

PREVALENCE OF ANTIBODIES TO HEPATITIS E IN TWO RURAL EGYPTIAN COMMUNITIES

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Abstract. A population-based serosurvey in two rural Egyptian communities was used to assess age-specific prevalence of antibody to hepatitis E virus (anti-HEV). One community is in the Nile Delta (11,182 inhabitants; 3,997 participants) and the other in Upper Egypt (10,970 inhabitants; 6,029 participants). Samples were tested for anti-HEV with a commercial enzyme-linked immunoassay (ELISA) based on antigens derived from open reading frame (ORF)2 and ORF3. Although there was a clear difference in sensitivity among the lots of the commercial test used, it was still possible to determine the seroprevalence. The seroprevalence of anti-HEV exceeded 60% in the first decade of life, peaked at 76% in the second decade and remained above 60% until the eighth decade. Prevalence of this magnitude is among the highest reported in the world, with an age-specific pattern more similar to hyperendemic hepatitis A virus transmission than generally described. Lot-to-lot variation in the sensitivity of the commercial ELISA kit highlights a problem when comparing seroepidemiologic studies of different populations.

INTRODUCTION

The successful sequencing of the hepatitis E virus (HEV) genome and the consequent ability to determine markers for infection with HEV have resulted in the recognition of HEV as the major cause of both epidemic and sporadic cases of enterically transmitted non-A, non-B viral hepatitis (ET-NANBH) in many developing countries.^{1–4} The prevalence of antibodies to HEV (anti-HEV) has been described for several populations, with levels as high as 40% in India.⁵ Another report from India, where hepatitis E epidemics are common, indicated seroprevalence as high as 60% in certain age groups.⁶ In Egypt, where outbreaks of hepatitis E have not been noted, reports have indicated that HEV infection is, nonetheless, endemic, with community seroprevalence as high as 60%.^{7,8} However, until now, there have not been any large studies to confirm these unusually high estimates. Here, we report a population-based study of anti-HEV prevalence in two communities in different regions of Egypt.

MATERIALS AND METHODS

Study population. In 1997, the Hepatitis C Prevention (HCP)-Egypt Project was initiated to investigate the epidemiology of hepatitis C virus (HCV) infection in Egypt, and samples collected for the HCP-Egypt Project were made available for HEV serological testing. The study encompassed two distinctly different rural villages in Egypt: (a) the village of Salaam and its satellite community, Ezbet Fateh El-Bab, in Upper Egypt, 15 km from the city of Assiut; and (b) the village of Aghour El Soghra in the Governorate of Qualoubiya in the southern Nile Delta.

The primary sources of drinking water for both villages are common deep wells (≥ 100 meters in depth), accessed predominantly by taps within the houses, or in the case of about 13%, accessed by a common tap outside. The water source for the rest of the population (about 9%) is shallower wells (about 10 meters deep). Neither community has a mu-

nicipal sewage system. For most households, sewage drains to an underground container that either leaks into the surrounding underground area or is periodically evacuated into a truck for removal of the waste.

Prior to the initiation of the HCP-Egypt Project in these villages in 1997, a census was taken in each village, and the households were surveyed to describe the population and to aid in recruitment of participants. Households were defined as groups of individuals who live in the same residence, sharing expenses and eating from a common pot, although not necessarily together. A questionnaire about demographic characteristics, household conditions, disease history, and potential risk factors for enterically and parenterally transmitted hepatitis was administered to all participants. Recruitment methods differed slightly in the two communities.

In the Nile Delta community, recruitment of a 50% sample of the 11,182 individuals identified in the census was attempted through systematic recruitment of every other household. Blood samples were collected from 75% of the target sample population (3,888 of the 5,156 residents who were five years of age or older), as well as an additional 111 of the 616 residents less than 5 years old. The mean ages of participants and nonparticipants five years and older were not significantly different for females or overall, but the mean age of participating males was slightly younger than nonparticipating males (24.5 and 26.8 years, respectively, $P = 0.001$). Nonparticipants were more likely than participants to be male.

