

Hepatitis C viraemic and seroprevalence and risk factors for positivity in northwest Cambodia: a household cross-sectional serosurvey

Emily Lynch (✉ emily.lynch@newyork.msf.org)

Epicentre <https://orcid.org/0000-0002-6811-1351>

Gregoire Falq

Epicentre

Chhorvy Sun

Medecins Sans Frontieres-France

PharmD Tek Bunchhoeung

Ministry of Health, Cambodia

Helena Huerga

Epicentre

Anne Loarec

Epicentre

Jean-Phillipe Dousset

Medecins Sans Frontieres-France

Tonia Marquardt

Medecins Sans Frontieres-France

Mickael Le Paih

Medecins Sans Frontieres

David Maman

Epicentre

Research article

Keywords: Hepatitis C, HCV, sero-prevalence, Cambodia, viraemia, MSF, serosurvey

Posted Date: September 4th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-15972/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published on February 26th, 2021. See the published version at <https://doi.org/10.1186/s12879-021-05826-0>.

Abstract

Background: Despite a dramatic reduction in HCV drug costs and simplified models of care, many countries lack important information on prevalence and risk factors to structure effective HCV services.

Methods: A cross-sectional, multi-stage cluster survey of HCV seroprevalence in adults 18 years and above was conducted, with an oversampling of those 45 years and above. 147 clusters of 25 households were randomly selected in two sets (set 1=24 clusters ≥ 18 ; set 2=123 clusters, ≥ 45). A multi-variable analysis assessed risk factors for sero-positivity among participants ≥ 45 . The study occurred in rural Moung Ruessei Health Operational District, Battambang Province, Western Cambodia.

Results: A total of 5,098 individuals and 3,616 households participated in the survey. The overall seroprevalence was 2.6% (CI95% 2.3-3.0) for those ≥ 18 years, 5.1% (CI95% 4.6-5.7) for adults ≥ 45 years, and 0.6% (CI95% 0.3-0.9) for adults 18-44. Viraemic prevalence was 1.9% (CI95% 1.6-2.1), 3.6% (CI95% 3.2-4.0), and 0.5% (CI95% 0.2 - 0.8), respectively. Men had higher prevalence than women: ≥ 18 years male seroprevalence was 3.0 (CI95% 2.5 - 3.5) versus 2.3 (CI95% 1.9 - 2.7) for women. Knowledge of HCV was poor: 64.7% of all respondents and 57.0% of seropositive participants reported never having heard of HCV.

Risk factor characteristics for the population ≥ 45 years included: advancing age ($p < 0.001$), low education (higher than secondary school OR 0.7 [95% CI 0.6-0.8]), any dental or gum treatment (OR 1.6 [95% CI 1.3-1.9]), historical routine medical care (medical injection after 1990 OR 0.7 [95% CI 0.6-0.9]; surgery after 1990 OR 0.7 [95% CI 0.5-0.9]), and historical blood donation or transfusion (blood donation after 1980 OR 0.4 [95% CI 0.2-0.9]); blood transfusion after 1990 OR 0.7 [95% CI 0.4-1.1]).

Conclusions: This study provides the first large-scale general adult population prevalence data on HCV infection in Cambodia. The results confirm the link between high prevalence and age ≥ 45 years, lower socio-economic status and past routine medical interventions (particularly those received before 1990 and 1980). This survey suggests high HCV prevalence in certain populations in Cambodia and can be used to guide national and local HCV policy discussion.

Background

Until very recently, the global Hepatitis C Virus (HCV) epidemic, responsible for an estimated 71 million chronic infections and 30% of the 1.34 million deaths due to viral hepatitis, was largely thought insurmountable (WHO, 2017). Though newer Direct Acting Antivirals (DAA) are safer, more effective and easier for patients than previous HCV treatment, the extremely high treatment cost (up to \$150,000 per patient) prohibited their widespread access and use, particularly outside of well-resourced health systems (WHO, 2017) (Rosenthal & Graham, 2016).

However, recent developments are restructuring the public health approach to HCV. Two common DAAs, Sofosbuvir (Sof) and Daclatasvir (Dac), have reduced dramatically in cost (Sof was \$84,000/treatment in 2013, and is only \$85 in 2019) (WHO, Guidelines for the Screening Care and Treatment of Persons with Chronic Hepatitis C Infection. Updated Version., 2016). Adjusted models-of-care in low-resource settings are treating more patients, as effectively, for a fraction of previous prices (cost-per-cure in a 2018 Cambodian

cohort dropped from \$1172 to \$370) (Iwamoto M, 2018). With these developments, widespread access to treatment is becoming a more realistic goal, encouraging Ministries of Health (MoH) and donors in low-resource settings to reconsider the expansion of HCV treatment as part of the global push towards the elimination of viral hepatitis by 2030, a goal adopted in 2016 by the World Health Assembly (WHO, Global Hepatitis Programme, 2017) (Boyd A, 2018).

The HCV burden in Cambodia has long been thought to be high. However, prevalence estimates in the general population are lacking. Several studies have attempted to understand the magnitude of HCV prevalence in Cambodia, but most have methodological limitations or focus on specific sub-populations rather than on the general population, with considerable variation in their estimates of prevalence rates (OI HS, 2009 Sep) (Yamada H, 2015 Apr) (van Griensven J, 2014 Feb 12) (De Weggheleire A, 2017). Anecdotal clinical evidence has suggested increased HCV risk based on historic exposures to unsafe transfusions or routine medical practices, but this hypothesis has not been tested in the general population.

In 2016, the Cambodian Ministry of Health (MoH) and MSF established an HCV screening and treatment project in Phnom Penh, with expansion to Moug Ruessei hospital in Battambang Province in April 2018. In total at the end of the third quarter of 2018, MSF had screened 36,029 patients for HCV (24,756 Phnom Penh & 11,273 Moug Ruessei) and initiated Direct Acting Antiviral (DAA)-based treatment for 9,731 patients (8,757 Phnom Penh & 974 Moug Ruessei).

