

Original Research Article

Sero Prevalance of hepatitis e virus in patients attending antenatal clinicAnurada. P^{1*}, Natarajan. V², Lakshmi Sarayu. Y³¹Final Year Post Graduate , Department of Department of Microbiology, Rajah Muthaih Medical College, Annamalai University, Chidambaram, Tamilnadu, India²Professor and Head, Department of Department of Microbiology, Rajah Muthaih Medical College, Annamalai University , Chidambaram, Tamilnadu, India³Professor, Department of Microbiology, Rajah Muthaiah Medical College, Annamalai University, Chidambaram, Tamilnadu, India***Corresponding author**

Anurada. P

Email: dr.ann.2904@ymail.com

Abstract: Human hepatitis E virus (HEV) was first reported in 1980 and is now considered a major cause of acute non-A, non-B hepatitis in humans. HEV infection is reported throughout the world and occurs in epidemic form in developing countries where the disease is endemic and is often associated with water contamination after heavy rains or flooding. Sporadic human HEV infections occur in industrialized countries where infected individuals contract the infection while traveling to endemic regions. It is important to have clear data about the prevalence rate of HEV in a country in order to facilitate the possible introduction of large scale national surveillance program and delivery of vaccination in the future and in consequence prevent the maternal and congenital mortality. This hospital based descriptive study was conducted in pregnant women for analysis of antenatal case for hepatitis E virus antenatal cases who attended the medicine department in Raja muthaiah medical college and Hospital, Chidambaram, during the period August 2015 till August 2016. Extensive data suggest that hepatitis E is a major contributor to disease and mortality across much of the Indian continent, with country-level variability, as expected. Still, it is challenging to make comparisons across these populations, given differing methodologies and assays used to determine HEV etiology. There was also lack of molecular techniques (HEV RNA detection) for confirmation of our results. All of the risk factors for the acquisition of HEV infection we're not be addressed in the questioner.

Keywords: Human hepatitis E virus (HEV), sporadic human HEV infection.

INTRODUCTION:

The fecal-oral route is considered the primary mode of HEV transmission in humans. HEV infection of pigs was discovered in 1997. Since 1997, sporadic HEV infections in industrialized countries have been reported in people who have not traveled abroad and are associated with HEV isolates genetically homologous to those found in domestic pigs. [1]. HEV infection of chickens and rats has since been documented in the U.S. and abroad. Evidence suggests that pigs serve as an important reservoir for HEV and thus exposure to pigs, pork products, or pig organs may pose a risk of zoonotic infection [2]. Swine HEV infection causes a subclinical, non-icteric hepatitis in growing pigs. Pigs experimentally-infected with swine HEV had no signs of clinical illness; however, they were viremic for 1 to 2 weeks, HEV was present in the liver of infected pigs, and the pigs shed a large amount of HEV in feces for

several weeks. Similar results were demonstrated when pigs were infected with human HEV. HEV also can be detected in pig manure storage facilities such as concrete pits and earthen lagoons and we demonstrated that HEV found in the pit manure is viable and infectious to pigs [3]. We attempted, but failed, to detect HEV in on-site drinking water or surface water on or near pig farms. These finding suggest a potential risk of contamination of water supplies by HEV in pig manure exists but evidence of this is lacking to date [4]. Cross-species infection with HEV among different species of animals has been demonstrated; swine HEV infects nonhuman primates, human HEV infects pigs, and chicken infects turkeys. Swine and avian HEV have been shown to be genetically distant with nucleotide homology of approximately 60%. We demonstrated experimental infection of pigs with avian HEV [5]. The avian HEV was found in the liver of

