Seroprevalence of hepatitis E virus (HEV) in a general adult population in Northern Norway: the Tromsø study

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Abstract

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis in many parts of the world but only a few cases have been diagnosed in Norway. To investigate the HEV exposure rate in a presumed low-risk area, we have conducted a population-based study of anti-HEV IgG seroprevalence in Northern Norway. A total of 1800 serum samples from 900 women and 900 men, age 40-79 years, were randomly selected from the 21083 participants in the 7th Tromsø Study, representing the 32591 inhabitants of the Tromsø municipality that were ≥40 years. All samples were analyzed by ELISA-1 (recomWell HEV IgG). Samples testing positive or borderline, as well as a 1.5-fold excess of negative samples, were retested by ELISA-2 (DiaPro HEV IgG). If still borderline or a result discordant from ELISA-1, the sample was retested by ELISA-3 (Wantai HEV IgG) and strip-immunoassay (recomLine HEV IgG). Anti-HEV IgG was detected in 205 individuals (11.4%), yielding an estimated seroprevalence of 10.4% in the age-matched population of Tromsø. Using logistic regression analysis followed by multivariable backward elimination analysis, increasing age (OR 1.036; $p<0.001$) and higher education (OR 2.167; $p<0.001$) were found as potential risk factors, whereas travel abroad or eating of red meat was not. Our results indicate that HEV-infection is common in Northern Norway and suggest that HEV testing should be included in the evaluation of elevated liver enzymes.

Keywords

hepatitis E virus

Immunoglobulin G

seroepidemiologic study

Enzyme-linked immunosorbent assay

hepatitis

Introduction

Hepatitis E virus (HEV) infection is a major cause of acute viral hepatitis worldwide (1-3). HEV infection may cause acute liver failure in pregnant women and patients with preexisting liver disease, as well as chronic hepatitis with rapid progression of liver fibrosis in immunocompromised patients (4). In addition, neurological manifestations, kidney injury, pancreatitis and hematological disorders have been reported (5, 6). However, most people infected by HEV are believed to have an asymptomatic self-limiting course (7).

HEV is a non-enveloped, single-stranded, positive sense RNA virus that belongs to the *Hepeviridae* family. Based on phylogenetic analysis of many HEV isolates, seven genotypes (GT) have been found in mammals, all belonging to a single serotype (8). Five of the genotypes have been shown to infect humans in different geographical locations and by different modes of transmission. HEV GT1 and GT2 are highly endemic in Asia, Africa, the Middle East and Mexico where they cause large outbreaks due to fecal-oral transmission from contaminated drinking water. HEV GT7 has been found in camels in the Middle East and was found to infect one person regularly consuming camel milk and meat (9). In Europe, HEV GT3 is the most prevalent genotype, followed by GT4. Both genotypes can infect animals such as domestic pigs, wild boar, and deer, and for GT3 also rabbits, and are sporadically transmitted to humans through direct contact with infected animals or indirectly by consumption of undercooked HEV-containing animal products (8). Transmission may also occur through consumption of shellfish, and contaminated berries and vegetables (10-12). HEV can be transmitted through blood and blood products, through solid organ transplantation, and by vertical transmission from mother to fetus. Recently, the United Kingdom, Ireland, France, Japan and the Netherlands have implemented universal, targeted or partial HEV screening of donor blood to avoid HEV transmission by blood transfusion (3).

Anti-HEV IgG seroprevalence and incidence of hepatitis E varies both between and within countries. In Europe, seroprevalence rates ranging from 0.035% to 60.9% have been reported (13, 14) with “hot-spots” in southwest France, the Netherlands, Scotland, western Germany, Czech Republic, central Italy and western/central Poland (3). There is limited knowledge about HEV in Norway. One study, investigating 1200 blood donors, 79 swine farm workers and 153 pig herds (15) found anti-HEV IgG antibodies in 14% of the blood donors, 30% of the swine farm workers and in 90% of the investigated pig herds. There is also one case report on acute HEV-infection in a 20-year young Norwegian man with no recent travel history (16).

The aim of this study was to determine the exposure to HEV in a presumably low-risk adult population in Northern Norway. Since commercially available serological immunoassays have been reported to vary in sensitivity and specificity (17-19), a testing, retesting and confirmation strategy was defined using four different immunoassays in accredited collaborating laboratories.

Materials and Methods

Study population and samples

The 7th Tromsø Study (Tromsø 7) is the latest of a series of population-based surveys since 1974 in the municipality of Tromsø in Northern Norway (20) and was carried out in 2015-16. Residents of age 40 and above were all invited, and 21 083 women and men (64.7% of eligible subjects) participated (**Table 1**). The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Ethical permission was obtained from the Regional Committee for Medical Research Ethics – South East Norway. Informed consent was obtained from all individual participants included in the study. To ensure a representative study population, we included serum samples from 1800 randomly selected participants, age 40-79 years, consisting of 900 women and 900 men in four age strata of equal size (**Table 2**). All samples were kept frozen at -20°C until tested for anti-HEV IgG in Tromsø. The samples were aliquoted in two additional tubes for retesting in collaborating labs.

Immunoassays for anti-HEV IgG detection

For detection of anti-HEV IgG, four commercially available HEV immunoassays were used in three different labs (**Table 3**), all according to the manufacturer´s instructions. RecomWell HEV IgG enzyme-linked immunosorbent assay (ELISA) was selected for the first test of all 1800 sera and this test is from now on denoted ELISA-1. For retesting of all ELISA-1 IgG positive or borderline samples and 1.5-times the number of ELISA-1 non-reactive samples, DiaPro HEV IgG ELISA (ELISA-2) was used. All samples with borderline result or discordant result between ELISA-1 and ELISA-2 were retested by Wantai HEV IgG ELISA (ELISA-3) and RecomLine IgG (strip-immunoassay). For ELISA-1 the antibody activity in units per mL (U/mL) was calculated by dividing the extinction value of the sample by the mean of the extinction values of the cutoff and multiplying this by 20, while for ELISA-2 and ELISA-3 antibody activity was calculated by dividing the extinction value of the sample by the mean of the extinction values of the cutoff (S/Co).

The strip-immunoassay consists of nitrocellulose membrane test-strips with seven purified recombinant HEV GT1 and GT3 antigens from open reading frame 2 (ORF2), encoding the viral capsid protein, and open reading frame 3 (OFR3), encoding a multifunctional protein (O2N: N-terminal part of ORF2 protein GT1 and GT3; O2M: middle part of ORF2 GT1 and GT3; O2C: C-terminal part of ORF2 protein GT1 and GT3 and O3: the full length ORF3

GT1). Antibodies to the different antigens were present if the respective band was of an intensity equal to or stronger than the intensity of the reference (cutoff) band. As defined by the manufacturer, the different bands gave scores from one to four and a total score of ≥4 defined the sample as positive. Of note, an O2C band gives a score of four.