This study establishes a robust estimate of the HCV burden and seropositivity risk factors for the general population in three rural districts of northwestern Cambodia.

Methods

Study setting

The survey was conducted from April to August 2018 in the health operational district of Moug Ruessei, Battambang province, located in northwestern Cambodia near the border of Thailand. The three surveyed (administrative) districts included 175 villages, 20 communes, and 13 health center catchment areas. The area population consisted of an estimated 202,790 individuals in 42,072 households; village sizes ranged from 139 to 3,979 inhabitants (29–822 households)[i].

Study population, survey design and sample size

This was a cross-sectional population-based survey, using a multi-stage cluster design with probability proportional to size (PPS) and random sampling of villages (using Emergency Nutrition Assessment – ENA software version 2011) and random sampling of households (25 per cluster). All consenting adults 18 years and above (including visitors[ii]) were eligible for inclusion in the survey. The sampling methodology enabled an oversampling of the population greater than or equal to 45 years old to account for higher expected prevalence in this population.

Sample sizes were calculated using EpiInfo software, with an estimated 7% HCV prevalence among adults 45 years and above and 1.6% HCV prevalence among all adults 18 years and above, at 95% confidence, precision = 1.0% (≥ 45 years) and 0.8% (≥ 18 years), a non-response rate of 15%, and an average household size of 4.7. A total of 147 clusters were selected (123 clusters targeting the population ≥ 45 and 24 clusters for the population ≥ 18). The final sample size required 4,784 individuals (1,610 aged ≥ 18 and 3,174 aged ≥ 45), 3,628 households (577 including ≥ 18 and 3,051 including ≥ 45).

Data collection

15 teams (each consisting of a surveyor plus a nurse) administered face-to-face standardized, pre-piloted electronic questionnaires to households and individuals. Questionnaires included information on socio-demography, migration, knowledge of HCV prevention and treatment, and individual history of HCV exposure and seropositivity risk factors. Data were entered and collected using electronic tablets and then exported to a secure Kobo platform.

Statistical analysis

Statistical analyses (description, prevalence calculation and seropositivity risk factor analysis) were conducted using probabilities/sampling weights calculated for each stage of the sampling: village, household and individual. The sampling stratum was considered the cluster, and analysis took into account the finite population correction factor.

We conducted a multivariate analysis to identify risk factors for HCV serological infection among the population ≥ 45 years. Risk factors for seropositivity identified a priori included demographic variables (age, gender, occupation, education level, ID poor card status^[iii]), spatial variables (health centers catchment area, distance to Moug Ruessei referral hospital, distance to the health center from the catchment area), medical variables (history of blood transfusion and blood donation, history and location/provider type for medical injections, surgery and delivery, dental and gum treatment, type of contraception, miscarriage and abortion) and behavioral variables (tattoos, piercing, IV drug use, pedicures, manicures and frequenting of barbershops). The association between the seroprevalence and the explanatory covariates was quantified by fitting a linear multivariate regression model. The multivariate analysis retained variables from the univariate analysis with p-value less than 0.2. Estimates of the regression coefficients of the model and odds ratios with their standard error are presented. Statistical analyses were conducted using R version 3.4.1 (R Development Core Team, 2014).

The list of villages and population data was provided by the Provincial Health Department (PHD), 2016 and 2017 census data. Household lists (official household registers or notebooks) were provided by chiefs of villages and updated to include any new or temporary residents.

Community engagement

Prior to the start of the survey, meetings were organized with local authorities at all levels to introduce the objectives of the survey and to discuss the timeline and the survey team's requests for the involvement of each area/village/authority in the survey. Mobilisers (identified by the chief of each village) visited selected households prior to the data collection to request the household's presence at home on the planned days of data collection.

Laboratory procedures

Sero-infection was assessed for all participants using the SD Bioline® HCV rapid diagnostic test (Labs), which was performed according to the manufacturer's instructions, using capillary blood collected by fingerpick by trained nurses.

Seropositive participants were invited to the nearest health center to assess their HCV viral load; HIV and HBV diagnostics were also offered at this time to ensure smooth linkage to clinical care. These results were not collected or tracked by the survey. Specimens were stored and transported to the MSF laboratory in Moug Russei hospital in cold chain (2-8°C). Samples were centrifuged the same day and stored in a refrigerator (2-8°C) before their analysis within 24h. Viral load was assessed using the Xpert® HCV viral load assay with GeneXpert® Instrument Systems (Cepheid, Sweden).

Linkage to treatment and care

Patients with detectable viral load were invited to the MSF/MoH HCV program at Moug Ruessei hospital to receive their results and initiate treatment, if desired (transport costs for this travel were reimbursed by the survey). Outside of the initial and final visits at the hospital, follow up care was provided at the closest health facility to the patient's place of residence.

Ethical considerations

Household and individual written consents were obtained before any inclusions in the survey. This study received ethical approval from the Médecins Sans Frontières Ethical Review Board (ID: 1816), as well as the Cambodian National Ethics Committee for Health Research (NECHR; 23 February 2018 NECHR meeting minutes).

[ii] Defined as any person who slept in the household the previous night

[iii] The ID poor card is a system in Cambodia to identify impoverished households eligible to receive public assistance. For the purposes of this survey, there were three possible types of ID poor card status: 1) ID poor card 1, 2) ID poor card 2 and 3) Poor letter (whereby the village chief or another local leader provided a letter confirming the impoverished status of the family). The difference between ID poor card 1 and 2 is a matter of severity of poverty levels; the poor letter is written in the absence of having an official designation as ID poor card 1 or 2, for example in sudden or unexpected circumstances

Results

Participation

A total of 5,098 (of 5,215) individuals and 3,616 (of 3,668) households participated in the survey, with an individual response rate of 97.8% and a household response rate of 98.6%. The percentage of households either absent or refusing to participate was equal, at 0.7%, respectively. The percentage of individual absenteeism or refusal was also equal, at 1.1%, respectively.