inoculated pigs and was shed in feces for at least 3 weeks. Rat HEV failed to replicate in pigs. These findings further support the growing concern that pigs are an important reservoir of HEV and emphasize the critical role of pigs in the epidemiology of HEV. Epidemiology studies have shown that HEV infection is quite common in some areas of the world such as India, Africa, and Southeast Asia, where it can represent the main cause of acute hepatitis. Outbreaks of HEV infection have been reported from Central and Southeast Asia [6]. Therefore, it is an important public health concern in many developing countries of Southeast and Central Asia, the Middle East, northern and western parts of Africa, and Mexico, where outbreaks have been reported. Evidence is accumulating that shows HEV infection to be an emerging disease in some developed countries as well. From studies of blood donors in developed countries, it would appear that the prevalence of HEV in these countries is around 3–5%. Conflicts and war have a devastating impact on public health and the availability of safe and healthy food and Water [7]. Therefore the prevalence of HEV is higher in these areas. Hepatitis E virus (HEV) is a small, non-enveloped virus, approximately 27-34 nm in diameter [8]. The virus has a polyadenylated, single-stranded RNA genome, approximately 7.2 kilo bases in length, with a positive polarity and a cap at its 5'-end. The viral genome contains short non-coding regions at both the 5' and the 3' ends, and contains three discontinuous and partially overlapping open reading frames. The largest open reading frame (ORF), known as ORF1, codes for viral non-structural proteins and contains several conserved domains, including putative methyltransferase, protease, helicase and RNA-dependent RNA polymerase [9]. The ORF2 codes for the viral capsid protein, and the ORF3 for a small phospho protein with uncertain function. HEV is the only member of the genus *Hepevirus* and is placed in the family *Hepeviridae*. The genus *Hepevirus* consists so far of two species: (i) mammalian HEV, which causes human disease and infects several other mammalian species, in particular pigs; and (ii) avian HEV, which is responsible for big liver and spleen disease in chicken, and is known to infect other birds such as turkeys. The avian HEV is believed not to be transmitted to man [10]. HEV is excreted in feces of persons infected with this virus, and is transmitted mainly through the fecal-oral route, though other routes have also been described. Routes of transmission of HEV infection include: (i) fecal-oral transmission due to fecal contamination of drinking water; (ii) food-borne transmission from ingestion of products derived from infected animals; (iii) zoonotic transmission from animals to humans from exposure to infectious body fluids of infected animals; (iv) transfusion of infected blood products; and (v) vertical (maternal-fetal) transmission. Of these, transmission through contaminated water is the most common. Zoonotic

transmission of HEV appears to be common in hyper endemic areas [11].

MATERIALS AND METHODS:

This hospital based descriptive study was conducted in pregnant women for analysis of antenatal case for hepatitis E virus antenatal cases who attended the medicine department in Raja muthaiah medical college and Hospital, Chidambaram, during the period August 2015 till August 2016. Ethical Committee Clearance: Ethical committee clearance was obtained from IHEC at Rajah Muthiah Medical College.

Inclusion criteria:

- The age group of patients was between 23-35 years.
- All Patients in Second and Third Trimesters were included
- All Patients with previous history of repeated/spontaneous abortion due to unknown reasons were included

Exclusion Criteria:

- Known cases of hepatitis B were excluded
- Known cases of liver disorder due to other viral /non-viral causes were excluded.

Method of sample collection: 3ml of blood was collected with a 3ml sterile disposable plastic syringe with aseptic precautions from the median cubital vein and transferred to labeled plain / clot-activator gel tubes for further storage and processing.

Method of sample processing:

1. The samples were centrifuged at 2500 rpm for 15 minutes to separate the serum.
2. The separated serum was pipetted out with precaution and transferred to labeled serum vials for further storage.
3. The serum was stored in the fridge at 4°C.
4. The collected samples were first subjected to a Rapid test and the results obtained were further verified by the ELISA method.

Rapid test method

1. Rapid immunochromatographic assay for detection of IgM antibodies to HEV in human serum - card test
2. Test kit used – Insight –HEV IgM device (Tulip Diagnostics (p) Ltd)
3. Which is a rapid, self – performing, immunochromatographic assay for detection of IgM antibodies to Hepatitis E virus in human serum?

