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A Serological Approach to Determine the Burden of Hepatitis E in Pregnant Women in Karachi, Pakistan

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An abstract of
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Abstract

A Serological Approach to Determine the Burden of Hepatitis E in Pregnant Women in Karachi, Pakistan

By Amanda Cecilia Howa

Background: Hepatitis E virus (HEV) is a self-limiting disease in the general population that causes epidemics of acute, viral hepatitis. However, in pregnant women, HEV causes more severe systems leading to obstetric complications, such as maternal death, antepartum hemorrhage, poor fetal outcomes, preterm delivery, and stillbirth.

Methods: A surveillance study conducted in Karachi, Pakistan from February 2015 to April 2016 recruited 221 mothers and their infants. Serum samples were collected from the mothers at delivery and their infants at birth, six weeks, and eighteen weeks and tested for the presence of IgG anti-HEV and IgM anti-HEV.

Results: At delivery, 75.1% of mothers were IgG anti-HEV positive. At birth, 74.8% of infants were positive for IgG anti-HEV, at six-weeks 59.1% were IgG anti-HEV, and at eighteen-weeks, 29.8% were IgG anti-HEV positive. At delivery, 7.4% of mothers tested positive for IgM anti-HEV. At birth, less than one percent of infants were positive for IgM anti-HEV. IgM anti-HEV increased to 2.3% in infants at six-weeks and decreased again to 0.7% at eighteen-weeks. Out of 21 total IgM anti-HEV positive individuals, 15 were also IgG anti-HEV positive.

Conclusion: The presence of IgG anti-HEV antibodies in infants decrease over time from birth to eighteen weeks. Coinciding IgG/IgM anti-HEV positivity signified acute HEV infection, which can have numerous implications and complications with pregnancy, including transfer of infection to the infant.

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Tables of Contents

I. Background and Literature Review

A. Clinical, Microbiological, and Transmission Characteristics of Hepatitis E

Hepatitis E virus (HEV) is a frequent cause of viral hepatitis worldwide.[1] HEV is part of the *Hepeviridae* family within the genera *Orthohepevirus*.[1] HEV has four major genotypes, HEV1-4.[2] HEV1 and HEV2 infections are limited to humans, while HEV3 and HEV4 can infect humans, pigs, and other mammalian species.[2] HEV pathogenesis involves three separate stages: the incubation period, acute hepatitis E, and a convalescent phase.[3]

HEV infection is asymptomatic or mild in the majority of infected individuals who do not show any liver related symptoms.[3] Acute icteric hepatitis, which occurs in 5-30% of infected individuals, consists of a prodromal phase. This phase lasts up to a week and includes symptoms such as malaise, fever, body aches, nausea, and vomiting.[3] The icteric phase is characterized by jaundice and dark-colored urine, which generally resolves on its own.[3] Chronic hepatitis E is most common in individuals who have received a solid organ transplant.[4]

Neurological manifestations have occurred with HEV.[5] Most commonly, Guillain-Barré syndrome, neuralgic amyotrophic, and encephalitis/myelitis have been noted.[5]

HEV transmission in developing countries is mainly caused by HEV1 and HEV2.[2] Infection is transmitted through water contaminated with fecal matter.[2] Person-to-person transmission is uncommon, but it has been suggested that household factors may play an important role in transmission.[6] HEV3 and HEV4 occur mainly through zoonotic transmission by having contact with infected animals[7] or consumption of contaminated food, although these strains of the virus have been detected in raw sewage.[8]

Hepatitis E is often indistinguishable from other forms of acute viral hepatitis, therefore testing is required for the presence of antibodies against HEV in the serum, or HEV RNA, capsid

antigen in the blood, or other bodily fluids.[3, 9] The incubation period for HEV ranges from 15-60 days following exposure, and averages 40 days.[9] Initially, HEV has a short-lived IgM response[2], which generally coincides with the increase of liver enzymes such as serum alanine aminotransferase.[3, 9] This response is followed by the more long-lasting IgG antibodies that can persist for several years.[2, 3] Detecting anti-HEV antibodies is still problematic. The Food and Drug Administration (FDA) has not approved any serologic test to diagnose HEV infection for use in the United States.[9] However, tests are available for research purposes.[9] Enzyme immunoassays are used with recombinant open reading frame 2 (ORF2) and ORF3 proteins from an HEV1 strain as antigens.[1] Anti-HEV IgM in the serum is an indicator of an acute infection, whereas the presence of only anti-HEV IgG is a marker for a past infection.[3] It has been suggested that the concentration of anti-HEV IgG could provide insight on the risk of reinfection.[10]

