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Screening US Vietnam Veterans for Liver Fluke Exposure 5 Decades After the End of the War

George Psevdos, MD,*† Florence M. Ford, RN, BSN,‡ and Sung-Tae Hong, MD, PhD§

Background: Clonorchiasis caused by *Clonorchis sinensis* and opisthorchiasis caused by *Opisthorchis viverrini* are prevalent in the Far East, including Vietnam. The causal relationship between the 2 liver flukes and cholangiocarcinoma (CCA) has been well documented. Cholangiocarcinoma, a rare tumor of bile ducts, usually occurs in the sixth-seventh decade of life. In the United States, 8000 people are diagnosed with CCA each year. US Vietnam Veterans, 5 decades after the end of the war, are being diagnosed with CCA. Although CCA is linked to several conditions, no study has been done to our knowledge linking *C. sinensis* or *O. viverrini* as the culprits for CCA diagnosis in Vietnam Veterans.

Methods: A pilot prospective epidemiological study was performed at the Northport Veterans Affairs Medical Center. We screened Vietnam Veterans for exposure to eating raw or undercooked fish from the rivers in Vietnam during their service. Serological testing was performed by the multiantigen enzyme-linked immunosorbent assay in Seoul National University of Medicine, South Korea.

Results: Of the 97 Veterans screened, 50 met the inclusion criteria with 1 Veteran being diagnosed with CCA. Of the 50 Veterans, 24% tested positive for serum IgG antibodies to *C. sinensis*. Those who tested positive had no detectable liver fluke parasites by fecal examination.

Conclusions: Our study is the first to show evidence of exposure to liver flukes in US soldiers during their service in the Vietnam War. Further research is needed to examine the possible link of liver fluke infection and risk for developing CCA in Vietnam Veterans.

Key Words: Vietnam Veterans, *Opisthorchis viverrini*, *Clonorchis sinensis*, cholangiocarcinoma

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Clonorchiasis and opisthorchiasis are diseases caused by liver flukes, *Clonorchis sinensis* and *Opisthorchis* species, respectively. *Opisthorchis viverrini* and *C. sinensis* are infamous helminths causing a major public health problem in Southeast (Thailand, Laos, and South Vietnam) and East Asia (North Vietnam, China, and Korea).¹ Infection by either of these parasites can lead to chronic inflammation in the human host's biliary tract. Epidemiological

and experimental data strongly characterize *C. sinensis* and *O. viverrini* as carcinogens and the culprits for the development of cholangiocarcinoma (CCA), a cancer of the bile ducts, often decades after primary infection.^{1–3} *C. sinensis* is widely distributed in the Northern provinces of Vietnam, whereas *O. viverrini* is present in the central and south provinces of Vietnam.⁴ Cholangiocarcinoma is not common. The American Cancer Society estimates that about 8000 people in the United States are diagnosed with bile duct cancer each year or 1 to 2 cases per 100,000 patients.^{5,6} Several conditions have been linked to CCA carcinogenesis and include primary sclerosing cholangitis, liver cirrhosis, hepatolithiasis, viral hepatitis B and C infection, Caroli disease, and the liver flukes.^{5,6} During the Vietnam War, over 2.5 million Americans served their country in the armed forces stationed in Vietnam. Vietnam Veterans, 5 decades after the end of the war, are being diagnosed with CCA. The Veterans groups have risen the possibility of being exposed to the parasites during their service and have made their cases public.^{7,8} To our knowledge, no studies have been done confirming exposure to liver flukes in Vietnam Veterans or a link for developing CCA. We performed a pilot study in our Veterans Affairs Medical Center screening Vietnam Veterans for exposure to liver flukes by history and serology.

