumption. Subsequent multivariable regression analysis further confirmed that a history of consuming raw fish is one of the independent risk factors for *C. sinensis* infection. Our study also found that the *H. pylori* infection rates among individuals with and without *C. sinensis* infection was 45.9% and 43.0%, respectively, with no statistically significant difference. However, the *H. pylori* infection rate among individuals with current *C. sinensis* infection reached 61.5%. Although this did not show statistical significance, it suggests that *H. pylori* may facilitate the invasion and pathogenicity of *C. sinensis* in the human body through certain mechanisms, such as raw fish consumption, poor hygiene. The antibody results might reflect prior exposure or involve serological cross-reactivity rather than active synergy. Additionally, selecting hospitalized patients may introduce bias toward more severe cases. Finally, the cross-sectional design limits causal inference. Future longitudinal and mechanistic studies are needed to clarify these relationships.

H. pylori infection often occurs in childhood and, if not eradicated, can persist for a lifetime. We can't help but wonder whether the colonization of *H. pylori* during childhood prepares the ground for the invasion of Clonorchiasis. *H. pylori* downregulates inflammation and controls the host's immune response through various virulence factors[33]. Its induced chronic active inflammation reduces the gastrointestinal mucosal barrier function, creating a suitable environment for the colonization and development of Clonorchiasis. Theoretically,

if an individual has a prior *H. pylori* infection, especially a CagA-positive infection, this may lead to a certain degree of immunosuppression or immune tolerance. When such an individual subsequently gets infected with Clonorchiasis, the immune microenvironment changes caused by the previous *H. pylori* infection may facilitate the persistence of Clonorchiasis and disease progression. Furthermore, *H. pylori* infection may also indirectly affect the host's susceptibility and response to Clonorchiasis by influencing the composition of the gastrointestinal microbiota. The gut microbiota is closely related to the development and function of the host immune system[34], and the dysbiosis caused by *H. pylori* infection may further impact the host's defense against other pathogens. Therefore, we speculate that *H. pylori* infection could be a factor influencing the clinical heterogeneity of symptoms after Clonorchiasis infection.

To clarify the interaction between the two, we selected 73 hospitalized patients with current *C. sinensis* infection from another hospital and used the Western blot method to detect *H. pylori* antibodies. Strain identification was performed based on the presence of CagA and/or VacA regions. The results showed that the *H. pylori* infection rate among patients with current *C. sinensis* infection was 61.6%, slightly higher than the rate of 44.0% in healthy individuals, although this difference was not statistically significant. However, the infection rate of CagA + strains in patients with current *C. sinensis* infection was 56.2%, significantly higher than the 28.0% in healthy individuals, indicating a significant association between CagA + *H. pylori* strains and current *C. sinensis* infection.

In the hamster model established by Dangtakot et al., the colonization rate of *H. pylori* in the hepatobiliary system in the co-infection group with *H. pylori* and *C. sinensis* was higher than that in the single infection group, and it increased the severity of hepatobiliary abnormalities, manifested as more severe periductal fibrosis, cholangitis, and biliary hyperplasia[35]. This may result from one or more of the following reasons: 1. The local inflammation and tissue damage in the hepatobiliary system caused by Clonorchiasis provide a more favorable environment for *H. pylori* colonization[36]; 2. The urease produced by *H. pylori* neutralizes gastric acid, creating a more suitable weakly acidic environment for its survival[37]. After Clonorchiasis infection, physiological changes such as bile stasis or alterations in bile duct pH facilitate the survival and proliferation of *H. pylori*. Additionally, bile acids can enhance the translocation of *H. pylori*'s CagA protein into host cells, amplifying the effects of CagA to promote inflammation and damage[38]; 3. Co-infection by both pathogens interferes with the host's immune response[39], weakening the ability to clear *H. pylori*; 4. Researchers speculate that *H. pylori* may exhibit a "ride-along" phenomenon in the lifecycle of Clonorchiasis, where L-fucose mediates *H. pylori* colonization in the Clonorchis intestine[40], followed by the transmission of *H. pylori* carried by Clonorchiasis larvae in the hepatobiliary system[41,42]; 5. *H. pylori* may first cause damage in the stomach and then infect the biliary system via ascending duodenal infection or through the portal venous circulation [43]; 6. Some virulence factors of *H. pylori* strains (CagA and VacA) may enhance their colonization ability in the hepatobiliary system[44]. Of course, the interactions between the two pathogens are not unidirectional. The inflammatory response induced by *H. pylori* infection may lead to changes in the biliary and intestinal mucosa, providing a suitable environment for the colonization and development of Clonorchis, while the Treg cells induced by the infection suppress the inflammatory response[45], thereby reducing the host's defense against Clonorchis infection.