The current gold standard in determining an acute HEV infection is detecting HEV RNA, which can be detected in blood, feces, and other bodily fluids.[3] HEV RNA is detectable in the blood and stool during the incubation period.[3] HEV RNA becomes undetectable in blood approximately three weeks after the onset of symptoms and approximately five weeks after the onset of symptoms in the stool.[2]

In the majority of cases, acute HEV does not require antiviral therapy.[3] However, in immunosuppressed individuals, intervention may be necessary. Reducing the dosage of immunosuppressant in solid organ transplant recipients with HEV infection can clear their infection.[11] Ribavirin has been used to treat HEV infection in kidney transplant recipients.[12] Additionally, pegylated α-interferon has been used to treat chronic HEV infection after liver transplantation.[13]

Although there is no FDA-approved vaccine for HEV, there is a vaccine that was recently approved for use in China.[9] A Phase III clinical trial in China showed that their vaccine, HEV 239, was well tolerated and effective in preventing acute infection in the general population.[14]

B. Hepatitis E in Low Income Countries

HEV is endemic in Asia, the Middle East, Africa, and Mexico.[15] Hepatitis E outbreaks in endemic areas are generally HEV1 or HEV2 and transmitted via the fecal-oral route.[16] Mathematical modeling has estimated that HEV1 and HEV2 caused 20.1 million new cases annually in Africa and Asia (95% credible interval: 2.8-37.0 million), which included 3.4 million symptomatic cases (95% credible interval: 0.5-6.5 million), 70,000 deaths from acute liver failure (95% credible interval: 12,400-133,000), and 3,000 stillbirths (95% credible interval: 1,900-4,400).[17]

HEV1 or HEV2 outbreaks often occur due to contaminated water supplies or utilizing water supplies such as rivers that have been contaminated with sewage.[18] There has been evidence of person-to-person transmission in a refugee camp in Northern Uganda where HEV was not detected in drinking water or zoonotic sources.[19] Vertical transmission, from mother to child, is well documented for HEV1 and HEV2.[20, 21] It has also been reported that HEV has been transmitted through blood transfusions in endemic areas.[22]

C. Hepatitis E in Pakistan

Pakistan has had three recent outbreaks of HEV. An epidemic of non-A, non-B hepatitis occurred in Sargodha, Pakistan in 1987.[23] The outbreak occurred at a local college and 133 clinical cases were reported. No deaths were reported but all cases required hospitalization.[23] The cause of the outbreak was due to an ineffective water treatment system.[23] An outbreak in 1998 at a military unit in Abbottabad, Pakistan required 109 men to be hospitalized.[24] The

source of the infection was found to be fecal contamination in the water system.[23] An additional outbreak in Islamabad, Pakistan starting in 1993 infected over 3,800 people with HEV.[25] The source of the outbreak was found to be caused by the malfunction of a water treatment plant and the implications of the potential consumption of untreated water.[25]

D. Hepatitis E in Pregnant Women

HEV can be transmitted vertically from mother to fetus and HEV has an increased severity among pregnant women.[26] However, the reason for the increased severity and mortality in pregnant women remains unclear.

HEV can cause fulminant hepatic failure and death in 15-20% of cases of pregnant women during outbreak settings.[26] Women with HEV infection were more likely to have obstetric complications, such as antepartum hemorrhage (relative risk 4.1, CI 1.7-10.2), intrauterine fetal death (RR 1.9, CI 1.3-2.7), poor fetal outcomes (RR 1.6, CI 1.2-2.0), preterm delivery (RR 1.2, CI 1.0-1.4), and stillbirth (RR 1.8, CI 1.2-2.5), than women with other forms of viral hepatitis.[27] There has been evidence for increased susceptibility to fulminant hepatitis in the second and third trimester, but women in all trimesters are at risk.[26]

