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Unexpectedly High Prevalence of Hepatitis C Virus Infection, Southern Laos

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During 2017–2019, a total of 88/753 (11.7%) of patients 5–90 years of age in hospitals in Saravan Province, Laos, were seropositive for hepatitis C virus antibodies. Viral RNA was found in 44 samples. Sequencing showed high diversity within genotype 6. We recommend exposure-risk investigations and targeted testing and treatment.

Hepatitis C virus (HCV) infection carries high risk for progression to chronic status and liver complications, such as cirrhosis and cancer. Transmission usually occurs through blood (e.g., during medical procedures, blood transfusions, tattooing, or intravenous drug use). Because those who clear the virus remain HCV antibody positive, testing for viral RNA is essential for diagnosis of chronic infection (1).

We conducted a cross-sectional, hospital-based study during May 2017–March 2019 to determine seroprevalence and genotyping of HCV in Saravan Province

in southern Laos. Saravan Province has a population of ≈400,000 distributed over 8 districts, 2 bordering Vietnam to the east and 2 bordering Thailand to the west. In 2017, only 8.5% of men and 6.9% of women had health insurance; 36.8% of the provincial population was in the poorest wealth index quartile; 17.8% of households had no electricity; and only 54.3% of men and 44.7% of women were literate, the lowest literacy rates in Laos (2).

We nonrandomly selected 753 participants from a larger study (Appendix, <https://wwwnc.cdc.gov/EID/article/28/1/21-1307-App1.pdf>) (3); participants were persons >5 years of age who were recruited for the larger study while seeking care at the provincial hospital or 1 of 3 district hospitals. Overall, 11.7% (88) participants were HCV antibody seropositive, compared with <2% in previous studies in Laos (4,5) (Figure; Appendix). Only 2 seropositive patients were at the hospital for hepatitis-related reasons; HCV seroprevalence was not significantly different regardless of whether or not participants sought care for reasons associated with hepatitis. After multivariate analysis, those >30 years of age had much higher seroprevalence (70/350, 20%) than those ≤30 years of age (18/403, 4.5%; odds ratio [OR] 4.2; $p < 0.001$). This higher seroprevalence indicates either that older adults are at higher risk for exposure or that the older adults were infected some time ago, during childhood or early adulthood. Participants who practice Animism had a slightly higher seroprevalence (81/495; 16.4%) than followers of Buddhism or other faiths (7/258, 2.7%; OR 3.0; $p = 0.02$), and married participants had slightly higher seroprevalence (81/485, 16.7%) than single participants (7/268, 2.6%; OR 2.7; $p = 0.04$), although the associated risk factors are unknown (Table; Appendix).

Whether the observed west–east increase in seroprevalence is related to the proximity of Samuoi district (24.4% anti-HCV seropositive) to the Vietnam border remains unclear (Figure; Appendix). Although HCV seroprevalence in Quang Tri, a bordering province in Vietnam, has been reported to be <1% (6), much higher rates were found in different groups at high risk in Vietnam, such as intravenous drug users (IDU) and men who have sex with men (MSM) (7). We could find no reported link between the Samuoi district population and the IDU or MSM communities in Vietnam, although this link remains possible.

Seroprevalence was significantly higher among the Pako ethnic group (66/265, 24.9% vs. 22/488, 4.5%; OR 5.1; $p < 0.001$), which makes up most of the population in Samuoi district but not elsewhere. The Pako practice nonsterile teeth filing and lacquering during early adolescence with shared equipment and associated bleeding, although this practice is

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in decline. Pako do not often practice tattooing, but the women have ear piercings, which could be another source of infection. Other risk factors, such as blood transfusions and practices of MSM and IDU, are thought to be rare in this population, but non-sterile injection of traditional medicine might occur (8; A. Sernsarae, Samuoi District Health Office, pers. comm., 2020 Jul 23).