We conducted a multifactorial regression analysis on the factors that may influence current *C. sinensis* infection. The results suggest that CagA-positive *H. pylori* infection is one of the independent risk factors for current *C. sinensis* disease. Virulence factors like CagA enable *H. pylori* to activate the production of inflammatory cytokines and chemokines, leading to leukocyte recruitment. Suyapoh. W et al. observed that in patients infected with both CagA-positive *H. pylori* and *C. sinensis*, there was a significant increase in leukocyte infiltration and lymphocyte aggregation, indicating a synergistic effect of these two carcinogens in inducing severe inflammatory responses, goblet cell metaplasia, and abnormal proliferation[39]. Notably, the polymorphism of the EPIYA sequence in the CagA protein is closely related to the severity of disease in patients with Clonorchiasis infection, with the EPIYA-AB'C type (containing the variant EPIYT B) leading to more severe periductal fibrosis, while CagA types containing multimerization (CagA multimerization, CM) sequences were significantly associated with the degree of fibrosis[42]. Other studies have shown that CagA also induces persistent advanced periductal fibrosis (APF) after praziquantel treatment, even leading to recurrence, affecting patient prognosis[46]. These findings emphasize the importance of monitoring the CagA protein in the management and treatment of biliary diseases in cases of co-infection with Clonorchiasis and *H. pylori*.

This study has some limitations. As a cross-sectional design, it cannot establish causality or the sequence of infections. The lack of mechanistic data, such as cytokine profiles and functional assays, limits our understanding of the underlying biological interactions. In addition, potential confounders such as socioeconomic status, comorbidities, and hygiene practices were not fully accounted for and may have influenced the results. Antibody-based testing may also reflect prior rather than active infection. These factors should be addressed in future longitudinal and mechanistic studies.

In summary, the infection rate of CagA-positive *H. pylori* in patients with current *C. sinensis* infection is significantly higher than that in healthy individuals, and CagA-positive *H. pylori* strains are independent risk factors for current *C. sinensis* infection, suggesting that *H. pylori* carrying the CagA gene may have a synergistic effect on the occurrence and development of Clonorchiasis.

Most current studies primarily focus on the relationship between *H. pylori* and Opisthorchis viverrini co-infections, likely due to the distribution characteristics of Clonorchiasis in their respective locations. Our study provides supplementary insights into the synergistic pathogenic effects of *C. sinensis* and *H. pylori*. Although we have observed a potential association between CagA-positive *H. pylori* and current *C. sinensis* infection, possibly promoting more severe pathological damage and even the development of cholangiocarcinoma, there are still some research limitations. There is a lack of in-depth understanding of the molecular mechanisms by which CagA-positive *H. pylori* and *C. sinensis* co-infections synergistically affect host cells. More research is needed to clarify how these pathogens jointly influence signaling pathways and gene expression in biliary epithelial

cells. The host immune response to *H. pylori* and *C. sinensis* infections is very complex and exhibits individual variability. The diversity of the host immune background may not have been adequately considered in studies, potentially affecting a comprehensive understanding of pathogen virulence. Furthermore, long-term follow-up studies on co-infected patients are crucial for understanding their relationship with the progression of *C. sinensis* and assessing the long-term effects of potential therapeutic interventions.

Data availability

Some of the data in this study are cited from publicly available literature, and the specific sources have been indicated in the text. Other datasets generated and analyzed during the research process are not publicly available due to containing patient privacy information. They can be obtained from the corresponding author upon reasonable request.

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Author contributions
C.L., Z.L. and J.C. jointly conceived the research plan. F.D. collected the basic data from the clinical frontline. Y.L. assisted in integrating resources and participated in data organization. Z.L. provided historical medical record data. Q.D. carried out preliminary data analysis. J.P. assisted in interpreting the results. Y.N. provided reference opinions based on clinical experience. F.H. wrote the first draft of the paper. J.H. reviewed and revised the professional content related to infection. S.T. and J.L., as corresponding authors, controlled the quality of the paper, guided the revision and communicated externally.

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Declarations

Competing interests
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Ethical approval
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Declaration of generative AI and AI-assisted technologies in the writing process
No generative AI and AI-assisted technologies were used in the writing process.

Additional information
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