A study that was based during the HEV outbreak that occurred in Delhi, India in the 1950s showed that miscarriage, stillbirth, or neonatal death occurred in 56% of infants born to mothers with HEV infection.[28] During a waterborne outbreak in Islamabad, Pakistan, eight total fatalities were reported, four were pregnant mothers, all in their third trimester, and four were newborn infants whose mothers had acute hepatitis.[25] Women with HEV infection were also more likely to have additional obstetric complications than women with other forms of viral hepatitis.[27]

II. Introduction

Hepatitis E virus (HEV) is one of the most common causes of jaundice and acute hepatitis in the world.[1, 2, 29] HEV is transmitted enterically, often due to contaminated water supplies or utilizing water supplies, like rivers that have been polluted with sewage.[18]

There are four genotypes that contribute to human disease.[3] Genotypes 1 (HEV1) and 2 (HEV2) are associated with waterborne transmission and are spread via the fecal-oral route.[16] HEV1 and HEV2 are endemic in developing countries and are responsible for large outbreaks.[30] Genotypes 3 (HEV3) and 4 (HEV4) are zoonotic viruses and can infect humans, pigs, and other mammalian species,[2] with pigs as the main reservoir.[3]

HEV is endemic throughout much of Asia, the Middle East, Africa, and Mexico.[15] More specifically, HEV is endemic to Pakistan[31] and consequently, epidemic HEV has been reported in multiple areas in Pakistan. Sargodha, Pakistan had an outbreak at a local college due to an ineffective water treatment system in the food preparation area on campus.[24] Abbottabad, Pakistan had an outbreak an a military facility that caused 109 hospitalizations due to fecal contaminants in the water system.[23] A water treatment plant malfunction in Islamabad infected over 3,800 people with HEV.[25]

Acute icteric hepatitis occurs in 5-30% of infected individuals and lasts up to a week. Symptoms such as malaise, fever, body aches, nausea, and vomiting are common.[3] HEV infection is generally asymptomatic or mild and the majority of infected individuals do not show any liver related symptoms.[3] However, women with HEV infection were more likely to have obstetric complications than women with other forms of viral hepatitis.[27] The mortality rate among pregnant women with HEV is higher than among pregnant women without HEV, especially during the third trimester, causing death in 15-20% of cases.[26]

Taking a serological approach to determining the prevalence of HEV in pregnant women is important in understanding the burden of infection in Pakistan and low-income areas. The presence of IgG anti-HEV will detect past infection of HEV, which will help determine the overall burden HEV has had on the population. The presence of IgM anti-HEV will detect active infection in pregnant women and their infants and possible transfer from mother to infant during pregnancy. These data will contribute to HEV disease management in women and infants.

III. Data and Methods

Surveillance:

Data were collected from the Prevention of Pertussis in Young Infants in Pakistan (PrePY) Baseline Surveillance Study[32] which was conducted from February 21, 2015 to April 12, 2016. Blood samples in this study were tested for a variety of antibodies, including HEV. Data were collected in four low income areas in Karachi, Pakistan: Rehri Goth, Ibrahim Hyderi, Bhains colony, and Ali Akbar Shah. The Aga Khan University has run primary health care centers in these areas for several years.

We enrolled 221 healthy, pregnant women on or after 27 weeks' gestation or mothers who gave birth within the previous 72 hours. Infants born to enrolled mothers were followed for eighteen weeks. Few subjects were lost to follow-up due to refusal for blood draw after deliver, molar pregnancy, still birth, maternal mortality, or refusal to continue in the study. Maternal characteristics, such as age, vaccination status, education, antenatal care, body mass index, and hypertensive status, were collected as the time of enrollment. Infants born to enrolled mothers were followed from birth to eighteen weeks of age. Gestational age, gender, weight, and head circumference were measured at the time of delivery.

This thesis presents preliminary analysis of the data.

Blood Specimen collection:

A maternal blood draw (~5.5 mL) was completed within 72 hours of delivery. Three blood draws were drawn from infants, one at birth from cord blood, the second was collected at six weeks of age, and the third sample was collected at 18 weeks of age (Figure 1). Approximately 4 mL of blood was collected from the infants at each blood draw by a trained phlebotomist.