Study population

The following section extrapolates the demographics of the surveyed population to the population as a whole to confirm the representativeness of the survey sample. The surveyed individuals and households represented a population of 112,398 inhabitants living in Moung Ruessei district consisting of 49,903 males and 62,494 females, with an overall sex ratio of 0.80 (Table 1). Overall, the age and gender distribution of the study participants was similar to the distribution of the Battambang province population[i].

Table 1: Weighted study population count and proportion, per sex and per age category

| Age group | Male | | Female | | Total | | Sex ratio |
|-----------|--------|-------|--------|-------|---------|-------|-----------|
| | N | % | N | % | N | % | |
| [18-24] | 8,591 | 17.2 | 8,302 | 13.3 | 16,892 | 15.0 | 1,03 |
| [25-34] | 11,767 | 23.6 | 14,149 | 22.6 | 25,916 | 23.1 | 0,83 |
| [35-44] | 8,663 | 17.4 | 10,540 | 16.9 | 19,203 | 17.1 | 0,82 |
| [45-54] | 7,816 | 15.7 | 10,777 | 17.2 | 18,593 | 16.5 | 0,73 |
| [55-64] | 6,828 | 13.7 | 9,941 | 15.9 | 16,769 | 14.9 | 0,69 |
| [65-74] | 4,325 | 8.7 | 5,978 | 9.6 | 10,304 | 9.2 | 0,72 |
| [75-Inf] | 1,914 | 3.8 | 2,807 | 4.5 | 4,721 | 4.2 | 0,68 |
| Total | 49,903 | 100.0 | 62,494 | 100.0 | 112,398 | 100.0 | 0,80 |

The percentage of the study population that never attended school was roughly 50%, irrespective of gender (Table 2). Women achieved higher levels of education than men (24.6% of women have attended higher than secondary school, versus 10.0% for men).

For both men (63.4%) and women (45.9%), farming was the predominant (53.7%) occupation in the study population, followed by small business (7.3%) and labor (4.5%) (Table 2).

Roughly a third (30.7%) of the population surveyed reported being part of an ID poor card program (e.g. receiving social welfare) (Table 2).

Table 2: Description of the weighted study population

| | Male | | Female | | 18 - 44 | | 45+ | | Total | |
|-----------------------------|--------|------|--------|------|---------|------|--------|------|--------|------|
| | N | % | N | % | N | % | N | % | N | % |
| Education level | | | | | | | | | | |
| Never Attended | 25,187 | 50.5 | 31,804 | 50.9 | 29,814 | 48.1 | 27,176 | 53.9 | 56,991 | 50.7 |
| Primary | 12,151 | 24.4 | 8,702 | 13.9 | 13,933 | 22.5 | 6,921 | 13.7 | 20,853 | 18.6 |
| Secondary | 7,414 | 14.9 | 6,610 | 10.6 | 11,911 | 19.2 | 2,112 | 4.2 | 14,024 | 12.5 |
| Higher | 5,152 | 10.3 | 15,378 | 24.6 | 6,353 | 10.2 | 14,177 | 28.1 | 20,530 | 18.3 |
| Occupation | | | | | | | | | | |
| Farmer | 31,634 | 63.4 | 28,683 | 45.9 | 31,980 | 51.6 | 28,337 | 56.2 | 60,317 | 53.7 |
| Laborer | 2,768 | 5.5 | 2,281 | 3.6 | 3,898 | 6.3 | 1,150 | 2.3 | 5,049 | 4.5 |
| Small Business | 2,560 | 5.1 | 5,639 | 9.0 | 4,837 | 7.8 | 3,363 | 6.7 | 8,200 | 7.3 |
| Factory Worker | 84 | 0.2 | 361 | 0.6 | 433 | 0.7 | 12 | 0.0 | 445 | 0.4 |
| Student | 1,011 | 2.0 | 1,733 | 2.8 | 2,743 | 4.4 | 0 | 0.0 | 2,743 | 2.4 |
| Housework | 964 | 1.9 | 10,664 | 17.1 | 5,920 | 9.6 | 5,708 | 11.3 | 11,627 | 10.3 |
| Construction | 1,346 | 2.7 | 84 | 0.1 | 1,227 | 2.0 | 202 | 0.4 | 1,430 | 1.3 |
| Cleaning/Maid | 0 | 0.0 | 84 | 0.1 | 72 | 0.1 | 12 | 0.0 | 84 | 0.1 |
| Pensioner | 727 | 1.5 | 1,085 | 1.7 | 72 | 0.1 | 1,740 | 3.4 | 1,812 | 1.6 |
| None | 2,536 | 5.1 | 5,586 | 8.9 | 1,805 | 2.9 | 6,318 | 12.5 | 8,123 | 7.2 |
| Other | 6,273 | 12.6 | 6,295 | 10.1 | 9,024 | 14.6 | 3,545 | 7.0 | 12,568 | 11.2 |
| ID poor card program | | | | | | | | | | |
| ID poor card 1 | 5,375 | 10.8 | 8,292 | 13.3 | 5,920 | 9.6 | 7,748 | 15.4 | 13,667 | 12.2 |
| ID poor card 2 | 7,556 | 15.1 | 10,923 | 17.5 | 9,313 | 15.0 | 9,167 | 18.2 | 18,479 | 16.4 |
| Poor letter | 204 | 0.4 | 132 | 0.2 | 217 | 0.3 | 119 | 0.2 | 335 | 0.3 |
| Not part of a poor program | 35,665 | 71.5 | 42,263 | 67.6 | 45,119 | 72.8 | 32,810 | 65.1 | 77,929 | 69.3 |

More than a quarter of the population reported being away from home sometime within the previous year (Table 3). The percentage of the population that was away from home for one month or longer was substantial: 9.0% of the population were away 1 to 6 months and an additional 7.0% were away more than 6 months. There were higher percentages of being away from home for men compared to women, and for those aged 18-44 compared to those aged 45 years and older. The primary reason (90.4%) given for absence was to “find a job” (Table 3).