In the Upper Egypt community, 10,970 individuals were identified by the census. Recruitment of all households was attempted, and 86.7% of households participated. Blood specimens were obtained from 6,014 (62.8%) of the 9,581 village inhabitants five years of age or older, as well from 19 of 1,646 children less than 5 years old. Again, the mean ages of participants and nonparticipants five years and older were not significantly different overall or for females, but the mean age of participating males was slightly older than nonparticipating males (26.5 and 24.5 years, respectively, P

< 0.001). As with the Nile Delta community, nonparticipants were more likely to be male.

The age-distribution of participants was similar in the two communities; two-thirds of participants in both communities were younger than 30 years of age. This distribution is representative of the entire population over the age of five in these communities, with the exception of slight under-representation of young to middle-aged adult males.

Informed consent was obtained from participants, or, in the case of minors, their parents. The Egyptian Ministry of Health and Population, Assiut University, and the Institutional Review Board of the University of Maryland reviewed and approved all procedures and forms.

Serologic testing. Sera from 6,029 individuals in Upper Egypt and from 3,997 in the Nile Delta were tested for anti-HEV. Testing for total anti-HEV was performed using the Abbott HEV enzyme-linked immunoassay (ELISA) kit (Abbott GmbH, Diagnostika, Germany), according to the instructions of the manufacturer. The kit is based on two recombinant antigens derived from the Burmese strain of HEV and expressed in *Escherichia coli* (327 amino acids from open reading frame [ORF]2 and 123 amino acids representing the full length of ORF3); its procedures are performed by the manufacturer's automated system. Cut-off values for categorization of serostatus as positive or negative were determined by the system's automated reader on the basis of the optical density (OD) results for the positive and negative control specimens included in the kit. In the event that the automated reader was unable to calculate the cut-off value (due to nonuniform performance of controls), the cut-off value was calculated by laboratory personnel on the basis of control OD values. Repeat testing was performed for all specimens from plates for which automatic determination of cut-off values was not possible. Repeat testing was also performed for all specimens with borderline results on initial testing (OD/cut-off value between 0.9 and 1.1). When the repeat test result differed from the initial result, a third test was performed to determine the final classification of serostatus.

Due to the large size of the study sample, it was necessary to use two production lots of the commercial test for initial analysis of the 10,026 sera. All of the initial testing for the Nile Delta community and for about one-fifth of the Upper Egypt community was performed with "Lot 1," and the balance of initial testing for Upper Egypt was performed with "Lot 2." Repeat testing of nullified test runs and initially borderline results was performed with a third lot, "Lot 3," for both communities. Final serostatus was determined with Lot 1 for 85% of the Nile Delta and 15% of the Upper Egypt community, with Lot 2 for 71% of the Upper Egypt community, and with Lot 3 for 15% of the Nile Delta and 14% of the Upper Egypt community.

The validity of the results of the commercial test was evaluated by testing a convenience sample of 300 specimens from the study communities at the Hepatitis Viruses Section, National Institutes of Health (NIH) with an ELISA based on recombinant HEV ORF2 from a Pakistani strain expressed in insect cells. This confirmatory test was demonstrated to be sensitive and specific for the detection of anti-HEV.^{9,10} The results of the NIH test were regarded as the standard

TABLE 1

Comparison of results obtained with commercial kits and with NIH test

Lot	No.	r*	Concordance† (%)	Sensitivity (%)	Specificity (%)
All	300	0.61	57.0	54.7	75.8
1	71	0.85	64.8	64.2	75.0
2	178	0.54	53.4	48.7	80.8
3	51	0.46	58.8	60.4	‡

* Spearman correlation coefficient; *P*-value for all coefficients ≤ 0.0001 .

† Percent agreement of results (positive-positive, negative-negative).

‡ Only 3 samples tested by Lot 3 were positive by the NIH test.

for determination of sensitivity and specificity of individual lots of the commercial test.

Analysis. Serostatus was determined by dividing the OD of the test sample by the OD of the cut-off value (index value); values ≥ 1.0 were considered positive for anti-HEV, and values < 1.0 were considered negative. Seroprevalence was determined for five year increments of age, with calculation of the 95% confidence intervals (95% CI). These calculations were performed for each community and for each lot of the kits used for testing. Comparison of proportions was performed with the χ^2 test.