Statistics

To measure inter-rater agreement between different assays, Weighted Kappa statistics was used (https://www.graphpad.com/quickcalcs/kappa1.cfm). Due to the binary outcome (anti-HEV IgG positive or anti-HEV IgG negative), logistic regression (IBM SPSS version 24) was used to look at associations between a positive anti-HEV IgG result and potential risk factors from the self-reported questionnaires in Tromsø 7. Age, body-mass index (BMI), traveling for more than one week outside the Nordic countries during the last 12 months, the number of times of eating moose meat per year, and the number of times of eating reindeer meat per year were used as continuous variables. The level of education, the household income, smoking habits, diabetes, gender, alcohol habits and eating of red meat were entered as categorical variables. Recoding of some original groupings were necessary due to small groups. Age-adjusted logistic regression was performed for each of the variables and variables with a p-value <0.25 were selected as candidates for further multivariable analysis. All candidate variables were included in the multivariable model and the variable with the least significant p-value was omitted. This backward elimination process with a re-fitted model at each step was repeated until all variables were statistically significant. The inclusion in the final model was based on a significance level of 0.05. The predicated probability of a positive test was calculated by transforming the regression coefficients from the log odds scale back to the probability scale. The probability plot was made using R/R-studio version 3.5.1.

Results

Selection of representative serum samples for the anti-HEV IgG seroprevalence study

At the time of planning and initiation of this study, there was no data available on anti-HEV IgG seroprevalence in Norway. In order to select a representative adult population in Tromsø, we assumed a minimum of 2% seroprevalence and included 1800 individuals (**Table 1**). Since 96.4% of the study participants of Tromsø 7 were 40 to 79 years old, we randomly selected 225 women and 225 men in each of the following age groups: 40 - 49 years, 50 - 59 years, 60 - 69 years and 70 - 79 years (**Table 2**). Comparing the demographics of the HEV study population and the entire Tromsø 7 revealed no significant differences in age, except for the age group 70 - 79 years that was overrepresented in our study.

Anti-HEV IgG activity in 1800 serum samples using ELISA-1

All 1800 serum samples were first tested by ELISA-1. The result showed 198 (11%) anti-HEV IgG positive samples (>24.0 U/mL), 1589 (88.3%) anti-HEV IgG negative samples (<20.0 U/mL), and 13 (0.7%) undetermined samples due to borderline results (20 - 24 U/mL) (**Fig. 1a**). The frequency histogram showed that the IgG positive samples ranged from 24.1 to 78.7 U/mL, giving one peak around 28 - 32 U/mL and one smaller peak around 64 - 68 U/mL (**Fig. 1b**). No correlation between anti-HEV IgG activity and age or gender was found (data not shown). Looking at the negative samples, 1557 samples (98%) had an IgG level below 8.0 U/mL Thus, ELISA-1 indicated an anti-HEV IgG seroprevalence of 11.0% in the study population.

Comparison of anti-HEV IgG activities of 513 samples using ELISA-1 and ELISA-2

The serum samples having an ELISA-1 positive results (n=198) or borderline (n=13) results were retested by ELISA-2. In addition, 302 ELISA-2 negative samples were retested, which included all 13 samples with a result between 10 and 19.9 U/mL. Of totally 513 retested serum samples, ELISA-2 yielded 178 positive samples (S/Co > 1.1), 319 negative samples (S/Co < 0.9), and 16 samples with borderline result (S/Co 0.9 - 1.1). Thus, the results of ELISA-1 and ELISA-2 were concordant for 476 samples (92.8%), but discordant for 37 samples (7.2%) **(Supplementary Table 1)**. The concordant results consisted of 174 anti-HEV IgG positive (36.6%), 5 borderline (1.0%), and 297 negative (62.4%) samples, while the discordant results consisted of 18 fully discordant (e.g. positive vs. negative), and 19 partial discordant samples (i.e. being positive or negative in one, and borderline in the other test). Based on these numbers, a weighted Kappa of 0.886 was calculated indicating a very good qualitative agreement between ELISA-1 and ELISA-2.

To compare the quantitative results of ELISA-1 and ELISA-2, the results of ELISA-1 were plotted on the X-axis (U/mL) and the results of ELISA-2 (S/Co) were plotted on the Y-axis (**Fig. 2a)**. For all samples with concordant positive (red dots), negative (green dots) and borderline result (yellow dots), the anti-HEV IgG activity in ELISA-1 appeared to correlate with the activity measured by ELISA-2. Moreover, all 24 fully or partly discordant samples that were positive in ELISA-1, showed relatively low IgG levels (mean values of 28.6 U/mL) compared to the mean value of all 198 ELISA-1 positive samples (47.3 U/mL). In contrast, two of the three fully discordant samples that were only positive in ELISA-2, had high IgG levels (mean value of 3.84 S/Co) compared to the mean value of all 178 ELISA-2 positive samples (3.62 S/Co). Thus, with the exception of these two samples, a good agreement between the quantitative results of ELISA-1 and ELISA-2 was found. Restricting the seroprevalence rate to only the concordant ELISA-1/ELISA-2 positive samples (n=174), the minimum estimate of anti-HEV IgG-positivity was 9.7%.

Testing of indeterminate and discordant sera by ELISA-3 and strip-immunoassay

To better define the HEV IgG status of the five samples with concordant borderline results and the 37 discordant samples, retesting with ELISA-3 and the strip-immunoassay was done. Samples were scored positive if positive in at least two of the four assays used. As summarized in **Fig. 2b**, the five samples with a concordant borderline result were positive in both ELISA-3 and the strip-immunoassay and hence defined as positive. Of the 18 fully discordant sera, the 15 ELISA-1 positive sera were all defined as positive since they were positive by the strip-immunoassay (14 were positive by ELISA-3), while the three ELISA-2 positive sera were all defined as negative since they were negative by both ELISA-3 and the strip-immunoassay.