Table 3: Migration pattern in the study population per sex and per age category

| | Male | | Female | | 18 - 44 | | 45+ | | Total | |
|------------------------------------|--------|------|--------|------|---------|------|--------|------|--------|------|
| | N | % | N | % | N | % | N | % | N | % |
| Time away from home | | | | | | | | | | |
| Never absent | 34,443 | 69.0 | 46,872 | 75.0 | 40,715 | 65.7 | 40,599 | 80.6 | 81,314 | 72.3 |
| Less than 1 month | 6,024 | 12.1 | 7,087 | 11.3 | 6,208 | 10.0 | 6,903 | 13.7 | 13,111 | 11.7 |
| 1 to 6 months | 5,509 | 11.0 | 4,608 | 7.4 | 8,157 | 13.2 | 1,959 | 3.9 | 10,117 | 9.0 |
| More than 6 months | 3,928 | 7.9 | 3,927 | 6.3 | 6,930 | 11.2 | 925 | 1.8 | 7,855 | 7.0 |
| Reason to be away from home | | | | | | | | | | |
| Find a job | 8,765 | 92.9 | 7,481 | 87.7 | 13,933 | 92.3 | 2,314 | 80.2 | 16,246 | 90.4 |
| Study | 168 | 1.8 | 289 | 3.4 | 433 | 2.9 | 24 | 0.8 | 457 | 2.5 |
| Other | 503 | 5.3 | 766 | 9.0 | 722 | 4.8 | 547 | 18.9 | 1,269 | 7.1 |

[i] Department of Economic and Social Affairs, 2017

Prevalence

Main findings

The overall seroprevalence for the entire adult population 18 years and above was 2.6% (CI95% [2.3-3.0]) (Table 4), with 5.1% (CI95% [4.6-5.7]) in adults 45 years and above, and 0.6% (CI95% [0.3 - 0.9]) in adults 18-44. In finer age categories, higher and similar results were found for categories of those 55 and older (Figure 1). The prevalence was, respectively, 6.0 (CI95% [5.2 - 6.8]), 7.28 (CI95% [6.1 - 8.4]), and 6.7 (CI95% [5.2 - 8.3]),

in the age categories 55-64, 65-74 and 75 and older (Figure 1). Men had a higher prevalence than women: 3.0 (CI95% [2.5 - 3.5]) vs. 2.3 (CI95% [1.9 - 2.7]), respectively (Table 1).

Table 4: Seroprevalence and viraemic prevalence per sex and per age category

| | Serology | | Viraemia | |
|---------|-----------|--------------------|-----------|--------------------|
| | N | Prevalence CI95% | N | Prevalence CI95% |
| Overall | 221/5,098 | 2.61 [2.25 - 2.96] | 157/5,098 | 1.88 [1.62 - 2.14] |
| [18-44] | 5/859 | 0.58 [0.27 - 0.89] | 4/859 | 0.47 [0.17 - 0.76] |
| [45+] | 216/4,239 | 5.10 [4.55 - 5.65] | 153/4,239 | 3.62 [3.22 - 4.01] |
| Male | 112/2,159 | 3.03 [2.54 - 3.52] | 84/2,159 | 2.37 [1.94 - 2.79] |
| Female | 109/2,939 | 2.27 [1.87 - 2.66] | 73/2,939 | 1.49 [1.22 - 1.76] |

Viraemic prevalence was also notably high in older adults. The prevalence for adults 18 years and above was 1.9 (CI95% [1.6 - 2.1]) (Table 4) and for adults 45 years and above it was 3.6 (CI95% [3.2 - 4.0]), while it was 0.5 (CI95% [0.2 - 0.8]) in adults 18-44 (0.5 [0.2 - 0.8]). Like the serological results, men have higher viraemic prevalence than women, at 2.4 (CI95% [1.9 - 2.8]) compared to 1.5 (CI95% [1.2 - 1.8]), however, with overlapping confidence intervals.

Geographical pattern

Among adults 45 years old and above, some geographic variation in seroprevalence was noted at the level of the health center catchment area, ranging from the lowest prevalence, in Prey Svay, of 3.0 (CI95% [1.3 - 4.6]) to the highest in Prey Toch, at 9.4 (CI95% [6.7 - 12.1]) (Table 5). Nevertheless, this analysis was unable to quantify any clear geographic patterns in seroprevalence. Exploratory analysis showed no identifiable trends in prevalence according to the distance from the catchment area to Moung Ruessei referral hospital, or to the health center (Figure 2).

Table 5: HCV seroprevalence per health center catchment area by age categories.

| | ≥ 18 years | | ≥ 45 years | |
|---------------|------------|--------------------|------------|---------------------|
| | N | Prevalence | N | Prevalence |
| Chrey | 23/443 | 3.17 [2.13 - 4.20] | 21/324 | 6.49 [5.21 - 7.77] |
| Kea | 16/351 | 4.59 [2.46 - 6.71] | 16/351 | 4.59 [2.46 - 6.71] |
| Keas Kralor | 17/391 | 2.99 [1.75 - 4.24] | 16/324 | 4.82 [3.30 - 6.33] |
| Kor Kos | 23/360 | 4.13 [3.28 - 4.97] | 22/295 | 7.57 [4.58 - 10.55] |
| Mong | 27/586 | 2.02 [1.05 - 3.00] | 26/392 | 6.56 [4.15 - 8.97] |
| Prek Chik | 14/321 | 2.86 [1.51 - 4.20] | 14/288 | 4.86 [3.54 - 6.19] |
| Prek Tralach | 16/578 | 1.75 [1.09 - 2.42] | 16/513 | 3.11 [2.35 - 3.87] |
| Prey Svay | 11/414 | 1.87 [1.20 - 2.55] | 11/378 | 2.95 [1.28 - 4.63] |
| Prey Toch | 17/225 | 3.87 [0.83 - 6.90] | 17/182 | 9.41 [6.72 - 12.09] |
| Robos Mongkol | 15/405 | 2.57 [1.25 - 3.89] | 15/370 | 4.05 [2.81 - 5.28] |
| Russei Kraing | 10/500 | 0.74 [0.51 - 0.97] | 10/335 | 2.99 [2.14 - 3.83] |
| Talars | 25/329 | 4.82 [3.46 - 6.18] | 25/292 | 8.56 [6.98 - 10.15] |
| Thitpdey | 7/195 | 3.59 [1.58 - 5.60] | 7/195 | 3.59 [1.58 - 5.60] |