OBSERVATION AND RESULTS

Totally 100 pregnant females are included in the study. Among them (18-24) Years was around

(34%) 25-30 Years (57%). Above 30 years was around (9%)(Fig-1).

Totally 100 pregnant females are included in the study. Among Them 25 members were drinking well water, 30 Members Were Drinking Ground Water, and 45 Members Were Drinking Pipe Line Water(Fig-2).

Totally 100 pregnant females are included in the study. Among them 33 patients were in II trimester and 67 patients were in III trimester(Fig-3).

Totally 100 pregnant females are included in the study. Among Them 60% Were HEV Positive And 40% Were HEV Negative(Fig-4).

Totally 100 pregnant females were include in the study. Among them 24 were primi gravida AND 76 were in multi gravid. Both primary and multi gravid were analyzed in the study. The p <0.001 value was statistically significant.(Fig-6)

Totally 100 pregnant females were include in the study. Among them 56 females were frequently prone for open sanitation and 44 were prone for closed sanitation. Both open and closed sanitation were analyzed in the study(Fig-8).

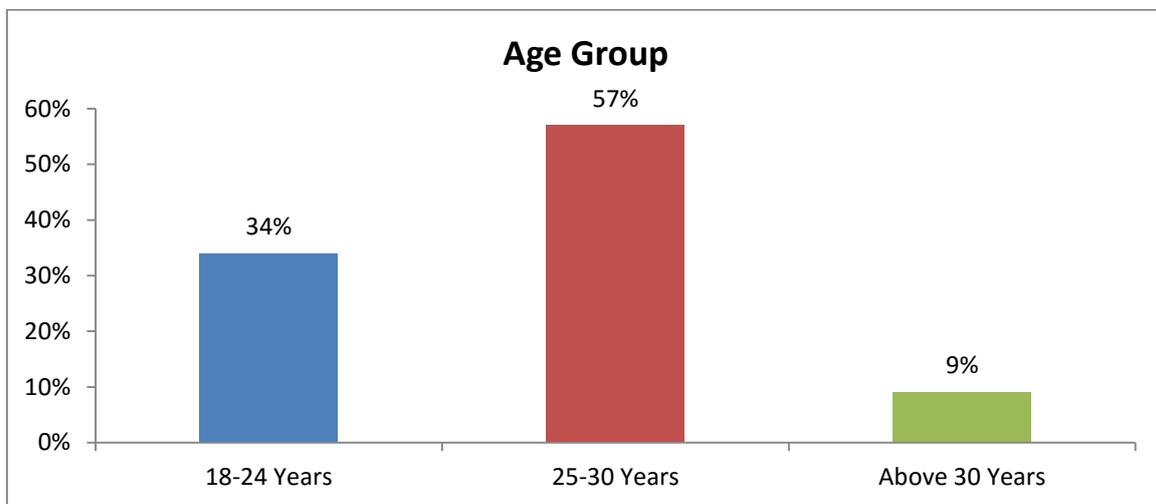


Fig 1: Shows Age Group distribution of pregnant women for analysis of antenatal case for hepatitis E virus antenatal cases (n=100)