The 10 partial discordant samples that were positive by ELISA-1 or ELISA-2, were all found to be positive as they were positive by ELISA-3 (eight were positive by strip-immunoassay). In addition, one sera borderline by ELISA-1 and negative by ELISA-2 was found to be positive by both ELISA-3 and strip-immunoassay (**Fig. 2b**). Based on the confirmatory testing by ELISA-3 and strip-immunoassay, 31 additional individuals were judged as being anti-HEV IgG positive. This increased the number of HEV-exposed individuals to 205 (11.4%) consisting of 98 (10.9%) women and 107 (11.9%) men. Compared to the first testing by ELISA-1, the seroprevalence in the study population increased by 0.4% from 11.0% to 11.4%. Of note, 27 of 29 samples (93%) testing positive by strip-immunoassay, had antibodies only against O2C. All O2C positive samples had a strong GT3 band and a weak

GT1 band, with the exception of one sample giving equally strong GT1 and GT3 bands. Moreover, two of the five samples with a slightly weaker O2C band than the cutoff, were found to be positive due to positive ELISA results.

Anti-HEV IgG seroprevalence in the age-matched population of Tromsø

In order to estimate the anti-HEV IgG seroprevalence in the age-matched population of Tromsø, the seroprevalence was calculated considering the number of residents in each age group (**Table 1**). This gave an average seroprevalence of 10.4%.

Potential risk factors for a positive anti-HEV IgG activity

Twelve potential risk factors for anti-HEV IgG were identified in the survey questionnaire of Tromsø 7 and answers registered (**Supplementary Table 2**). An age-adjusted logistic regression analysis was performed for each of these variables (**Table 4**). Six factors were found to have a p-value below 0.25 and were included in a backward multivariable regression analysis. The only factors remaining in the final adjusted model were increasing age and education for ≥4 years in college or university (**Table 5**). The predicted probability for a positive anti-HEV IgG test translated into a smooth, almost linear increase with age (**Fig. 3a**). Thus, the odds of having a positive anti-HEV IgG test, increases by a factor of 1.036 for each additional year above 40 years of age (95% CI: 1.021-1.051, $p<0.001$). In addition, the odds of a positive anti-HEV IgG test was 2.167 times higher in the highest education category compared to the lowest education category (95% CI: 1.415-3.319, $p<0.001$). The predicted probability for a positive anti-HEV IgG test shows a steeper increase with increasing age for those with more than 4 years of college or university compared to those with only primary school (**Fig. 3b**). In conclusion, only increased age and higher education were found to be factors associated with a positive anti-HEV-IgG test result.

Discussion

In this population-based study, the anti-HEV IgG seroprevalence of adults in a presumably low-risk geographic area in Northern Norway was examined. By analyzing 1800 serum samples from participants age 40-79 years in the Tromsø 7 study, the anti-HEV IgG seroprevalence was determined to be 10.4% in the age-matched population of Tromsø municipality. No significant difference in seroprevalence was seen between woman and men. When analyzing 12 potential risk factors obtained from the self-reported questionnaires (return rate >90%) using logistic regression analysis followed by multivariable backward elimination analysis, only increasing age (OR of 1.036 per year; 95% CI: 1.021-1.051, p<0.001) and higher education (OR 2.167; 95% CI: 1.415-3.319, p<0.001) were found to be significantly associated with anti-HEV IgG.

The anti-HEV IgG seroprevalence of 10.4% is surprisingly high but in the lower range of the ones previously reported from other European countries. A meta-analysis including 73 European studies conducted between 2003 and 2015 concluded that HEV seroprevalence varied between and within countries, depended on the assay used, was higher in individuals exposed to pigs and/or wild animals and increased with age (21). In agreement with the geographic variation described within countries, we found a lower seroprevalence than the 14.0% reported in the study of 1200 Norwegian blood donors mainly from South Norway (15). This difference can not be explained by age, as the median age of the blood donors was 45 years (range from 18 to 83 years, data available from only 999 blood donors), compared to the median age of 59.3 years for the participants in our study. The seroprevalence for blood donors above 50 years was about twice as high as what we found for the similar age groups in our study (51-60 years: 22% and 61-83 years: 28% versus 50-59 years: 10.4%; 60-69 years: 13.3% and 70-79 years:15.1%) arguing for a higher exposure rate with increasing age in South Norway. Although we cannot exclude that the difference is at least in part due to the use of different assays, we suspect that many of the blood donors were from areas where industrial agriculture and pig farming is more common than in Northern Norway.

Given the reported difference in sensitivity of different serology assays and the absence of gold standards defining false-positive results (specificity), we developed a careful testing, retesting and confirmation strategy with up to four different immunoassays, which were performed in different accredited laboratories. Scoring samples positive only if at least two of the four assays were reactive, 205 participants were anti-HEV-positive yielding an anti-HEV seroprevalence of 11.4% in the examined population. Using a stricter definition scoring fully discordant samples positive only if both confirmatory assays were reactive, 196 participants were anti-HEV-positive yielding a

seroprevalence of 10.9%, which is not significantly different than 11.4% (p=0.63). Although the strip-immunoassay contains seven recombinant HEV antigens from four different epitopes, according to the manufacturer it is sufficient to have a single O2C band (GT1 or GT3) above the reference intensity for the test to be positive. As detailed in the results, all 29 positive samples had antibodies against O2C, and only two of these samples had antibodies against other antigens, thereby questioning the added value of these antigens. Others reported similar findings when using the previous version of the assay (18). Moreover, it can be questioned whether the manufacturer's scoring system is too strict since two samples with an O2C band slightly weaker than the reference band get the score zero but are positive by ELISA. Unfortunately, the strip-immunoassay using antigens from GT1 and GT3 can not be used for genotyping, probably since both genotypes belong to the same serotype.

Although symptomatic acute HEV-infection is reported to mainly affect males above 50 years (22) we found no association between anti-HEV IgG positivity and gender. Similar results have been found in other studies (23, 24), indicating that men are not more frequently infected than women but may be more likely to become symptomatic. Whether or not this is due to gender differences or hepatotoxic insults remains to be examined. We did not find an association between seropositivity and diabetes, BMI, smoking, alcohol consumption, travelling outside the Nordic countries for more than one week the last 12 months, household income or eating of meat. Since a high frequency of HEV with a high genetic similarity to GT3 and GT4 has been reported in moose in Sweden (25), another Scandinavian country, and since people in the arctic region eat moose- and reindeer meat, we looked for an association with yearly intake of these. However, no association with anti-HEV seropositivity was found. Since moose meat is not readily available in markets, intake of moose meat can be a surrogate marker of hunting big game. In other European regions, hunters have an increased risk of HEV infection (26-28). This does not seem to be the case in Tromsø municipality, probably since wild boar and deer are not part of the fauna.