Knowledge of HCV

Most participants, 3,302/5,103 (64.7%), reported never having heard of HCV[i]. This percentage was lower amongst seropositive participants (57.0%). Among those who reporting having heard of HCV, knowledge of HCV transmission was similarly reported between seropositive and seronegative participants (55.8% versus 53.2%). The percentage of all survey participants who had both heard of HCV and accurately reported all of the transmission pathways among the options given ranged from 12.6% to 17.5%. Among the 1,801 participants who had heard of HCV, 1,139 participants (63.2%) responded that they know how HCV can be prevented. Although most participants correctly reported HCV prevention methods (“not sharing needles or syringes with other people,” “using a condom,” and “using sterile or unused medical devices”: 93.1%, 80.0%, 88.2%, respectively), a majority (85.4%, 82.2%) also reported that “getting a vaccine,” and “washing your hands thoroughly,” were other possible ways to prevent HCV transmission.

Seropositivity risk factor analysis

Table 6 presents socio-demographic characteristics and risk factors associated with seroprevalence in the multivariate linear regression model for adults 45 years and above. Seroprevalence increased with age ($p < 0.001$). Seroprevalence was also associated with socio-economic status: seroprevalence was lower

among people who achieved an education level higher than secondary school than those who never attended school (OR 0.7 [95% CI 0.6-0.8]); there was also some evidence that seroprevalence was also lower among people with an ID poor card 1 compared to those not part of a poor program (OR 0.8 [95 % CI 0.6-1.0], $p < 0.01$) . Exposure to routine medical care was associated with HCV seroprevalence: Seroprevalence was higher among people who had their first injection (for a medical need) before 1990 (injection after 1990 OR 0.8 [95% CI 0.6-0.9]); people who had their first surgery before 1990 (surgery after 1990 OR 0.7 [95% CI 0.5-0.9]); people who donated blood for the first time before 1980 (blood donation after 1980 OR 0.4 [95% CI 0.2-0.9]); people who had a blood transfusion for the first time before 1990 (blood transfusion after 1990 OR 0.7 [95% CI 0.4-1.1]); and people who had dental and gum treatment (OR 1.6 [95% CI 1.3-1.9]). The degrees of freedom of the model was high (34) given the number of events (216). Nevertheless, the goodness of fit of the model (Hosmer Lemeshow test) was less than 0.05.

Table 6 - Socio-demographic characteristics and risk factors, adults ≥ 45 years old (p-value per level and odds ratio).

| | | β (Se) | p-value | | OR (CI95) |
|---------------------------------------|----------------|-----------------|---------|--------|-------------------------|
| | (Intercept) | -2.69 (0.49) | | | 0.07 [0.03- 0.18] |
| | age | 0.03 (0.00) | <0.001 | <0.001 | 1.04 [1.03- 1.04] |
| Level of education | Primary | 0.17 (0.09) | 0.068 | <0.001 | 1.20 [0.99- 1.45] |
| Reference: Never Attended | Secondary | 0.05 (0.16) | 0.738 | | 1.06 [0.77- 1.46] |
| | Higher | -0.38 (0.10) | <0.01 | | 0.68 [0.55- 0.84] |
| ID poor card | ID poor card 1 | -0.27 (0.13) | <0.05 | <0.01 | 0.76 [0.59- 0.99] |
| Reference: Not part of a poor program | ID poor card 2 | 0.12 (0.10) | 0.235 | | 1.13 [0.93- 1.37] |
| | Poor letter | 0.86 (0.45) | 0.064 | | 2.36 [0.96- 5.80] |
| | Don't Know | 0.67 (0.35) | 0.058 | | 1.96 [0.99- 3.89] |
| Health center | Kea | -0.51 (0.25) | <0.05 | <0.001 | 0.60 [0.36- 0.99] |
| Reference = Chrey | Keas Kralor | -0.32 (0.21) | 0.135 | | 0.72 [0.47- 1.10] |
| | Kor Kos | 0.24 (0.24) | 0.315 | | 1.27 [0.80- 2.04] |
| | Mong | -0.13 (0.22) | 0.568 | | 0.88 [0.56- 1.37] |
| | Prek Chik | -0.22 (0.18) | 0.234 | | 0.80 [0.56- 1.15] |

| | | β (Se) | p-value | | OR (CI95) |
|--|-------------------------|------------------|---------|--------|-------------------------|
| | Prek Tralach | -0.75 (0.16) | <0.001 | | 0.47 [0.34- 0.64] |
| | Prey Svay | -0.85 (0.31) | <0.01 | | 0.43 [0.23- 0.79] |
| | Prey Toch | 0.37 (0.20) | 0.067 | | 1.45 [0.98- 2.16] |
| | Robos Mongkol | -0.56 (0.20) | <0.01 | | 0.57 [0.38- 0.84] |
| | Russei Kraing | -0.78 (0.18) | <0.001 | | 0.46 [0.32- 0.65] |
| | Talars | 0.24 (0.14) | 0.092 | | 1.28 [0.96- 1.69] |
| | Thitpdey | -0.72 (0.34) | <0.05 | | 0.48 [0.25- 0.94] |
| Injection – threshold 1990 Reference = injection before 1990 | No injection | -0.24 (0.17) | 0.155 | <0.01 | 0.78 [0.56- 1.09] |
| | Injection 1990 or after | -0.29 (0.09) | <0.01 | | 0.74 [0.62- 0.89] |
| | Don't Know if injection | -0.36 (0.59) | 0.544 | | 0.70 [0.22- 2.23] |
| Surgery – threshold 1990 Reference = Surgery before 1990 | No surgery | -0.68 (0.14) | <0.001 | <0.001 | 0.50 [0.38- 0.67] |
| | surgery 1990 or after | -0.42 (0.15) | <0.01 | | 0.65 [0.48- 0.88] |
| | Don't Know if surgery | -12.65 (0.43) | <0.001 | | 0.00 [0.00- 0.00] |
| Blood donation – threshold 1980 Reference = blood donation before 1980 | No blood donation | -1.04 (0.32) | <0.01 | <0.001 | 0.35 [0.19- 0.66] |