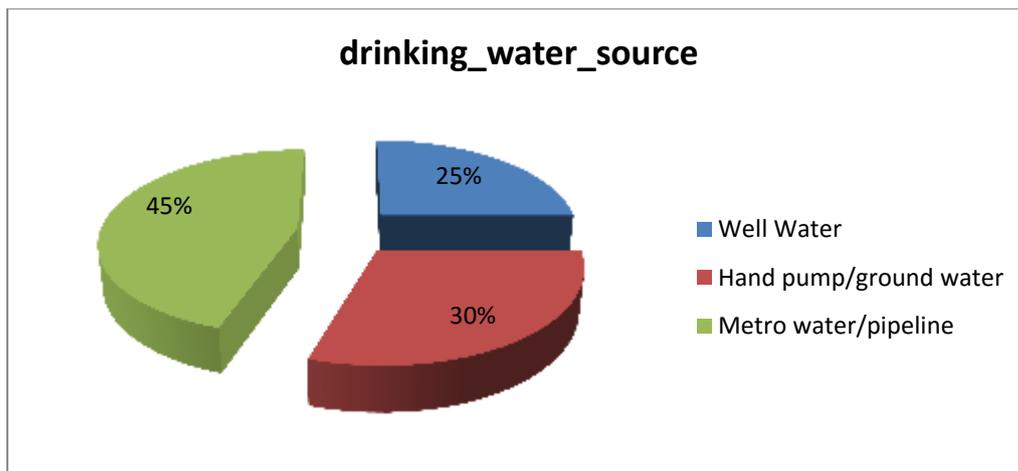


Fig 2: Shows Drinking_Water_Source of Pregnant Women for Analysis of Antenatal Case for Hepatitis E Virus Antenatal Cases (N=100)

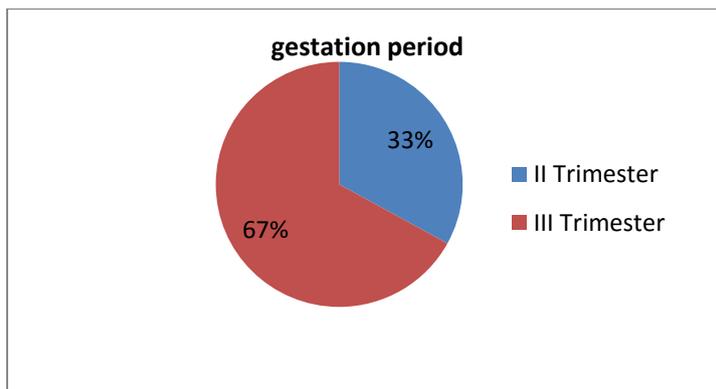


Fig 3: Shows gestation period distribution of pregnant women for analysis of antenatal case for hepatitis E virus antenatal cases (n=100)

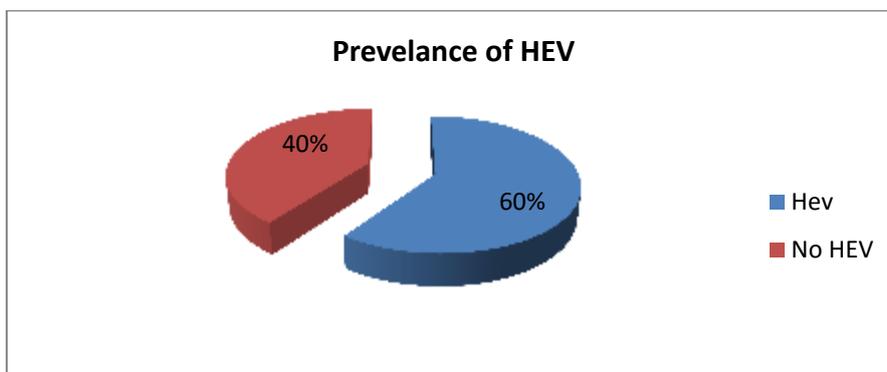


Fig 4 Shows HIV Infection Positive and Negative among Pregnant Women for Analysis of Antenatal Case for Hepatitis E Virus Antenatal Cases (N=100)