The two independent risk factors found in our population-based study were increasing age and having more than four years of college or university education. The odds of a positive anti-HEV IgG test increased by 3.6% for each additional year above 40. An increased seroprevalence with increasing age has also been found in several other studies (24, 29, 30). Of note, the levels of the anti-HEV IgG titers (**Fig. 1b**) did not decrease with increasing age as has been reported for some viruses such as BK polyomavirus (31, 32). This suggests that the cumulative increase in HEV IgG seropositivity may be due to infection/re-infection throughout life, even in elderly with a more senescent immune response. However, the detailed long-term dynamic of naturally acquired anti-HEV IgG is

largely unknown (reviewed in (33)), and should be investigated using consecutive samples from patients with acute HEV-infection. The finding that people with higher education were more frequently anti-HEV IgG positive than people with less education is in contradiction to a study from the Netherlands (34). One explanation could be that many people with higher education living in Tromsø may originate from other areas inside or outside Norway where HEV is more prevalent and have moved to Tromsø due to studies or work at the local university or university hospital. Alternatively, this may reflect yet undefined food or travel habits. Unfortunately the Tromsø 7 study did not include participants below 40 years of age, which would have enabled a more comprehensive study of the population of Tromsø.

Autochthonous cases of HEV in Western European countries are mainly caused by HEV GT3 and some small outbreaks have been directly linked to consumption of undercooked or raw pork products or shellfish [35, 36]. GT3 was probably also the cause of infection in most of our seropositive participants, but this would be difficult to prove due to the normally very short viraemic phase and the lack of a genotype-specific serology test. Although the majority of the participants (72.5%) did report eating meat weekly, this was not found to be a risk factor for anti-HEV IgG seropositivity. This as opposed to a study from the Netherlands reporting significantly higher seroprevalence among blood donors consuming meat on daily basis compared to vegetarian donors [37]. Although extensive, the Tromsø 7 questionnaire did not distinguish between eating beef, mutton or pork and did not identify exclusive vegetarians, which may have affected the outcome. In 2016 the red meat consumption per Norwegian inhabitant was calculated to be 51 kg per year and about half of this was pork meat (38). Although the consumption of red meat has increased by about 15% the last 30 years, it is still lower than reported from Sweden, Finland, Denmark, France, Finland, England and USA. In the municipality of Tromsø, there are no pig farms, wild boars, wild rabbits or deer. Together, this supports that Tromsø is a low-risk geographic area.

What could be the source explaining a 10% HEV-exposure rate among adult inhabitants of Tromsø? Some commonly suggested risk factors missing in the questionnaire were consumption of shellfish, received blood transfusion and transplantation. In addition, the consumption of agricultural products such as unpeeled fruits, berries, raw vegetables such as lettuce, should have been investigated. These food items have been suggested to be a source of HEV (10, 12, 39) and are frequently imported to Northern Norway. Recently, an anti-HEV IgG seroprevalence of 56.6% and 32.2% was found in dogs and cats in Germany (40). Since no viral RNA could be

isolated, the genotype is still not known. However, if HEV from dogs and cats can infect humans, this could very well be an important source of HEV infection in Tromsø.

In conclusion, this carefully performed population-based study indicates an anti-HEV IgG seroprevalence of 10.4% among adults in Tromsø municipality. The seroprevalence was similar for women and men and increased with increasing age. Despite attempts to better define the risk for HEV infection, the primary source of HEV infection if existing, is undefined. Our data justify to recommend HEV testing in all patients with elevated liver enzymes, which is not the present routine in most Norwegian hospitals.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure legends

Fig. 1. ELISA-1 anti-HEV IgG reactivity in 1800 participants of the population-based study Tromsø 7.

a. The results are presented as total number of samples with negative (white), borderline (grey) or positive (black) anti-HEV IgG detection.

b. A frequency histogram of the results divided in 4 U/mL intervals. The results are presented as negative (white), borderline (grey) or positive (black) anti-HEV IgG detection.

Fig. 2. Anti-HEV IgG reactivity in 513 selected samples from the population-based study Tromsø 7.

a. The qualitative and quantitative results of ELISA-1 and ELISA-2 are compared by plotting the results of ELISA-1 on the x-axis as U/mL and plotting the result of ELISA-2 on the Y-axis as S/Co. A polynomial trend-line gives a R-squared value of 0.7534. Concordant positive results are presented as red dots, concordant negative results are presented as green dots, and concordant borderline results are presented as yellow dots. Fully discordant results are shown as black dots while partly discordant samples are shown as blue dots.

b. A flowchart showing the result of ELISA-1, ELISA-2, ELISA-3 and strip-immunoassay. Samples with a borderline result (border n=5), a fully discordant result (n=18) or a partly discordant result (n=19) after ELISA-1 and ELISA-2, where resolved by the use of ELISA-3 and strip-immunoassay. The results of ELISA-1 and ELISA-2 are presented as positive (pos), borderline (border) and negative (neg) anti-HEV IgG detection. The results of ELISA-3 and the strip-immunoassay are shown in red circles for positive results, green squares for negative results and white triangles for borderline results. ELISA-3 and strip-immunoassay confirmed or detected 31 additional positive samples giving a total number of 205 positive samples.

Fig.3. Predicted probability of a positive anti-HEV IgG test using the data from 1800 participants of the population-based study Tromsø 7.

a. The predicated probability of a positive anti-HEV IgG test with increasing age (n=1800). The 95% confidence interval is also shown.

b. The predicated probability of a positive anti-HEV IgG test with increasing age for those with ≥4 years university or college education (n=498) compared with those with only primary school (n=453). The 95% confidence interval is also shown.

TABLE 1 Invited for Tromsø 7 study (T7) and participating in T7 and HEV study, respectively

	Total			Women			Men		
	Invited for T7	Participating in T7 (% of invited)	Participating in HEV study (% of participants in T7)	Invited for T7	Participating in T7 (% of invited)	Participating in HEV study (% of participants in T7)	Invited for T7	Participating in T7 (% of invited)	Participating in HEV study (% of participants in T7)
Residents (≥40 years)	32591[†]	21083 (64.7%)	1800 (8.5%)	16539	11074 (67.0%)	900 (8.1%)	16052	10009 (62.4%)	900 (9.0%)
Age 40 - 49	10757	6432 (59.8%)	450 (7.0%)	5195	3378 (65.0%)	225 (6.7%)	5562	3054 (54.9%)	225 (7.4%)
Age 50 - 59	8861	6035 (68.1%)	450 (7.5%)	4534	3245 (71.6%)	225 (6.9%)	4327	2790 (64.5%)	225 (8.1%)
Age 60 - 69	7129	5179 (72.6%)	450 (8.7%)	3586	2677 (74.7%)	225 (8.4%)	3543	2502 (70.6%)	225 (9.0%)
Age 70 - 79	3898	2676 (68.7%)	450 (16.8%)	2001	1361 (68.0%)	225 (16.5%)	1897	1315 (69.3%)	225 (17.1%)
Age 80 - 104	1946	761 (39.1%)	0 (0%)	1223	413 (33.8%)	0	723	348 (48.1%)	0

[†]Total population in Tromsø 01.01.2016 was 74541 residents. All residents ≥40 years were invited.