| | | β (Se) | p-value | | OR (CI95) |
|---|---------------------------------|---------------|---------|--------|------------------|
| | Blood donation 1980 or after | -0.83 (0.34) | <0.05 | | 0.43 [0.22-0.85] |
| | Don't Know if blood donation | -14.94 (0.75) | <0.001 | | 0.00 [0.00-0.00] |
| Blood transfusion – threshold 1990 | No blood transfusion | -0.65 (0.21) | <0.01 | <0.001 | 0.52 [0.34-0.79] |
| Reference = blood transfusion before 1990 | Blood transfusion 1990 or after | -0.42 (0.25) | 0.092 | | 0.65 [0.40-1.07] |
| | Don't Know if blood transfusion | -13.56 (0.37) | <0.001 | | 0.00 [0.00-0.00] |
| Dental and Gum treatment | Yes | 0.44 (0.08) | <0.001 | <0.001 | 1.56 [1.33-1.83] |
| Reference = No | Don't Know | -12.25 (0.54) | <0.001 | | 0.00 [0.00-0.00] |

[i] Knowledge of HCV was assessed by the following questions: 1) Have you ever heard of Hepatitis C Virus or HCV? 2) Do you know how the hepatitis C virus is transmitted? 3) Can Hepatitis C virus be transmitted through: droplets (coughing, sneezing, respiratory secretions)? Food? Blood? Sexual contact? Handshake with an infected person? Sharing household objects like razors or toothbrushes? Sharing needles or syringes? touching items in public places (doorknobs, handles in transport, unhygienic toilets)? 4) Are there any other ways Hepatitis C virus can be transmitted that I haven't mentioned? Please specify. 5) Next I am going to ask you a few questions about the disease that is caused by hepatitis C virus. Please answer based on what you already know. Is it possible to have the hepatitis C virus but not have any symptoms? 6) Are there medications available to treat Hepatitis C virus infections? 7) Do you know how the hepatitis C can be prevented? 8) Can Hepatitis C virus infection be prevented by: getting a vaccine? using a condom? not sharing needles or syringes with other people? washing your hands thoroughly? using sterile or unused medical devices? 9) Are there other ways to prevent Hepatitis C virus infection that I haven't mentioned? Please specify.

Discussion

This HCV seroprevalence survey is the first of its kind in size and rigor in Cambodia. In this survey we found that HCV prevalence is higher in people 45 years and older (with prevalence increasing with older age) and among those with less education, that there is sometimes wide geographic variability in HCV estimates, and that HCV disease is poorly understood even among those who are seropositive.

The prevalence rates in this study were similar to estimates from some studies conducted in the country, but substantially different from others. The 2.6% prevalence seen in this population was far lower than the 14.7% seen in blood donors in 2009, and was half of the rates (5.8% and 5.2%) seen in observational studies of people living with HIV (PLHIV) (van Griensven J, 2014 Feb 12) (De Weggheleire A, 2017) (Yamada H, 2015 Apr). The only other survey of a general population cohort, from Siem Reap in 2012 (across only three northern villages; n=483), found double (5.2%) the seroprevalence of this cohort, though our survey confirmed several findings of that survey as well (higher prevalence in older cohorts, geographic variability in seroprevalence, similar viraemic prevalence) (S, 2019). Notably, our results diverge from that smaller survey in the gender differences in seropositivity risk that we found, and the fact that the Siem Reap survey associated blood transfusions and surgical history with a higher risk of viremia, while ours associated these factors with seroprevalence.

Surveys in nearby Thailand have similarly found a wide range of HCV prevalence among different regions and groups. A recent large-scale survey of the general population in Phetchabun of adults aged 35-64 found 6.9% anti-HCV positive (Wasitthanasem R, 2020), though an earlier study found a 15.5% anti-HCV positivity rate in Phetchabun, compared to 3.6% in neighboring Khon Kaen Province (Wasitthanasem R V. P., 2017). More similarly to our results among older adults (but dissimilarly as it was among a sub-group), a 5.5% HCV seroprevalence was identified among HIV cohorts in Cambodia in a systematic review and meta-analysis (Martinello M, 2016).

Predictably, HCV seroprevalence was especially linked (among those 45 years and older) to routine medical care (dental work), and certain medical procedures that occurred prior to 1990 and 1980: injections, surgery, blood donation and transfusion. Though these findings may speak to historic trends, the fact that the study cannot determine when transmission occurred in this population means that it is critical to generally reinforce infection prevention and control (IPC) measures in healthcare facilities and among health care staff at all levels.

Furthermore, programs for HCV screening and treatment should particularly consider older populations. Systematic testing of those aged 45 years and older, and treatment for the viraemic positives would contribute to the goal of HCV elimination in Cambodia and could substantially reduce the existing reservoir of HCV in the general population. Increasing overall awareness of HCV in the general population through information, education and communication (IEC) will also be a critical component of decreasing transmission and prevalence.

There have been many obstacles on the road to HCV elimination. For many years, effective drugs were lacking; once effective and safer DAA emerged, their cost was prohibitive. Now, simplified care models make HCV interventions more efficient and able to treat more people more cost effectively. Establishing baseline

prevalence rates and identifying groups at highest risk for seropositivity using statistically rigorous methods like the ones described here provides the necessary evidence for HCV elimination programs.