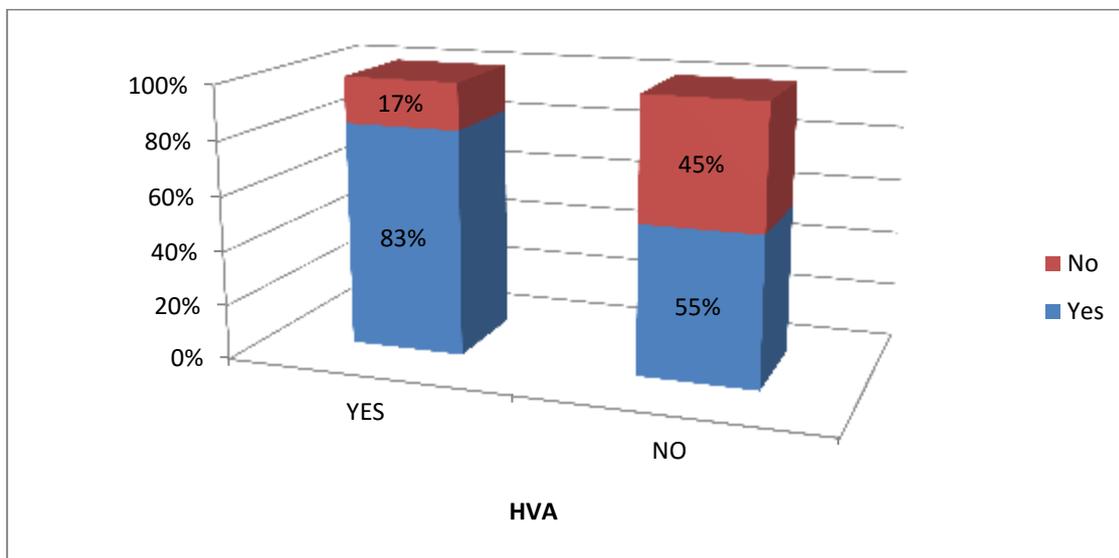


Fig 5 shows the prevalence of co infection of HAV among pregnant women with HEV infection