Correlations of the results of the commercial test kits with those of the NIH test were determined using the Spearman correlation coefficient. Concordance was calculated as the number of specimens for which the results of both tests (commercial and NIH) were in agreement (positive-positive, negative-negative) divided by the total number of specimens tested by both methods, expressed as percent. Analysis was performed with the SAS System for Windows[®], release 6.12 (SAS Institute, Inc., Cary, NC).

RESULTS

A comparison of results obtained with the commercial test and with the NIH test is summarized in Table 1. The overall correlation of index values was moderate, with Lot 1 having the strongest correlation with the NIH test results. Likewise, concordance with the NIH test was best for Lot 1, which also demonstrated the highest sensitivity of the three lots. As only a few samples tested with Lot 3 were positive with the NIH test, specificity for that lot is not presented. Very few of the specimens that tested positive by the Abbott kit tested negative by the NIH assay: 2.3% of those tested by Lot 1, 6.3% tested by Lot 2, and 6.5% tested by Lot 3.

Seroprevalence by age and manufacturer's production lot for both communities, restricted to those individuals for whom serostatus was determined by Lot 1 or 2, appears in Figure 1. Lot 1 was the basis for final serostatus determination of 3,377 of 3,997 participants in the Nile Delta and 906 of 6,029 participants in Upper Egypt. Lot 2 was the basis for final determination of serostatus for 4,284 of 6,029 participants in Upper Egypt. The seroprevalence for Lot 1 in Upper Egypt approximated or attained the levels found in the Nile Delta with the same lot. Within the Upper Egypt cohort, seroprevalence on the basis of Lot 2 results was consistently and substantially below that obtained with Lot 1 across all age groups except those under five years, suggesting lot-to-lot variation in sensitivity. Among those youn-

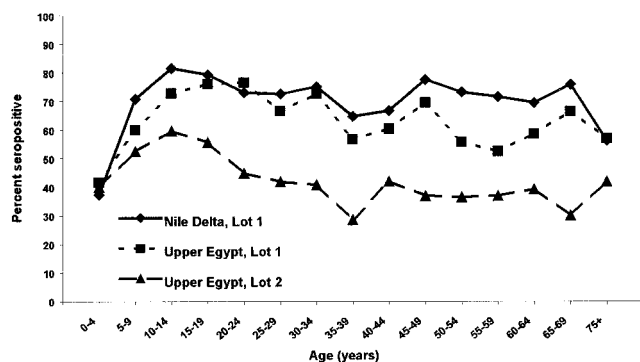


FIGURE 1. Age-specific seroprevalence by community and lot of test kit.

ger than five years, the seroprevalence of those in the Nile Delta community tested with Lot 1 (37.3%, 95% CI 27.6, 48.1) was similar to those in Upper Egypt tested with both Lot 1 (41.7%, 95% CI 16.5, 71.4) and Lot 2 (40.0%, 95% CI 7.3, 83.0).

Because of the clear difference in sensitivity between Lots 1 and 2, and in light of the stronger correlation of the results of Lot 1 with the NIH results, the sensitivity of Lots 2 and 3 was adjusted to the level of Lot 1. This was performed by adjusting the cutoff level for Lots 2 and 3 so that the seroprevalence in the second decade of life obtained with these lots equaled that obtained with Lot 1 in the second decade of life in Upper Egypt. With this adjustment, the peak level of seroprevalence is similar for the two communities, as is the pattern of age-specific seroprevalence (Table 2, Figure 2). The seroprevalence was high for all groups, peaking in the second decade at 75.6%. The overall seroprevalence was 67.7% (95% CI 66.7, 68.6) for both communities combined, 69.8% (95% CI 68.4, 71.2) for the Nile Delta community, and 66.2% (95% CI 65.0, 67.4) for the Upper Egypt community.