Only 44 of the samples we tested were positive for HCV RNA. The relatively low rate of chronic in-

fection could indicate exposure early in life; persons infected at <25 years of age are thought to have much lower risk for chronic infection (1). A substantial proportion of children in our study were also infected, either by mother-to-child transmission or through the same routes as the adults in the study.

We obtained sequence data for 39 samples. All belonged to genotype 6 (Appendix). The sequence diversity does not suggest any recent large-scale transmission events, because no identical sequences

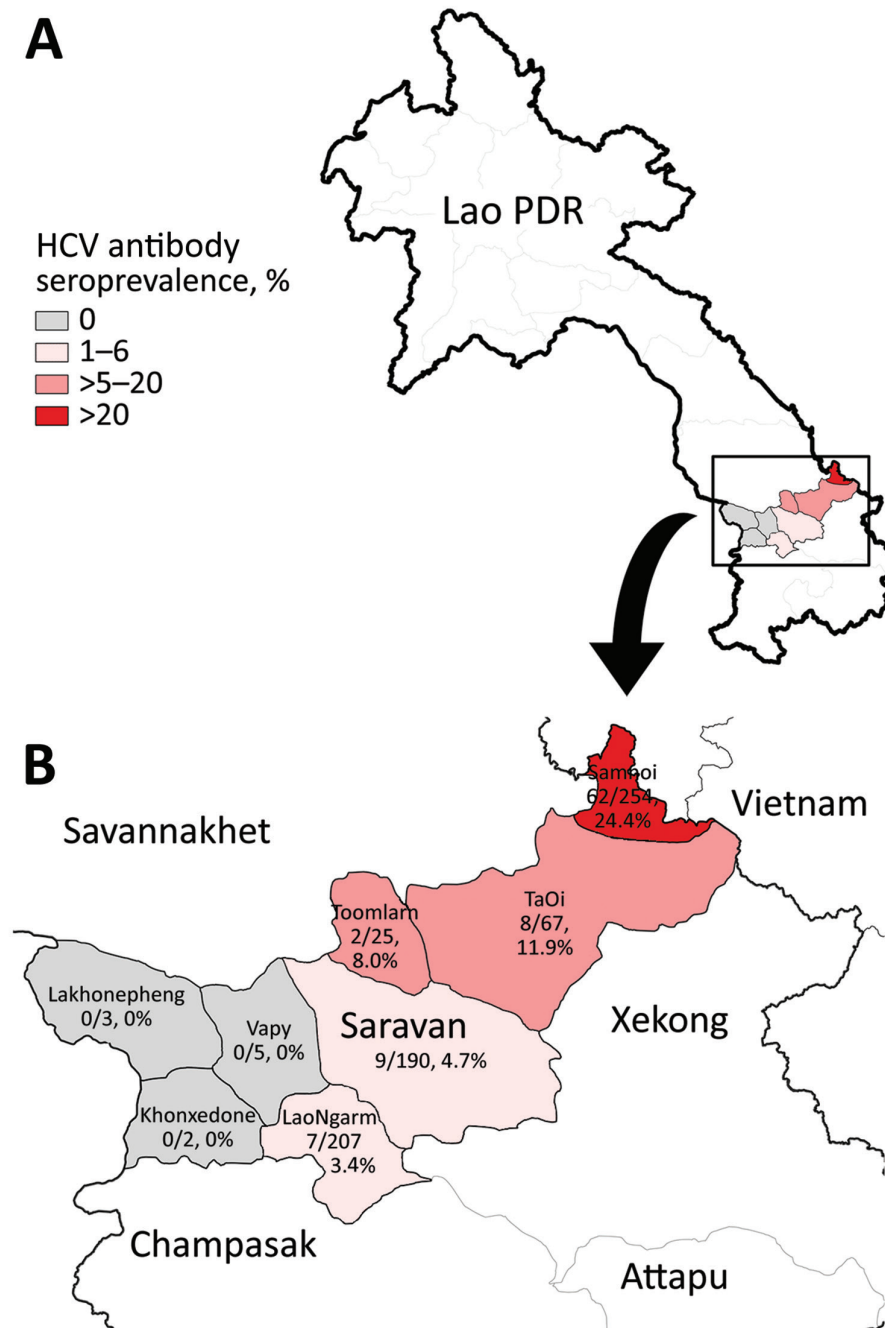


Figure. HCV seroprevalence, Laos, May 2017–March 2019. A) Location of Saravan Province; B) districts of Saravan Province. Colors represent seroprevalence levels. HCV, hepatitis C virus.