TABLE 2 Participants in HEV study by age and gender

	Participants	Gender		Mean age (years ± SD)				Median age			
						50 percentile		25 percentile		75 percentile	
	All	F†	M‡	F	M	F	M	F	M	F	M
Total	1800	900	900	59.3 (0.370)	59.3 (0.374)	59.5	59.5	49.3	49.3	69.8	69.8
Age 40 - 49	450	225	225	44.7 (0.187)	44.6 (0.177)	45.0	45.0	42.5	42.0	47.0	47.0
Age 50 - 59	450	225	225	54.6 (0.194)	54.5 (0.182)	55.0	55.0	52.0	52.0	57.0	57.0
Age 60 - 69	450	225	225	64.5 (0.197)	64.5 (0.190)	64.0	64.0	62.0	62.0	67.0	67.0
Age 70 - 79	450	225	225	73.3 (0.183)	73.7 (0.178)	73.0	73.0	71.0	71.0	75.0	76.0

†F=Female
‡M=Male

TABLE 3 Assays used for anti-HEV IgG detection

Assay	Producer	Antigen for coating	Serostatus interpretation
recomWell HEV IgG (indirect sandwich-ELISA)	Mikrogen Diagonostik[†]	Recombinant HEV ORF2 (Genotype 1 and 3)	Negative: <20 U/mL Borderline: 20–24 U/mL Positive: >24 U/mL
HEV IgG ELISA (indirect sandwich-ELISA)	Dia.Pro[‡]	Recombinant HEV antigens: Four synthetic peptides representing epitopes from ORF2 and ORF3 from the Burmese and Mexican HEV strains	Negative: S/Co < 0.9 Borderline: S/Co 0.9–1.1 Positive: S/Co >1.1
Wantai HEV IgG ELISA (indirect sandwich-ELISA)	Sanbio[§]	Recombinant HEV ORF2 C-terminal part (Genotype 1)	Negative: C.O.<0.9 Borderline: C.O.<0.9–1.1 Positive: C.O. >1.1
recomLine HEV IgG (strip-immunoassay)	Mikrogen Diagonostik[†]	Recombinant HEV antigens: ORF2 N-terminal part (Genotype 1 and 3), ORF2 C-terminal part (Genotype 1 and 3), ORF2 mid part (Genotype 1) and ORF3 (Genotype 1 and 3)	Negative: ≤2 Borderline: 3 Positive: ≥4

[†]Mikrogen GmbH, Neuried, Germany; [‡]Dia.Pro Diagnostic Bioprobes srl, Italy; [§]Sanbio B.V., Uden, The Netherlands.

TABLE 4 Age-adjusted logistic regression analysis for each variable

Variables	OR	95% CI	p-value
Age (per year)	1.027	1.013-1.041	<0.001‡
Gender			0.507
Female	Reference	-	
Male	1.104	0.824-1.479	
Diabetes			0.591
Never or previously	Reference	-	
Currently	0.829	0.418-1.645	
BMI†	1.028	1.962-1.029	0.761
Smoking			0.178‡
Never	Reference	-	
Previously	0.772	0.565-1.054	0.104
Currently	0.706	0.427-1.167	0.174
Eating red meat (beef, mutton, pork)			0.796
Never or rarely	Reference	-	
Weekly or more	0.958	0.692-1.327	
Travel outside Nordic countries last 12 months >1 week	1.104	0.984-1.238	0.091‡
Eating reindeer meat (times/year)	1.013	0.999-1.027	0.066‡
Eating moose meat (times/year)	1.003	0.989-1.017	0.645
Education			0.004‡
Primary school	Reference	-	
High school	1.468	0.950-2.267	0.084
<4 years in college/university	1.407	0.865-2.291	0.169
>=4 years in college/university	2.167	1.415-3.319	<0.001
Total yearly income in the household			0.019‡
<450.000 NOK	Reference	-	
451.000-750.000 NOK	1.657	1.094-2.510	0.017
>751.000 NOK	1.844	1.183-2.872	0.007
Alcohol consumption			0.792
Never	0.827	0.464-1.477	0.521
Monthly or less	0.788	0.537-1.157	0.224
2-4 times a month	Reference	-	
2-3 times a week	0.902	0.617-1.320	0.595
4 or more times a week	0.815	0.416-1.599	0.553

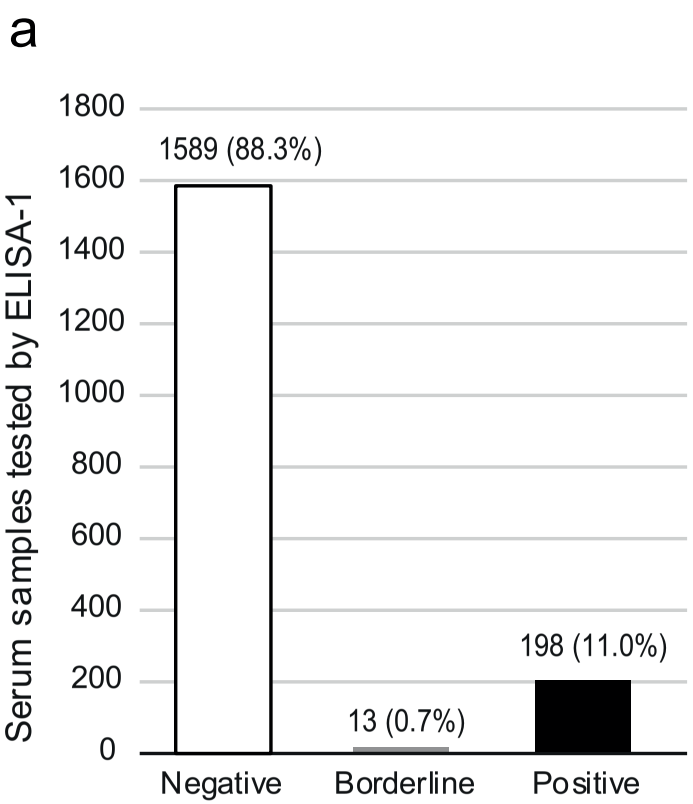
†BMI (Body-mass index, kg/m^2)