After each specimen collection, blood samples were transported under cold chain maintenance to the Infectious Disease Research Laboratory at the Aga Khan University Hospital. Sera were then separated at the research laboratory and stored in cryovials at -80˚C. The sera were then continuously transported under cold chain maintenance to the Microbial Pathogenesis and Immune Response Laboratory at the Centers for Disease Control and Prevention in Atlanta, Georgia, USA, for analysis.

Laboratory procedures:

Sera samples were analyzed for IgG and IgM anti-HEV using the DS-EIA-ANTI-HEV-G[33] and DS-EIA-ANTI-HEV-M[34] kits, respectively.

Statistical analysis:

The primary outcomes were antibody titers for IgG and IgM anti-HEV found in the infant's umbilical cord blood and serum at birth, six weeks, and eighteen-week time intervals. The data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA.)

Funding:

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IV. Results

There were 221 women in their third trimester of pregnancy with their 221 infants enrolled in the study. Serum samples were collected from 217 mothers within 72 hours of delivery (98.2%). Out of the 221 infants enrolled into the study, we obtained 206 samples at birth (93.2%). One hundred and thirty-two serum samples were collected at the six-week follow up (59.7%) and 141 serum samples were collected at the eighteen-week follow up (63.8%) (Table 1, Table 2). These methods have previously been described in detail.[32]

At delivery, 75.1% of mothers were IgG anti-HEV positive (Table 1). At birth, 74.8% of infants were positive for IgG anti-HEV, at six-weeks 59.1% were IgG anti-HEV, and at eighteen-weeks, 29.8% were IgG anti-HEV positive (Table 1). At delivery, 7.4% of mothers tested positive for IgM anti-HEV (Table 2). At birth, less than one percent of infants (0.5%) were positive for IgM anti-HEV. IgM anti-HEV increased to 2.3% in infants at six-weeks and decreased again to 0.7% at eighteen-weeks (Table 2).

Out of the 21 total IgM anti-HEV positive individuals, 15 were also IgG anti-HEV positive. Thirteen of the IgM/IgG anti-HEV positive participants were mothers and two were infants. Two of the IgM/IgG anti-HEV positive cases were a mother-infant pair where the infant tested positive at six-weeks. The remaining infant tested positive for IgM/IgG anti-HEV at birth, however, the mother was only IgG anti-HEV positive.

V. Discussion

We determined the presence of IgG anti-HEV and IgM anti-HEV antibodies in pregnant mothers in their third trimester and their infants in Karachi, Pakistan at delivery for the mothers and at birth, six weeks and eighteen weeks after birth for the infants. We found that the presence of IgG anti-HEV antibodies in infants decrease over time from birth to eighteen weeks (Figure

2). These findings are consistent with the literature where it was shown that the IgG anti-HEV decreases over time in infants after birth.[20]

Coinciding IgG and IgM anti-HEV positivity signified an acute HEV infection or the end of an acute infection.[35] The proportion of mothers that were both IgG anti-HEV and IgM anti-HEV positive at delivery indicates the possibility that they had an active HEV infection during pregnancy. Acute HEV infection during pregnancy can have numerous implications. HEV can cause fulminant hepatic failure and death in up to 20% of cases in outbreak settings.[26] Pregnant women are more likely to have complications such as antepartum hemorrhage, intrauterine fetal death, preterm delivery, poor fetal outcomes and preterm delivery.[27]

The percentage of IgG anti-HEV positive mothers at delivery was similar to the percentage of IgG anti-HEV positive infants at birth, indicating that the transfer of IgG anti-HEV might be similar. The proportion of IgM anti-HEV positive mothers indicates the proportion of infected women in the population. This high percentage (7.4%) suggests that the case fatality ratio might be overestimated in Pakistan, considering that we are identifying a relatively large number of pregnant women with an evidence of recent infection, without corresponding high proportion of high adverse outcomes.

It has been shown that an IgG anti-HEV and IgM anti-HEV positive mothers can transfer infections vertically to their infants.[21] There has been one case of potential vertical transmission of HEV from mother to infant, however it is of interest that the infant did not test IgM anti-HEV positive at birth, but at six-weeks, which indicates a potential lab error or an outside source of the HEV infection. There has been an instance where HEV has been isolated from breastmilk[36], so the possibility exists that HEV could be transmitted this way, but additional study is needed to determine the viability of this potential transmission method.