Table. Bivariate and multivariate analysis of HCV antibody positive cases, Laos, May 2017–March 2019*

Variable	Positive no./total no. (%)	Bivariate		Multivariate	
		OR (95% CI)	p value	OR (95% CI)	p value
Sex					
F	42/417 (10.1)	Referent	NA	Referent	NA
M	46/336 (13.7)	1.4 (0.9–2.2)	0.126	1.5 (0.9–2.6)	0.08
Age group, y					
<30	18/403 (4.5)	Referent		Referent	NA
>30	70/350 (20)	5.3 (3.1–9.2)	<0.001	4.2 (2.1–8.5)	<0.001
District†					
Lao Ngarm	7/207 (3.4)	Referent		NA	NA
Saravan	9/190 (4.7)	1.4 (0.5–3.9)	0.495	NA	NA
Samuoi‡	62/254 (24.4)	9.2 (4.1–20.7)	<0.001	NS	NS
Ta Oi	8/67 (11.9)	3.9 (1.3–11.1)	0.012	NS	NS
Toomlarn	2/25 (8)	2.5 (0.5–12.7)	0.274	NA	NA
Vapy	0/5 (0)	NA	NA	NA	NA
Khongxedone	0/2 (0)	NA	NA	NA	NA
Lakhonepheng	0/3 (0)	NA	NA	NA	NA
Marital status					
Single	7/268 (2.6)	Referent	NA	Referent	NA
Married or other	81/485 (16.7)	7.5 (3.4–16.4)	<0.001	2.7 (1.0–7.3)	0.04
Occupation					
Student or other	7/231 (3)	Referent	NA	NA	NA
Employee	14/67 (20.9)	8.5 (3.3–21.9)	<0.001	NS	NS
Farmer	67/455 (14.7)	5.5 (2.5–12.2)	<0.001	NS	NS
Ethnicity					
Non-Pako	22/488 (4.5)	Referent	NA	Referent	NA
Pako	66/265 (24.9)	7 (4.2–11.7)	<0.001	5.1 (2.7–9.7)	<0.001
Religion					
Buddhist or other	7/258 (2.7)	Referent	NA	Referent	NA
Animism	81/495 (16.4)	7 (3.2–15.4)	<0.001	3 (1.2–7.6)	0.02
Place of birth					
At hospital or unknown	57/440 (12.9)	Referent	NA	NA	NA
At home	31/313 (9.9)	0.7 (0.4–1.2)	0.2	NA	NA
Diagnosis					
Hepatitis non-related or unknown	86/702 (12.3)	Referent	NA	Referent	NA
Related to hepatitis	2/51 (3.9)	0.2 (0.1–1.2)	0.09	0.2 (0.1–1.1)	0.07

*NA, not applicable; NS, not significant; OR, odds ratio.

†District was not included in the multivariate analysis because it reduced the power of the model.

‡Samuoi district correlated strongly with Pako ethnicity (Pearson correlation coefficient, $R = 0.48$, $p < 0.001$).

were obtained, and the many genetically diverse clusters even in the same district (Samuoi) might indicate different infection sources. However, we cannot rule out a more distant large-scale transmission event. The new strains added to the genetic diversity of genotype 6 viruses found in a previous study from central and northern Laos provinces (5); this increased diversity has potential consequences for the use of commercial assays (9) and treatment strategies (10).