‡$p<0.25$

TABLE 5 Final results from backward stepwise regression

Variables	OR	95% CI	p-value
Age (per year)	1.036	1.021-1.051	<0.001*
Education			0.004
Primary school	Reference	-	
High school	1.468	0.950-2.267	0.084
<4 years in college/university	1.407	0.865-2.291	0.169
>=4 years in college/university	2.167	1.415-3.319	<0.001*

*p<0.001



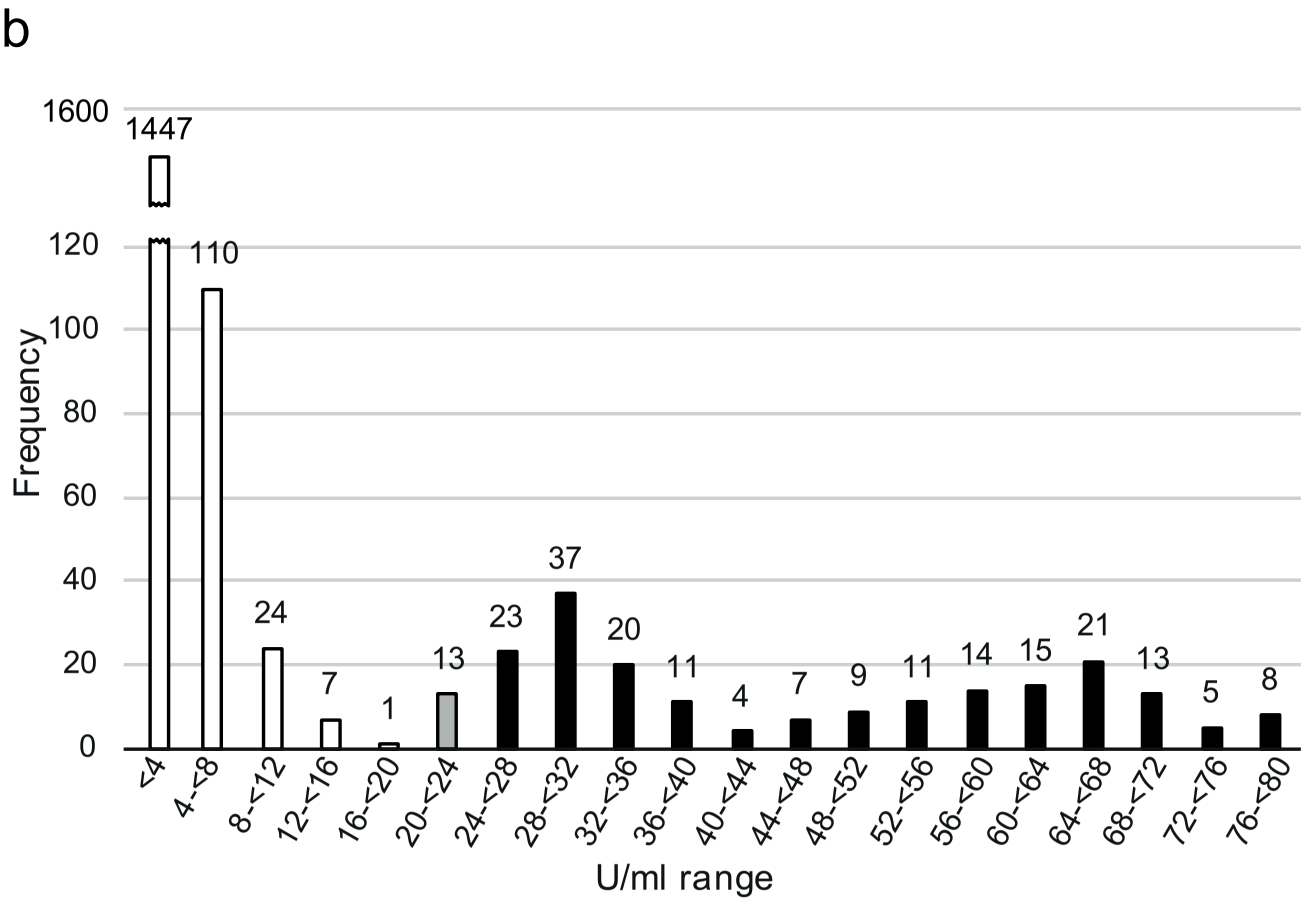
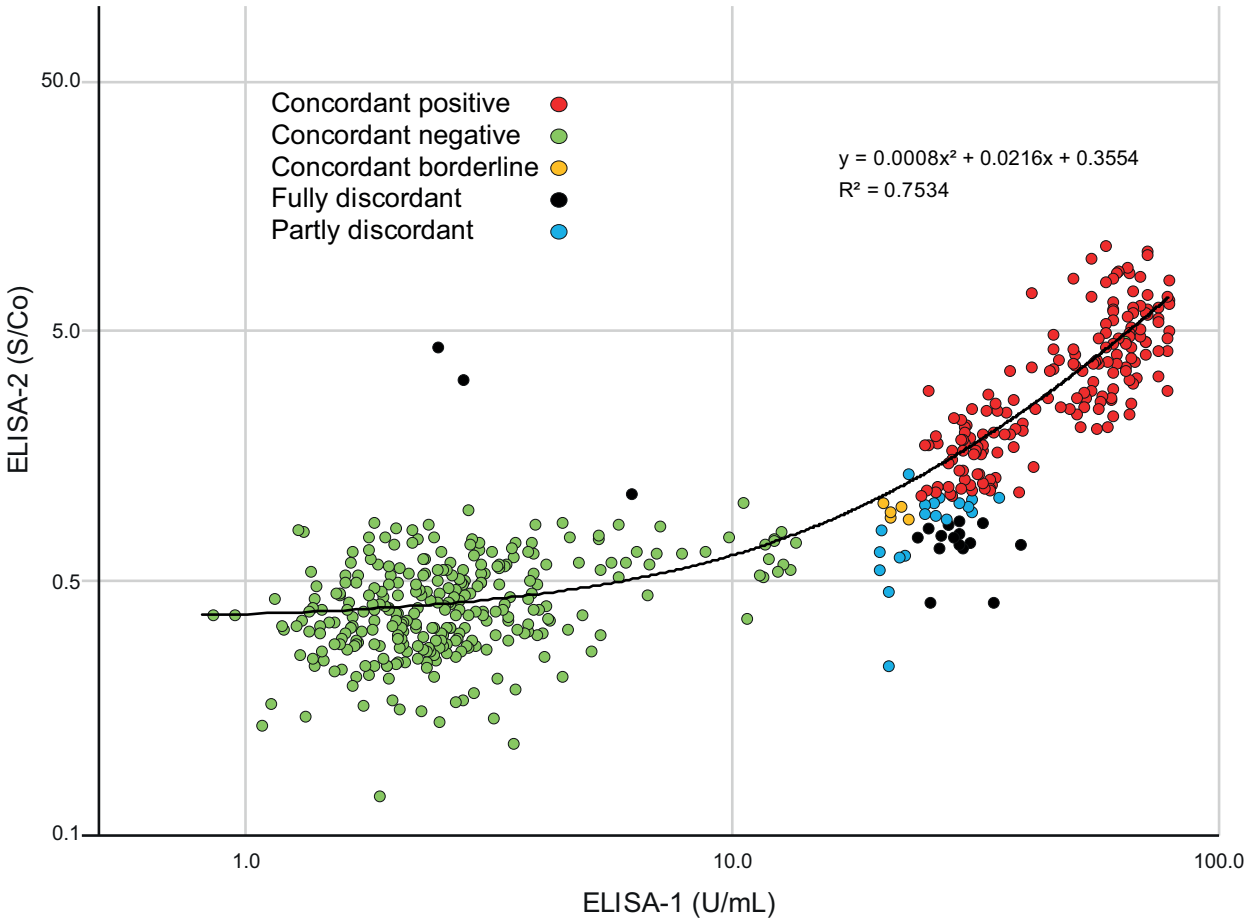