A limitation of this study is that we did not follow up on clinical symptoms, such as jaundice in the infants to compare the tests. These data are from Karachi, which is a main metropolitan city, and there is heterogeneity in the water sources, so caution should be exercised before generalizing these data. These data also cannot be generalized to HEV infections in developed areas due to the epidemiological differences in HEV.

VI. Public Health Implications

The World Health Organization's Millennium Development Goals are all interconnected, especially ensuring sustainable access to safe drinking water and basic sanitation and maternal health.[37] This thesis identifies topics within both of these Millennium Development goals by addressing the importance that sanitation facilities and access to clean water has in the prevention of infectious diseases, like HEV.

The prevalence of HEV infections in pregnant women highlights the conditions that certain populations in developing countries face that may lead to infections and outbreaks, such as untreated water sources.[23-25] As HEV is mainly transmitted through the fecal-oral route[16], preventing drinking water contamination and maintaining proper water treatment techniques is critical in maintaining safe drinking water, especially in areas where HEV is endemic, such as Pakistan.

VII. Tables and Figures

Table 1. Prevalence of Hepatitis E Virus IgG Antibody in Mothers and Infants				
	Mothers	**Infants**		
		At Birth	At 6 Wk	At 18 Wk
	n *(%)*	n *(%)*	n *(%)*	n *(%)*
Positive	163 *(75.12)*	154 *(74.76)*	78 *(59.09)*	42 *(29.79)*
Negative	54 *(24.88)*	52 *(25.24)*	54 *(40.91)*	99 *(70.21)*
	217	206	132	141

Table 2. Prevalence of Hepatitis E Virus IgM Antibody in Mothers and Infants				
	Mothers	**Infants**		
		At Birth	At 6 Wk	At 18 Wk
	n *(%)*	n *(%)*	n *(%)*	n *(%)*
Positive	16 *(7.37)*	1 *(0.49)*	3 *(2.27)*	1 *(0.71)*
Negative	201 *(92.63)*	205 *(99.51)*	129 *(97.73)*	140 *(99.29)*
	217	206	132	141

Figure 1.