The results of this study are sufficiently representative, demographically and geographically, to provide policy-relevant guidance on HCV screening strategies, treatment and elimination in Cambodia, and to contribute to global conversations on HCV epidemiology, treatment and disease reduction or elimination (S, 2019).

Limitations

There are several limitations to this study. There were not enough seropositive participants under 45 years to conduct a meaningful analysis of risk factors for infection in this age group, and children were not included. The timing of initial HCV infection cannot be identified by either seropositivity or viral load and therefore it was not possible to describe historical HCV epidemiology from this survey.

Moreover, the survey is not representative of all Battambang Province or of other regions in Cambodia, and the surveyed geographic area may have had unique characteristics (such as a low distance [$<20\text{km}$] from most health centers to the hospital or largest town) and should not be considered representative of other regions. Care should be taken when interpreting results, though these findings point to important potential infection trends and are the most robust rural HCV estimates in the country to date.

Conclusion

This study provides the first large-scale general adult population prevalence data on HCV infection in Cambodia. The primary conclusions fill gaps in the understanding of HCV epidemiology in Cambodia with more precision and power than currently existing data. The results show high prevalence in adults age 45 years and above and confirm the link between high prevalence and increasing age, lower socio-economic status and past routine medical interventions (particularly those received before 1990 and 1980). This survey serves as an alert to the potentially high prevalence of HCV infection in Cambodia and can be used to guide national and local HCV policy discussion.

Abbreviations

| | |
|-----|---|
| CI | Confidence interval |
| DAA | Directly Acting Antivirals (drug) |
| ENA | Emergency Nutrition Assessment software |
| ERB | Ethical Review Board |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C Virus |
| HIV | Human Immunodeficiency Virus |

| | |
|-------|---|
| IEC | Information Education and Communication |
| IPC | Infection Prevention Control |
| MOH | Ministry of Health |
| MSF | Médecins Sans Frontières |
| NECHR | National Ethics Committee for Health Research |
| OR | Odds ratio |
| PHD | Provincial Health Department |
| PLHIV | People living with HIV/AIDS |

Declarations

Ethics approval and consent to participate

This study has received ethical approval from the Médecins Sans Frontières Ethical Review Board (ID: 1816), as well as the Cambodian National Ethics Committee for Health Research (NECHR; 23 February 2018 NECHR meeting minutes).

Consent for publication

No individually identifying data is included in this publication.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by Médecins Sans Frontières-France and Unitaid.

Authors' contributions

EL and HH designed the study protocol, advised by KP and DM. MLP, JPD, TMand PTB added program and local context to the protocol design. GF and CS implemented the study in Battambang, with remote support from EL. GF and CS coordinated the data collection and cleaning and GF performed the data analysis. EL and GF (results and methods) drafted the manuscript. All authors reviewed the manuscript.

Acknowledgements

The study team first and foremost thanks the study participants for their contributions that made this research possible. We especially thank the Ministry of Health of Cambodia (notably, the Bureau of Communicable Disease Control), the Battambang Provincial Health Department, and the Health Operational District of Moug Ruessei for their collaboration and support of the study from start to completion. We are very grateful for the funding support from Unitaid and Médecins Sans Frontières, France, and the comprehensive support and enthusiasm of the MSF teams in Phnom Penh and in Moug Ruessei.

In memoriam: Madam Sok Kimleng, survey nurse and previous MSF staff.

References

1. Boyd A, D. L. (2018). Research Gaps in Viral Hepatitis. . *Journal of the International AIDS Society.*, 21.
2. De Weggheleire A, A. S. (2017). A cross-sectional study of hepatitis C among people living with HIV in Cambodia: Prevalence, risk factors, and potential for targeted screening. *PloS One.* 2017, 12(8):e0183530.
3. Iwamoto M, D. J. (2018). Identifying optimal care for hepatitis C and overcoming barriers to scale-up: MSF pilot programme. London: Médecins Sans Frontières UK Scientific Day: Innovation 2018.
4. Labs, A. (n.d.). SD Biotec HCV Rapid Diagnostic Test: 02FK10. South Korea.
5. Martinello M, A. J. (2016). Prevalence and Disease Burden of HCV Coinfection in HIV Cohorts in the Asia Pacific Region: A Systematic Review and Meta-Analysis. . *AIDS Rev.* 2016, 18(2):68-80.
6. Ol HS, B. B. (2009 Sep). Prevalence of hepatitis B and hepatitis C virus infections in potential blood donors in rural Cambodia. *Southeast Asian J Trop Med Public Health*, 40(5).
7. Rosenthal, E., & Graham, C. (2016). Price and affordability of direct-acting antiviral regimens for hepatitis C virus in the United States. *Infectious agents and cancer*, 1(1), 24.
8. S, W. (2019). How feasible is the global elimination of HCV infection? *The Lancet*, 393(10178):1265-7.
9. van Griensven J, P. L. (2014 Feb 12). Hepatitis B and C co-infection among HIV-infected adults while on antiretroviral treatment: long-term survival, CD4 cell count recovery and antiretroviral toxicity in Cambodia. *PLoS On.* *PLoS One*, 9(2):e88552.
10. Wasitthanasem R, P. N. (2020). Prevalence of Hepatitis C Virus in an Endemic Area of Thailand: Burden Assessment toward HCV Elimination . [*published online ahead of print, 2020 May 11*]. *Am J Trop Med Hyg.* , 10.4269/ajtmh.19-0817. doi:10.