The high rates of death and illness associated with chronic HCV infection suggest that a large proportion of the Saravan population will experience liver-related complications in the future. Despite a reduction in costs of direct-acting antiviral drugs, access to testing and treatment remains low in Laos. An in-depth case-control study to determine sources of infection and associated risk factors is warranted. Furthermore, evaluations of infection prevention, screening, and control measures in healthcare facilities and blood banks, as well as the general population, are needed.

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Dr. Black is the head of a research laboratory in the Institut Pasteur du Laos, Vientiane, Laos. His current research focuses on the immunology and public health burden of vaccine-preventable infectious diseases in Lao PDR.

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Limited Propagation of SARS-CoV-2 among Children in a Childcare Center, Canada, 2021

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An outbreak of severe acute respiratory syndrome coronavirus 2 with no definitive source and potential exposure to variants of concern was declared at a childcare center in Ontario, Canada, in March 2021. We developed a robust outbreak management approach to detect, contain, and interrupt this outbreak and limit propagation among children.

On March 1, 2021, an infant enrolled at a childcare center in the Kingston, Frontenac, Lennox, and Addington region in Ontario, Canada tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); however, no acquisition source was identified. The next day, another 7 children and staff at the facility tested positive, and an outbreak was declared.

We immediately searched for potential transmission events and deployed a public health inspector and nurse team. The infant had last attended the childcare center >3 days before symptom onset, beyond the 48-hour window for exposure risk according to standard guidance (1). Furthermore, the assessment team identified no travel, occupational, or other contact risks. Out of an abundance of caution, we extended the period of communicability (POC) from 48 to 96 hours, which defined the childcare center as an outbreak setting. We identified staff who had recently traveled to regions with high proportions of SARS-CoV-2 variants of concern (VOC). We were concerned that the increased transmissibility and virulence of a potential VOC outbreak in a childcare center could rapidly spread through the community, given recent studies demonstrating SARS-CoV-2 infection and transmission among children (2,3).

Case investigators gathered symptom profiles, onset dates, detailed exposure histories, risk

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Appendix

Participant Recruitment

Because of limited serum sample volume and reagent availability, a subset of 753 samples from a larger study on infectious diseases involving 2,500 participants (1) were tested for hepatitis C virus (HCV) antibodies. Initial testing indicated a high seroprevalence in Samouï district, Saravan Province, Lao People's Democratic Republic. Therefore all 254 samples from Samuoi district were selected, and an additional 499 samples were randomly selected from other districts.

Participants >5 years of age were recruited while seeking care at the provincial or one of 3 district hospitals. Both inpatients and outpatients were asked to participate in the study by healthcare staff and gave informed consent after reading the information sheet. In the case of participants unable to read Lao language, the healthcare workers read the form, and for participants unable to sign, a fingerprint was taken with the signature of a witness. The parents or guardians gave consent for children <18 years of age. Patients with jaundice or hepatitis-related complications were not excluded from participation. The study was approved by the Lao National Research Ethics Committee (ref. no. 005/2018 NECHR), and the data have been reported to the Lao Ministry of Health.

Questionnaire and Serologic Testing

After collecting demographic information (age, sex, hometown, place of birth, religion, occupation, ethnicity, reason for hospitalization, marital status), 5 mL blood specimens were taken from participants. The blood was allowed to clot, and serum specimens were separated by centrifugation. Serum specimens were stored at -20°C at the hospital and then sent to Institut

Pasteur du Laos where they were stored at -80°C until use. HCV antibody testing was done by ELISA (Diasorin, <https://www.diasorin.com>) according to the manufacturer's instructions.

PCR and Sequencing

All HCV antibody positive samples were sent to the Luxembourg Institute of Health for reverse transcription PCR (RT-PCR) and sequencing. The diagnostic PCR was done by using the REALSTAR HCV RT-PCR KIT 2.0 (Altona Diagnostics, <https://www.altona-diagnostics.com>) according to the manufacturer's instructions. Amplification and sequencing of the core/E1 and NS5B regions of the HCV genome were performed by using previously described primers (2,3). MEGA version 7.0.14 (4) was used for the phylogenetic analyses and to calculate genetic distances between sequences.