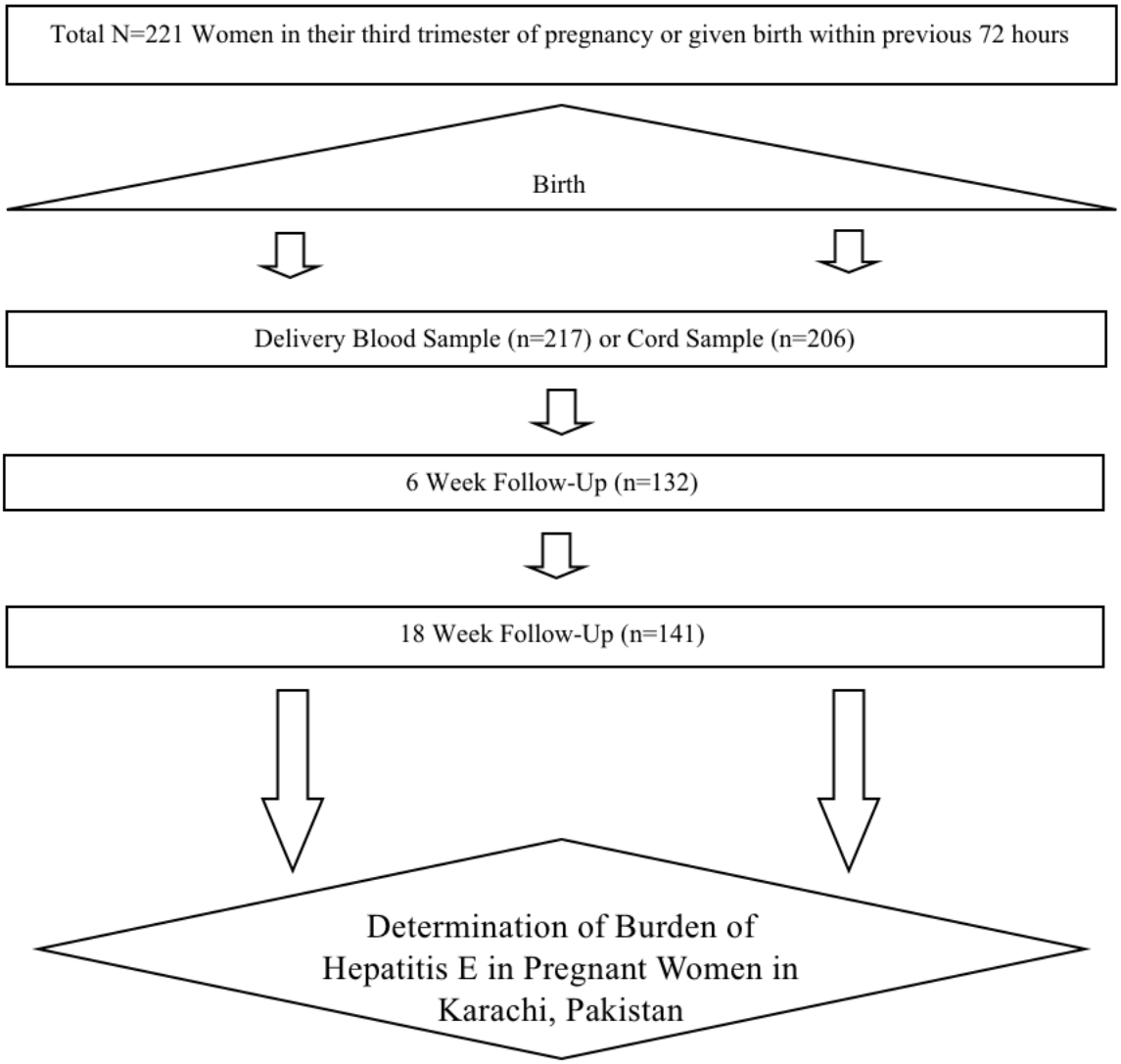
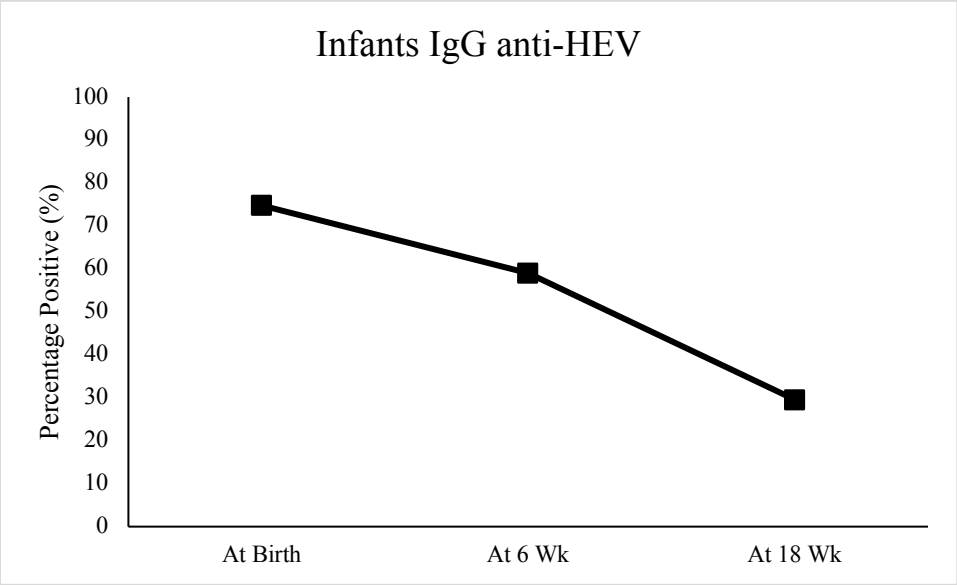

Total N=221 Women in their third trimester of pregnancy or given birth within previous 72 hours
Birth
Delivery Blood Sample (n=217) or Cord Sample (n=206)
6 Week Follow-Up (n=132)
18 Week Follow-Up (n=141)
Determination of Burden of Hepatitis E in Pregnant Women in Karachi, Pakistan

Figure 2.


Infants IgG anti-HEV
Percentage Positive (%)
100
90
80
70
60
50
40
30
20
10
0
At Birth
At 6 Wk
At 18 Wk

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