MATERIALS AND METHODS

A prospective pilot study was conducted at Northport Veterans Affairs Medical Center in 2017. A total of 97 Vietnam Veterans were screened for eating raw or undercooked freshwater fish from the rivers in Vietnam during their service. Diagnosis of CCA was also part of the inclusion criteria. There is no commercially available test for clonorchiasis in the United States. Blood specimens were transported to Seoul National University College of Medicine, South Korea for the enzyme-linked immunosorbent assay (ELISA) testing using a multihelminth-antigen assay that included *C. sinensis*. The present pilot study protocol was reviewed and approved by the Institutional Review Board of Northport Veterans Affairs Medical Center (approval number 00473). Informed consent was obtained from all subjects enrolled in the study.

RESULTS

Of the 97 Veterans, 50 (all men) met the study's inclusion criteria with 1 Veteran having had a diagnosis of CCA. Twelve (24%) of them tested positive for exposure to *C. sinensis*. The demographic characteristics of the subjects are shown in Table 1. The Veterans were deployed south to the demilitarized zone in Quang province and extended to the Mekong River Delta. Half (50%) of the Veterans who tested positive were deployed in the area between 14th and 16th parallels north (Fig. 1). Stool examination for ova and parasites was performed in those who screened positive; none showed liver flukes, and 1 showed *Entamoeba histolytica*. In addition, abdominal imaging (ultrasound or computed tomography) did not show abnormal findings in the extrahepatic or intrahepatic bile ducts. One positive patient had a dilated

From the *Division of Infectious Diseases, Veterans Affairs Medical Center, Northport; †Division of Infectious Diseases, Stony Brook University School of Medicine, Stony Brook; ‡Nursing Service, Veterans Affairs Medical Center, Northport, NY; and §Department of Parasitology and Tropical Medicine, Seoul National University of Medicine, Seoul, South Korea.

Correspondence to: George Psevdos, MD, Chief of Infectious Diseases, Veterans Affairs Medical Center, 79 Middleville Rd, Northport, NY 11768. E-mail: George.Psevdos@va.gov.

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TABLE 1. Demographic Characteristics of the Veterans Tested for Liver Fluke

Number met inclusion criteria:	50
Median age:	70 y
Race:	Caucasian, 45 (90%) African American, 4 (8%) Asian, 1 (2%)
Branch of service:	Army, 33 (66%) Marines, 13 (26%) Navy, 2 (4%) Air force, 2 (4%)
Years of service:	1965–66, 3 1966–67, 8 1967–68, 9 1968–69, 14 1969–70, 9 1970–71, 7
Fish exposure (raw/undercooked)	From local vendors (villagers), 29 Fishing (with grenade, knife, net, pole), 13 Unrecalled details, 8
Drunk unpurified water from the river:	45
Medical problems:	Diabetes, 14 (28%) CCA, 1 Hypertension, 35 (70%) PTSD, 42 (84%) Hyperlipidemia, 36 (72%)
History of cholecystectomy:	6
History of malignancy (not CCA):	No history, 31 Melanoma, 4 Basal cell skin cancer, 4 Lymphoma, 2 Bladder cancer, 1 Lung cancer, 1 Prostate cancer, 2 Thyroid cancer, 1 Rectal cancer, 2
Number tested positive for <i>Clonorchis</i> :	12 (24%)
Stool ova and parasites:	Positive for <i>Clonorchis</i> , none Positive for <i>Entamoeba histolytica</i> , 1
Area of service for positive test:	Corps I, 6 Corps II, 2 Corps III, 3 Corps IV, 1
Size of common bile duct:	All 12 Veterans with <1 cm

Corps I to IV are tactical zones of South Vietnam drawn by US Army. PTSD indicates post-traumatic stress disorder.

pancreatic duct (9 mm); histologic evaluation of the pancreatic duct showed focal mucinous metaplasia. The other patient who had CCA tested negative for *C. sinensis* on 2 separate specimens. Second testing for this Veteran was approved by our institutional review board, because the patient requested confirmation of the initial negative result.