The first decade was biased toward the later years since 91.8% of the subjects were five years or older, with 48.6%

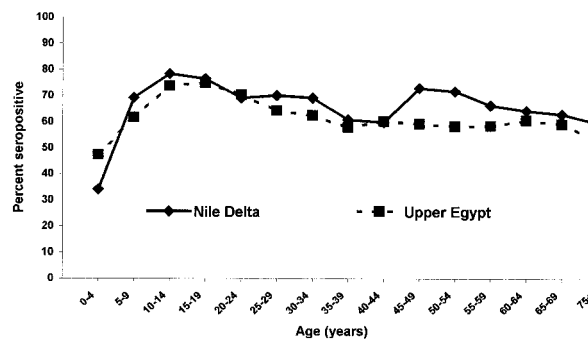


FIGURE 2. Seroprevalence for all participants and by community, with correction of sensitivity for Lots 2 and 3. Seroprevalence was calculated using all results for both communities, with inclusion of results for Lots 1, 2, and 3, after adjustment of sensitivity of Lots 2 and 3.

in the eighth or ninth year of life. Almost all of the 130 children younger than five years were in their third or fourth year, and seroprevalence in this group was 36.2%. The seroprevalence for those five years and older in the first decade was 64.7%.

Seroprevalence was similar for males and females (data not shown), and without notable differences for the combined sample population, for each community and for each age group (Table 2). There was no association between serostatus and a self-reported history of either liver disease (just over 1% in both seronegative and seropositive, chi-square P -value = 0.3) or viral hepatitis (less than 1% for both seronegative and seropositive, chi-square P -value = 0.2).

DISCUSSION

Endemicity of HEV infection has been described as predominantly restricted to areas in which outbreaks have been reported.¹¹ In this regard, Egypt is an exception since outbreaks of enterically-transmitted hepatitis have not been re-

TABLE 2
Seroprevalence, by age and community, using adjusted index values

Age (years)	Percent seropositive					
	All		Nile Delta		Upper Egypt	
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)
0-4	130	36.2 (28.0, 45.1)	111	34.2 (25.7, 43.9)	19	47.4 (25.2, 70.5)
5-9	1600	64.7 (62.2, 67.2)	721	69.2 (65.3, 72.8)	879	61.6 (58.3, 64.9)
10-14	1944	75.6 (73.6, 77.5)	755	78.4 (75.3, 81.3)	1189	73.8 (71.1, 76.2)
15-19	1438	75.5 (73.1, 77.6)	539	76.4 (72.6, 79.9)	899	74.9 (71.9, 77.6)
20-24	949	69.9 (66.8, 72.8)	350	69.1 (64.0, 73.9)	599	70.3 (66.4, 73.9)
25-29	694	66.4 (62.8, 69.9)	267	70.0 (64.0, 75.4)	427	64.2 (59.4, 68.7)
30-34	667	65.1 (61.3, 68.7)	265	69.1 (63.1, 74.5)	402	62.4 (57.5, 67.2)
35-39	673	59.0 (55.2, 62.7)	255	60.8 (54.5, 66.8)	418	57.9 (53.0, 62.7)
40-44	480	60.0 (55.5, 64.4)	199	59.8 (52.6, 66.6)	281	60.1 (54.1, 65.9)
45-49	439	64.7 (60.0, 69.1)	177	72.9 (65.6, 79.2)	262	59.2 (52.9, 65.1)
50-54	351	63.8 (58.5, 68.8)	145	71.7 (63.5, 78.7)	206	58.3 (51.2, 65.0)
55-59	262	61.5 (55.2, 67.3)	101	66.3 (56.2, 75.3)	161	58.4 (50.4, 66.0)
60-64	261	62.1 (55.9, 67.9)	109	64.2 (54.4, 73.0)	152	60.5 (52.3, 68.3)
65-69	150	60.7 (52.3, 68.4)	62	62.9 (49.7, 74.6)	88	59.1 (48.1, 69.3)
70-74	79	48.1 (36.8, 59.6)	33	51.5 (33.9, 68.8)	46	45.7 (31.2, 60.8)
≥75	39	71.8 (54.9, 84.5)	19	73.7 (48.6, 89.9)	20	70.0 (45.7, 87.2)

CI = confidence intervals.