Figure 1a and b

Figure 2a and b

a



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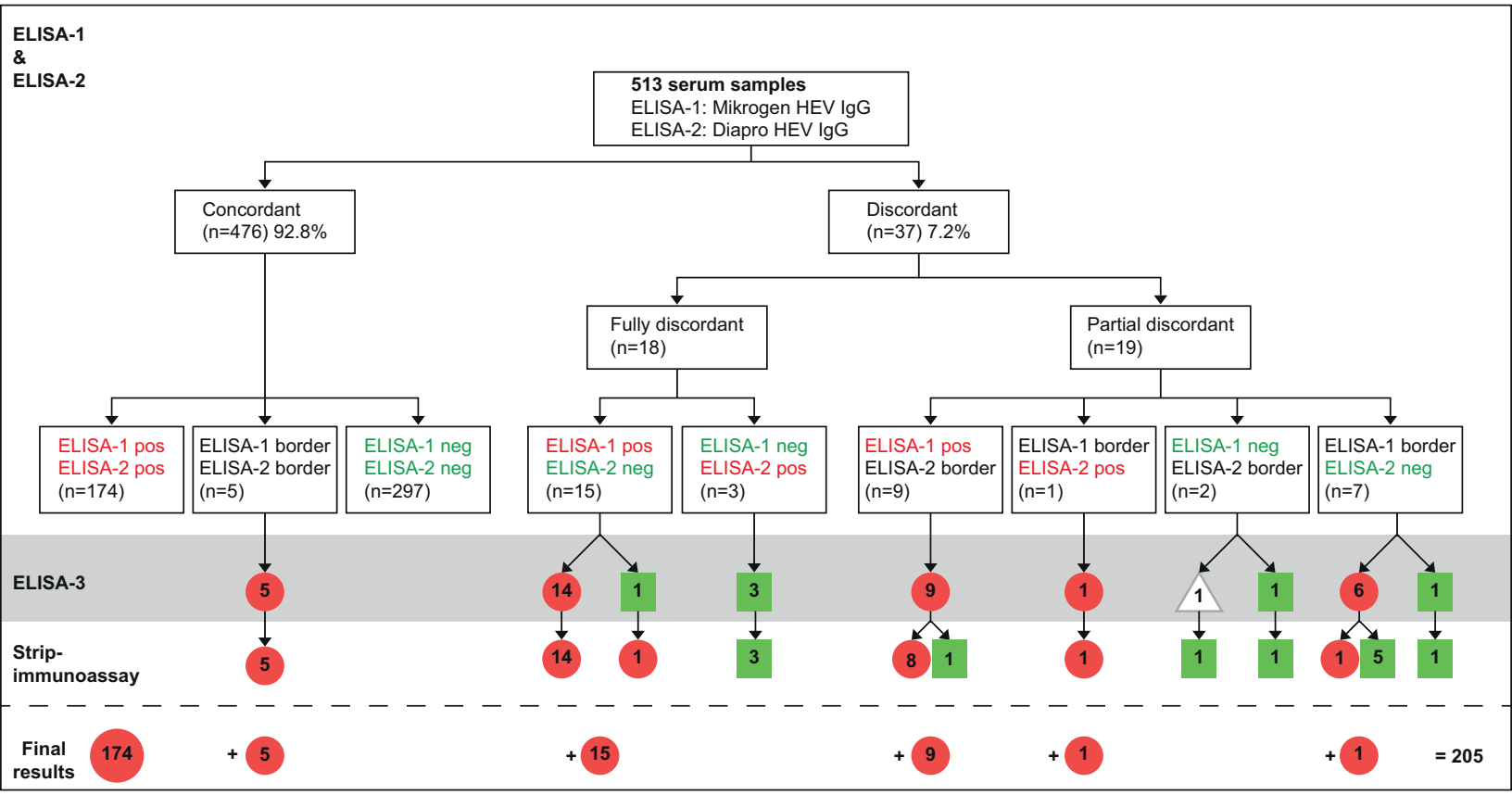