DISCUSSION

The life cycle of the liver flukes is complex (will not be reviewed in detail here). Humans are the definitive hosts. Humans acquire the parasitic infection by ingesting raw or inadequately cooked freshwater fish.⁹ In humans, the adult fluke inhabits the biliary tract, generally localizing within the intrahepatic bile ducts. The adult fluke has a life span of 20 to 25 years, which explains the persistent infection for a long duration.⁹ Five decades have passed from the Vietnam War; therefore, it is not likely to detect ova in the stool as direct evidence of the liver fluke infection in the Veterans. Nevertheless, the Veterans may have been infected by the liver fluke while they were in Vietnam because most of them reported eating raw or undercooked fish. Because our Veterans served in the area where *Opisthorchis* species were prevalent, they might have been infected by *O. viverrini* and not by *C. sinensis*, which is prevalent in Northern Vietnam. Our study screened their serology with crude antigen of *C. sinensis* because the multiantigen ELISA routinely includes *C. sinensis*, but not *O. viverrini*.¹⁰ A specific *O. viverrini* antigen is not yet developed in our reference research laboratory. We can estimate that the 12 ELISA positive Veterans were likely exposed to *O. viverrini* because the 2 liver flukes share common antigens that can cross react with each other.¹¹ Cross reaction with other helminthic antigens is also possible.¹¹ Another possible limitation is the time lapse of 5 decades since the end of the war; this time lag may not allow for the antibodies raised by

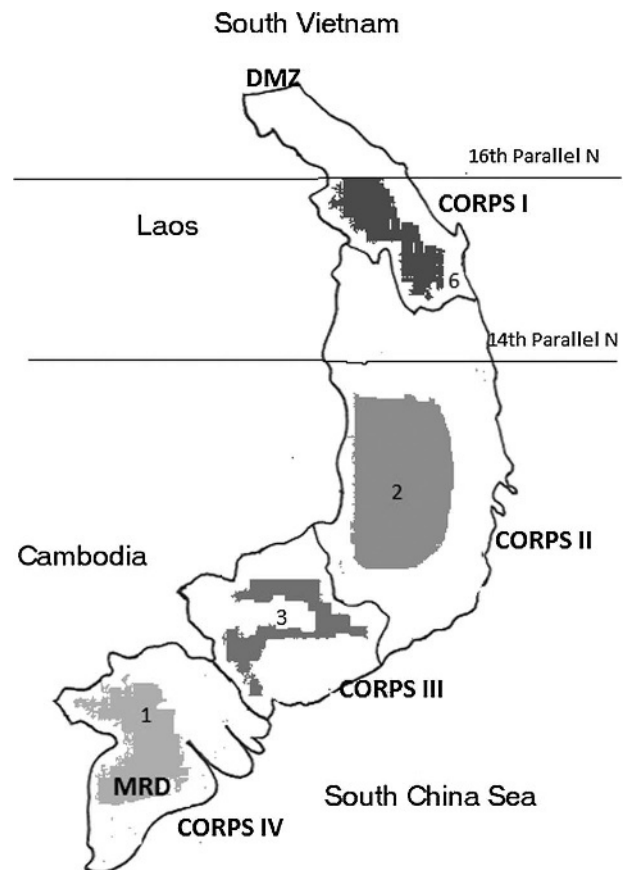


FIGURE 1. Map of South Vietnam during the war and number of positive liver fluke screens among US Vietnam Veterans. Corps I to IV are tactical zones of South Vietnam drawn by the US Army. DMZ, demilitarized zone; MRD, Mekong River Delta.

O. viverrini infection to remain detectable by ELISA. The 1 patient with CCA was ELISA negative twice, but this result may not definitively exclude the possibility of liver fluke infection because of the long time lag and the patient's weakened immune system due to the prolonged cancer treatment.

Our study is the first to show evidence of exposure to the liver fluke parasites in US soldiers during their service in the Vietnam War. Based on our pilot findings, we recommend an epidemiological study using a larger patient sample for serological testing using a specific *O. viverrini* antigen. Follow-up of liver fluke-exposed and unexposed Veterans in time will aid to determine whether liver fluke infection during combat service in the Vietnam War is a risk factor for developing CCA.

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