11. Wasitthanasem R, V. P. (2017). Assessment of hepatitis C virus infection in two adjacent Thai provinces with drastically different seroprevalence. *PLoS One*, 12(5):e0177022. Published 2017 May 5. doi:10.1371/journal.pone.0177022.
12. Weggheleire A De, A. S. (2017). A cross-sectional study of hepatitis C among people living with HIV in Cambodia : Prevalence , risk factors , and potential for targeted screening. *PLOS ONE*, 1–15.
13. WHO. (2016, April). *Guidelines for the Screening Care and Treatment of Persons with Chronic Hepatitis C Infection. Updated Version*. Retrieved from World Health Organization.: <http://www.who.int/hepatitis/publications/hepatitis-c-guidelines-2016/en/>
14. WHO. (2017). *Global Hepatitis Programme*. Retrieved from Global Hepatitis Report.
15. World Health Organization, Global Hepatitis Programme. (2017). *Global hepatitis report, 2017*. Retrieved Nov 26, 2019, from <http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1>
16. Yamada H, F. M. (2015 Apr). Seroprevalence, genotypic distribucon and potential risk factors of hepatitis B and C virus infections among adults in Siem Reap, Cambodia. *Hepatol Res.*, 45(4):480-7.

Figures

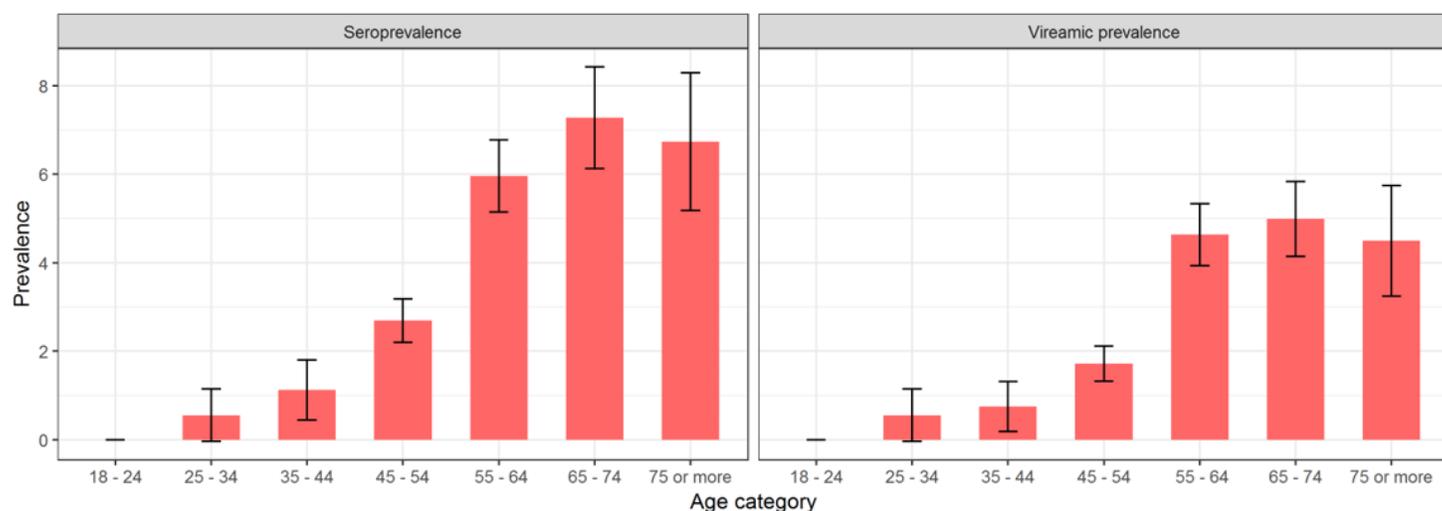


Figure 1

HCV seroprevalence and viraemic prevalence by age categories with 95% confidence intervals.

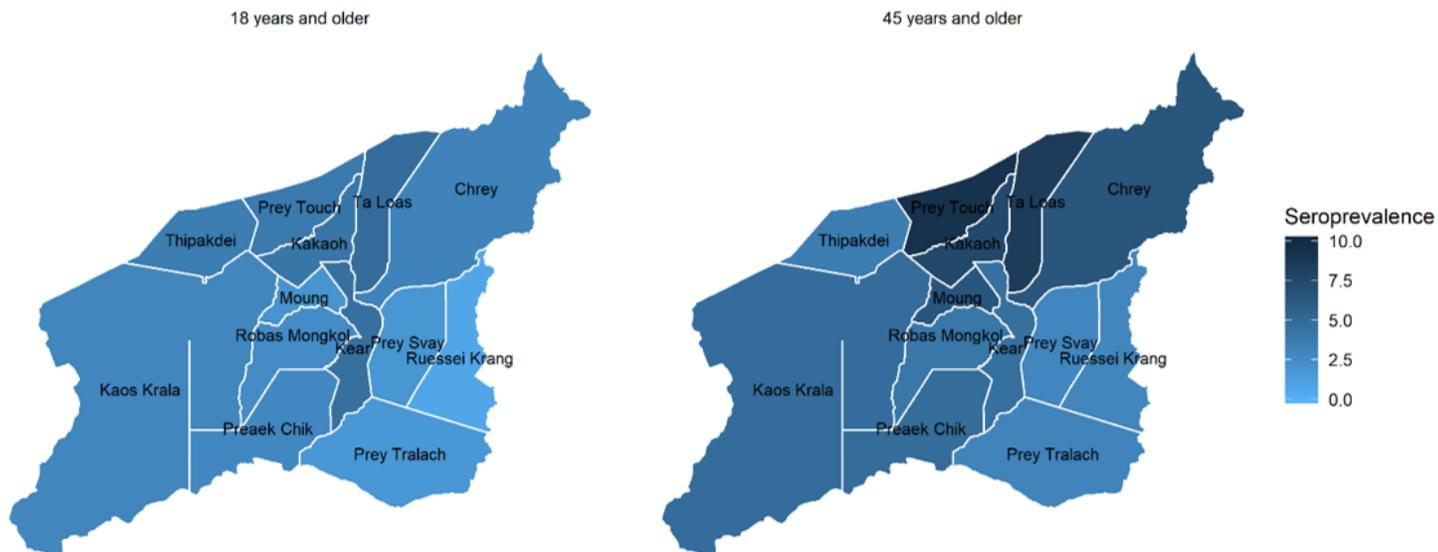


Figure 2

HCV serological prevalence by health center catchment area.