Data Analysis

The data were described by descriptive and analytical biostatistics. Summary statistics were calculated. Bivariate and multivariable analyses were performed for the independent variables (sex, age, ethnicity, occupation, marital status, religion, place of birth, and diagnosis) and the dependent variable (HCV antibody seropositive). In bivariate analyses, the Chi2 test or the Fisher exact test were used as appropriate. Variables with a p value <0.2 in bivariate analysis were included in the multiple logistic regression model. Binary regression was conducted by using a stepwise method for removing variables one by one, and the Akaike Information Criterion of the model was calculated to determine the appropriate variables to include in the final model. A p value <0.05 was considered statistically significant. Statistical analyses were performed by using Stata version 14 (5). Maps were created using QGIS software version 3.4.14 (6).

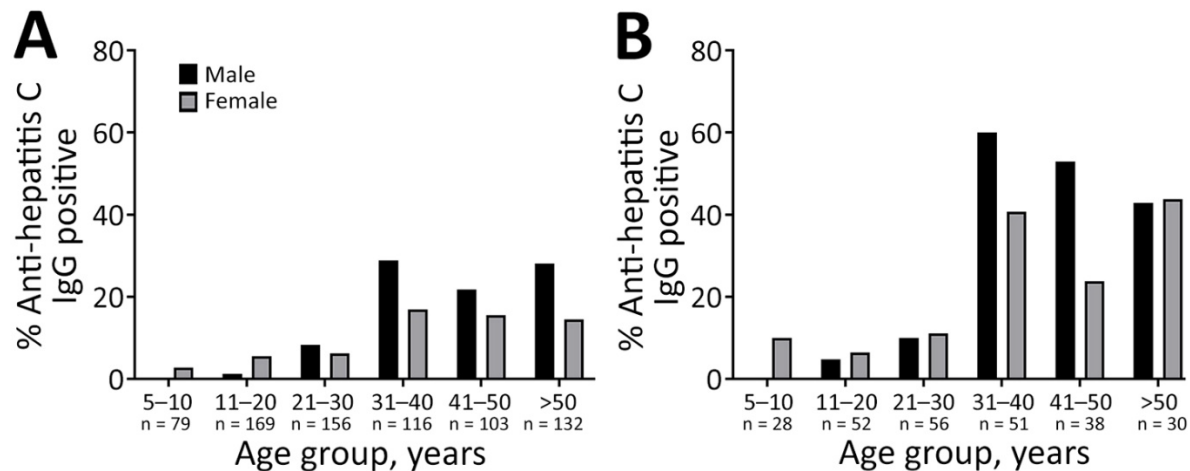
Population Characteristics

Of the 753 participants selected for HCV antibody testing, the median age was 32 (range 5–90) years and 55.4% were females. They were from 275 villages; 33.7% of participants were from Samuoi district, 27.5% from Lao Ngarm, 25.2% from Saravan, 8.9% from Ta Oi, 3.3% from Toomlarn, 0.7% from Vapy, 0.3% from Khongxedone, and 0.4% from Lakhonepheng