ported in this country. There is, however, ample evidence for endemicity of non-A, non-B hepatitis (NANBH). Numerous studies of the etiology of sporadic acute hepatitis among both children and adults have indicated that NANBH is the cause of 30% to 50% of cases.¹²⁻¹⁴ Consistent with those earlier reports, when serological tests for anti-HEV became available, HEV was found to be the etiology of 15% to 40% of cases of acute viral hepatitis.¹⁵⁻¹⁸ The lack of association of HEV seropositivity with a history of liver disease or viral hepatitis in the two communities described herein is consistent with the absence of outbreaks and suggests most infections occurred early in life and were asymptomatic or caused only mild illness. Such is the case for HAV infection which is so prevalent in Egypt that, in rural areas, infection occurs very early in life, with virtually 100% seroprevalence of anti-HAV after the first years of life.⁸ Therefore, this age-specific pattern of anti-HEV prevalence is much more like the pattern of hyperendemic hepatitis A virus (HAV) infection than that seen in previous studies of HEV, and suggests a greater community endemicity of HEV infection than reported from other communities.

There have been reports of age-specific anti-HEV prevalence from convenience samples,^{11,19-21} mixed population samples,⁵ and some population-based studies.²²⁻²⁷ Although seroprevalence has differed among the studies, these reports have consistently demonstrated relatively lower seroprevalence in the first decade, rising in the second decade, and then leveling off. However, a recent study from India reported high seroprevalence in the first decade, peaking in childhood and decreasing somewhat in adults, similar to the pattern seen in this report.⁶

There have been two publications describing anti-HEV prevalence in Nile Delta communities based on the same commercial kit used in the present study. One of these, by Kamel and others,⁷ assessed anti-HEV seroprevalence in a large (N = 1,259), population-based sample, and found an age-stratified pattern consistent with that found in other populations in which the virus is endemic, with relatively low levels in the first decade, increasing levels in the second decade, and peak levels of about 30% in the third decade, leveling off thereafter. The other study, by Darwish and others,⁸ used a smaller convenience sample, and demonstrated a pattern of age-specific anti-HEV suggesting hyperendemic transmission similar to our findings. High seroprevalence (57%) was demonstrated in the second half of the first decade, with levels remaining fairly stable for the older age groups.

Comparison of various assays for anti-HEV revealed problems with specificity in populations with low prevalence.¹⁰ However, the seroprevalence in Egypt is of such a magnitude that this should not be a factor, and using the NIH test as the standard, the commercial test demonstrated good specificity. On the other hand, the clear differences in sensitivity of the commercial test lots used in this large serosurvey emphasize the problem of comparing epidemiologic studies of seroprevalence using different tests for anti-HEV and even different lots of the same test. The problem is aggravated by lack of a confirmatory test for anti-HEV. This finding is an unanticipated result of testing this very large cohort, which necessitated using kits from more than one production lot, enabling comparison of performance of

the test lots for serum samples from the same population, which should have similar levels of age-specific prevalence. Although a cause for concern, the difference in results by lot is unlikely to be due to technique in performing the test, but, rather, the result of variability of the test's reagents from lot to lot. These lots were tested in the same laboratory, by the same personnel, and with the same laboratory equipment over numerous occasions for both lots. The differences in seroprevalence are consistent and systematic, with similar patterns of prevalence by age for both lots. It is unlikely that there would have been such a consistent, parallel pattern had the differences been due to variability in technique of performing the tests.

However, the similar seroprevalences of those under five years in both communities, regardless of whether Lot 1 or Lot 2 was used needs explanation. If real, this could reflect more recent infection in this age group relative to those in older groups. Those more recently infected are more likely to have higher anti-HEV titers and to have antibodies to the ORF3 antigen, which tend to be more transient than those to the ORF2 antigen.²⁸ This could result in similar sensitivity of the two lots if they are biased toward detecting antibody to the ORF3 antigen rather than to the ORF2 antigen. This phenomenon would be expected to fade in older age groups, who may have been infected at an early age. In addition, although the three point estimates for seroprevalence in this first age group are similar, there were relatively few individuals in this youngest age group (in Upper Egypt only 19 were tested using Lot 1 and 12 using Lot 2), resulting in wide confidence intervals that allow for true seroprevalences consistent with the differences observed in the older age groups.

Issues of precise seroprevalence notwithstanding, it is clear that the levels of anti-HEV in these two communities are the highest reported to date. The relatively unique finding of high seroprevalence in the first decade, similar to the pattern of hyperendemic HAV transmission in rural Egypt, requires further study.

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