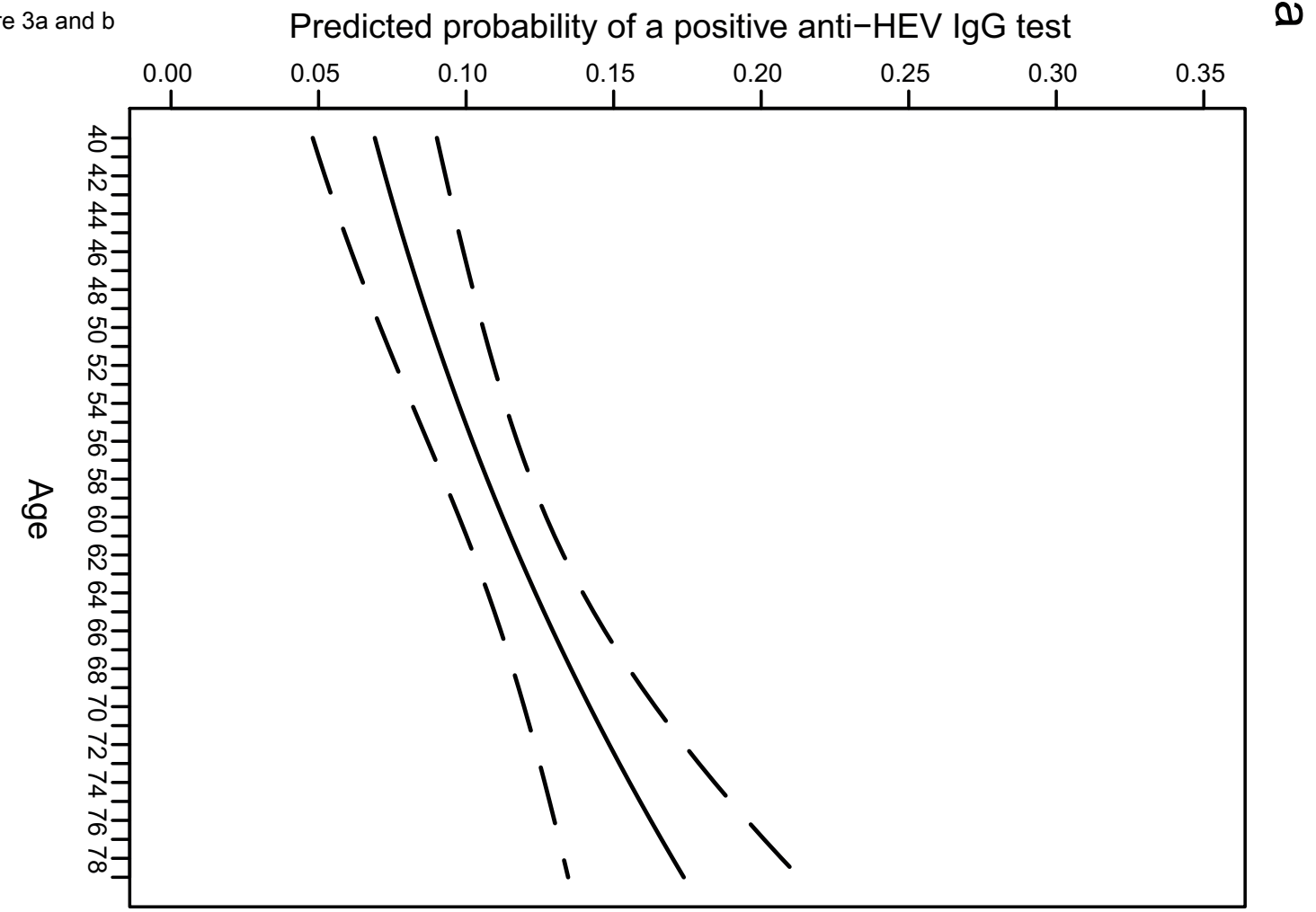
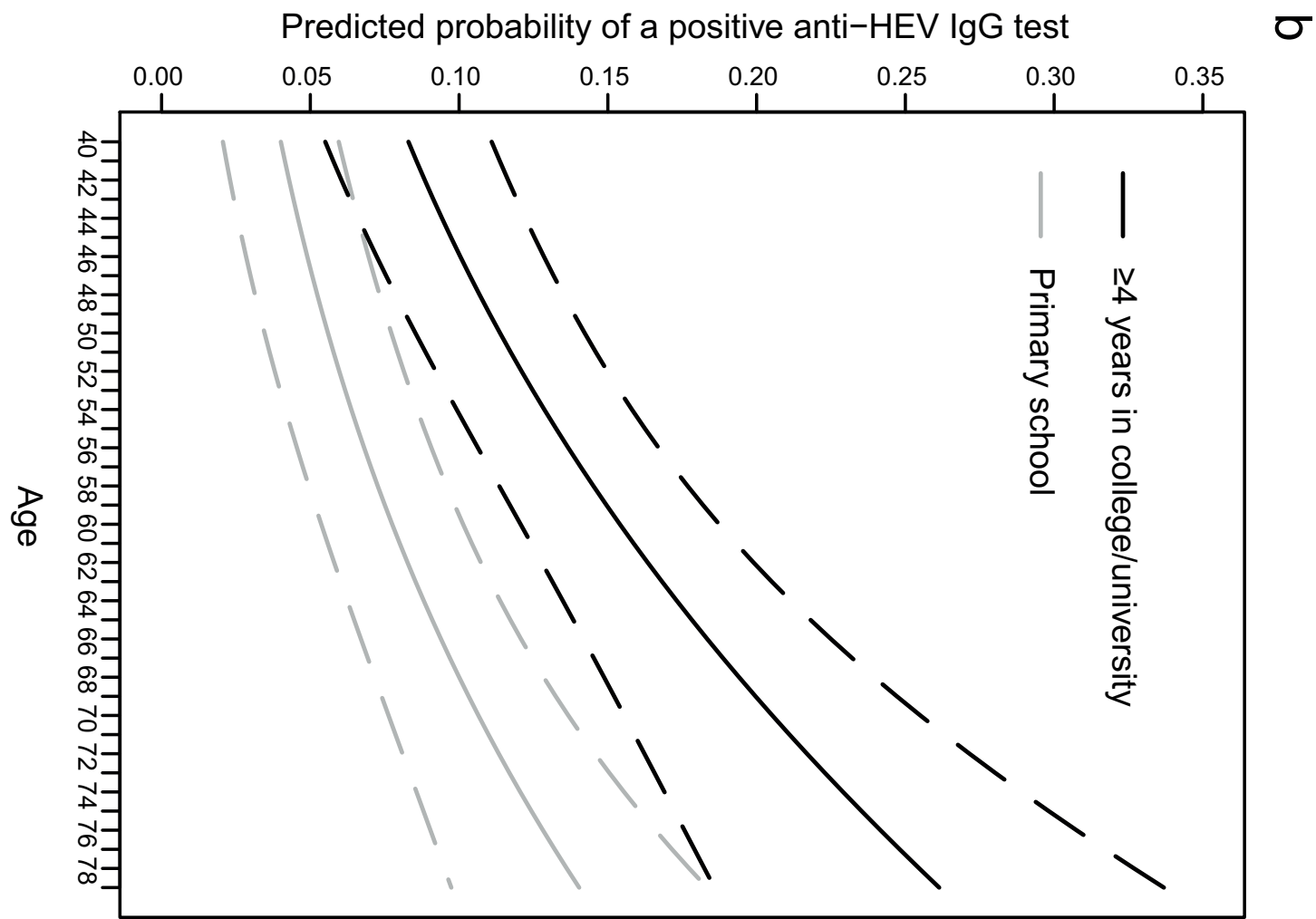


Figure 3a and b





Supplementary Table 2