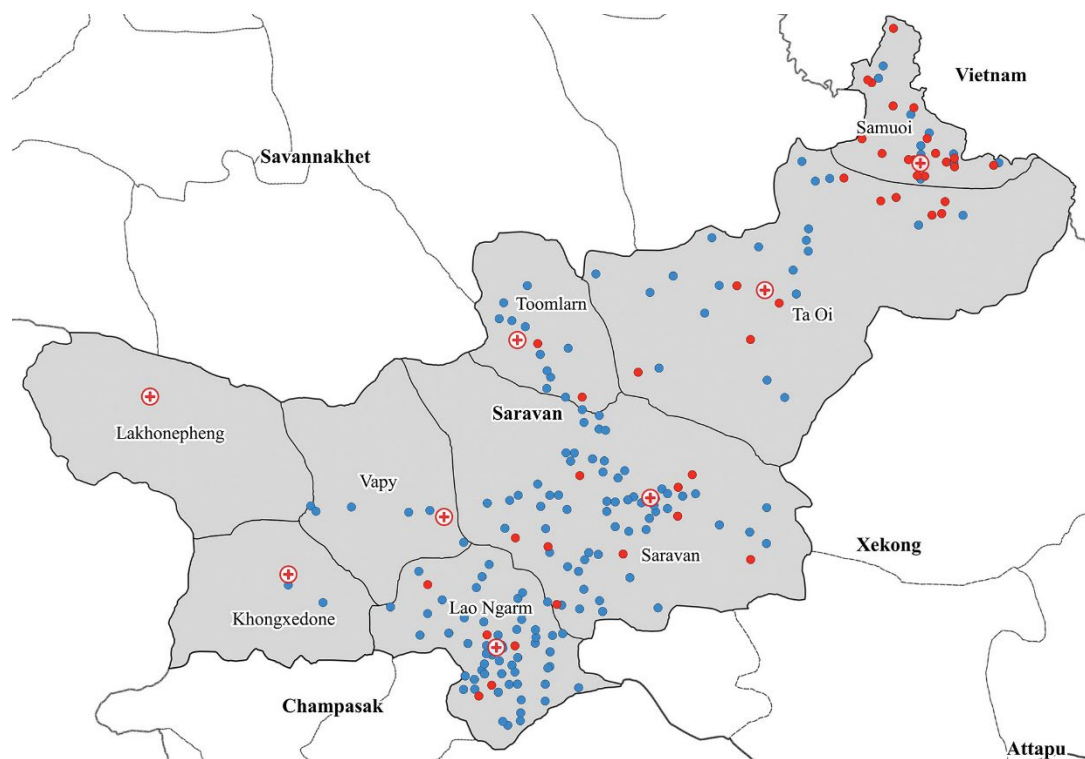
districts. There were >9 ethnic groups represented, including Pako (35.2%), Lao (10.9%), Taoi (10.1%), and others. Most participants from Samuoi were of the Pako ethnicity (250/254 [98.4%], as compared with only 15/499 [3%] from the other districts). Most (62.9%) participants were married and the main occupations were farmers (60.4%) and students in primary, secondary, and further education (26.4%). Only 51 participants (6.8%) were in the hospital for hepatitis-related illness (acute hepatitis, fever, fever of unknown origin, or hepatocellular carcinoma).

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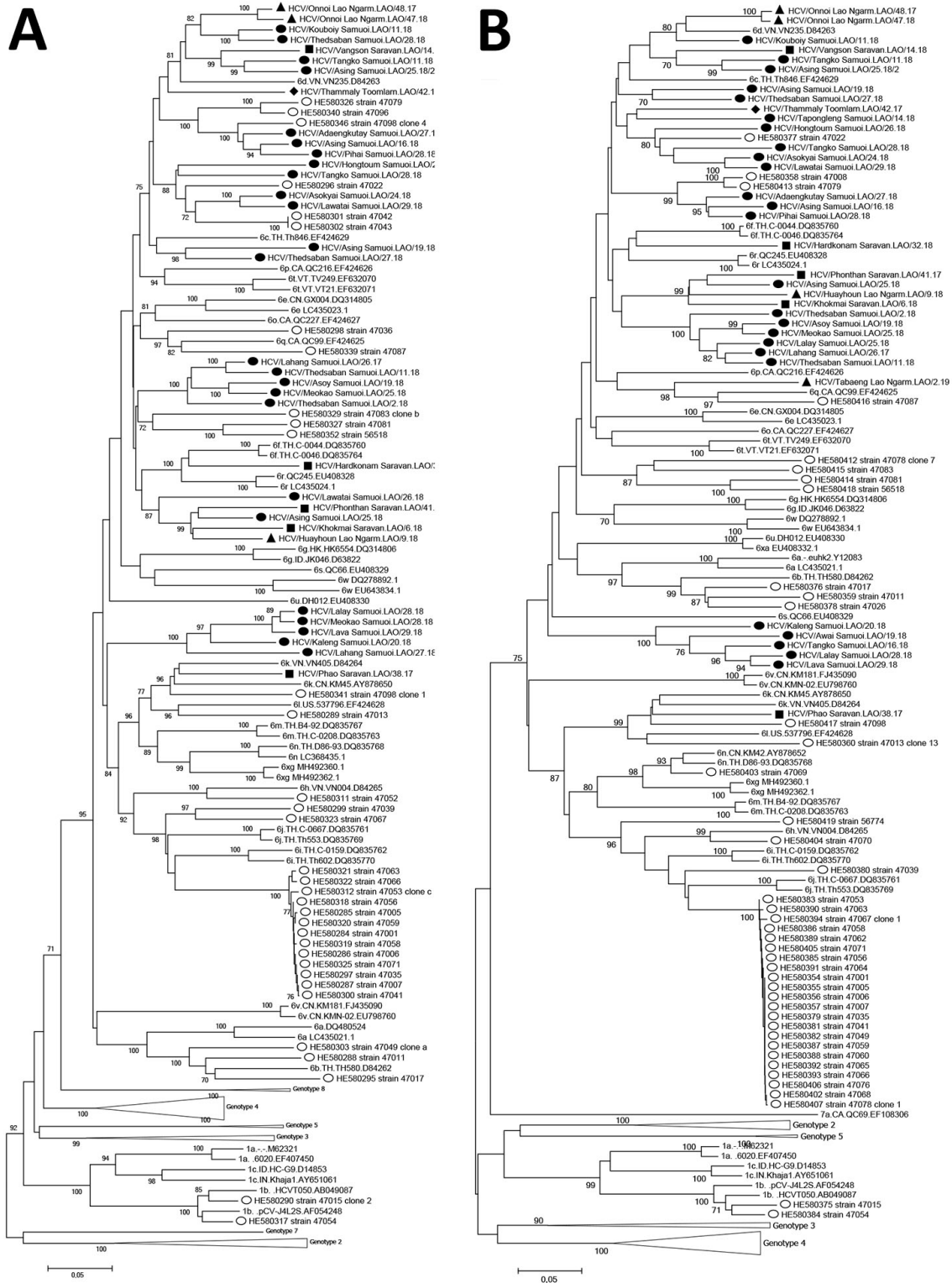
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Appendix Figure 1. Hepatitis C virus antibody seropositivity by age and sex in A) Saravan Province and B) Samuoi district. The numbers below the bars represent the total number of serum specimens that were tested per age group.



Appendix Figure 2. Location of villages with ≥ 1 hepatitis C virus antibody seropositive participant (red dots) and villages where all participants were hepatitis C virus antibody seronegative (blue dots). Participating hospitals are marked with a red cross. Participants were asked their place of residence, including village and district. The geolocation was determined according to data from the Lao National Statistics Bureau and plotted on QGIS (6).



Appendix Figure 3. Phylogenetic trees based on A) 793 nt of the core/E1 gene and B) 340 nt of the NS5B region. The analyses were done with the Kimura 2-parameter model and the Neighbor-Joining algorithm. Only bootstrap values of ≥ 70 are shown. New sequences are marked with a black symbol,

previously published sequences from Lao People's Democratic Republic are marked with a white dot. The different shaped black symbols denote the different districts: dot, Samuoi; triangle, Lao Ngarm; square, Saravan; diamond, Toomlarn. Reference sequences are shown with the suggested subtype in front. There were no identical sequences and the genetic distances ranged from 2.3 to 33.6% in the core/E1 gene region and from 1.5 to 42.3% in the NS5B region. Most of the sequences formed new clusters and only a few clustered interspersed with previously reported sequences (7). Most of the sequences were from Samoi district, and they formed several different groups. Within individual clusters, mainly sequences from the same district and some from the same village grouped together.