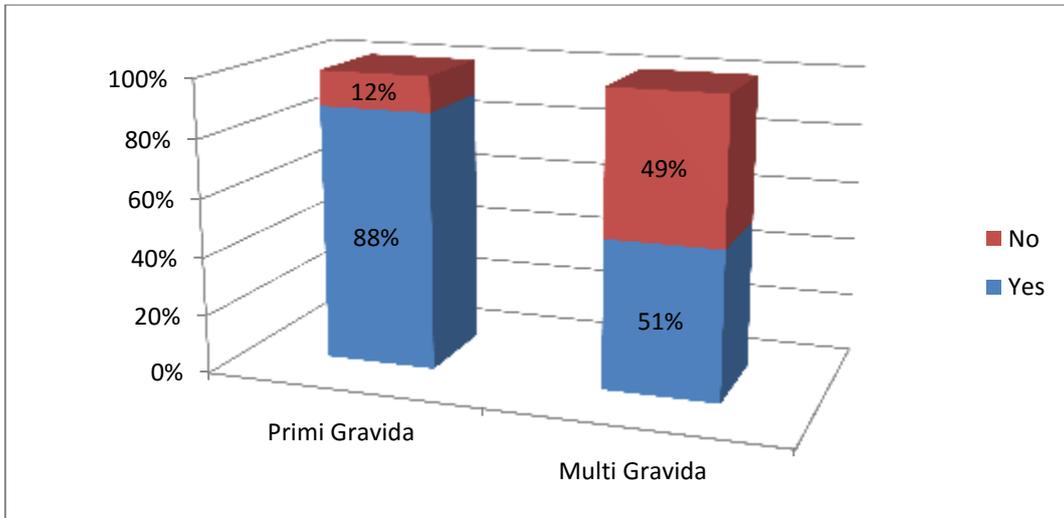


Fig 6: shows the prevalence of gravinda % among pregnant women

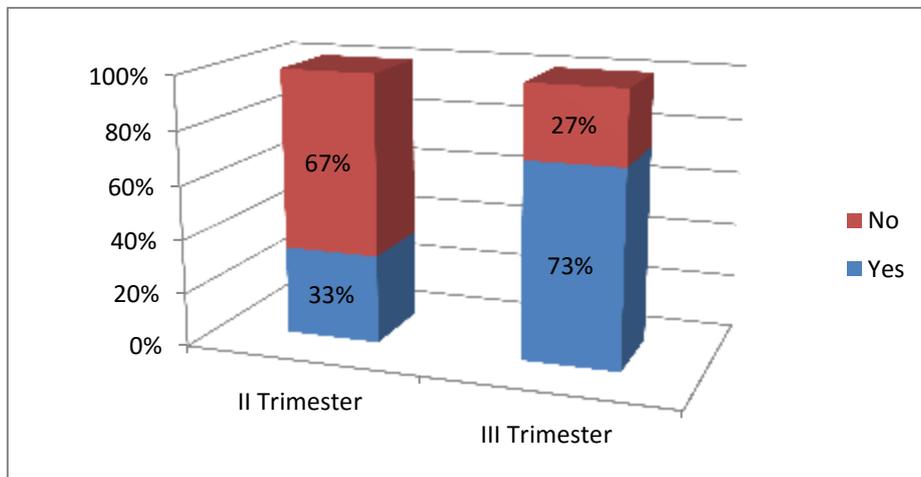


Fig 7: Shows trimester distribution of pregnant women for analysis of antenatal case for hepatitis E virus antenatal cases (n=100)

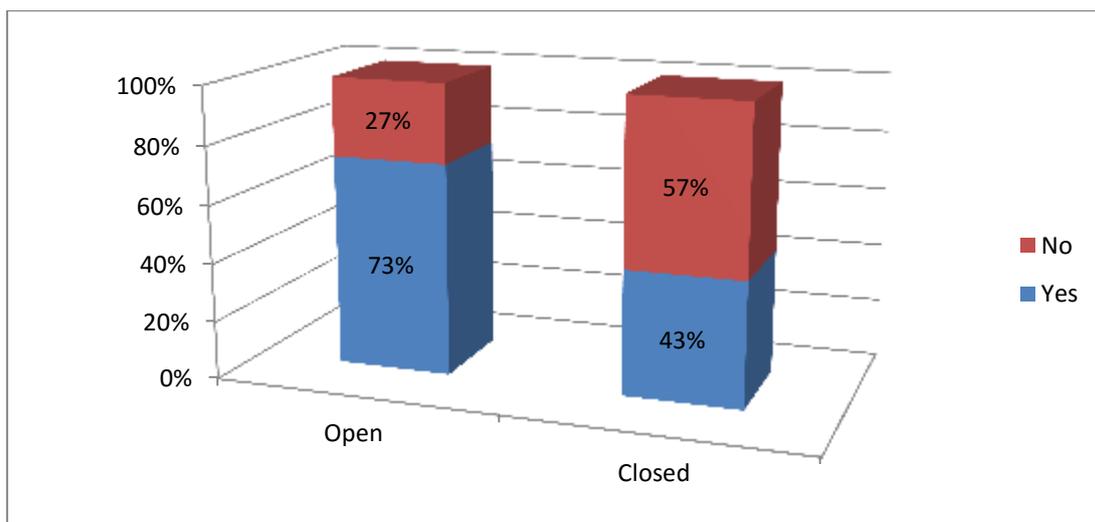


Fig 8: Shows distribution of type of sanitation for analysis of antenatal case for hepatitis E virus antenatal cases (n=100)

DISCUSSION:

The replication strategy of the HEV is poorly understood. Several strategies for experimental propagation and production of HEV to study the molecular biology and facilitate vaccine development have been published, but their reproducibility and feasibility need confirmation. A recent *in vivo* cell culture experiment used *in vivo* infected highly differentiated primate liver cells for the *in vitro* replication of HEV in a serum-free medium supplemented with growth factors and hormones [12]. A strand-specific RT-PCR technique was used for monitoring. Both positive strand and negative-strand HEV RNA were detected in cellular RNA of the culture cells and the positive-strand HEV RNA was detected in the culture medium (indicating shedding of virus-like particles into the culture medium). No cytopathic effects were observed. Thereafter, using the identical culture system, primary hepatocytes were infected with non-inactivated tissue culture-derived viruses and replication of HEV RNA was demonstrated in this model [13]. A neutralizing anti-HEV antibody directed against the ORF-2 encoded putative capsid protein blocked the infection of the liver cells. New Chinese strains of HEV have been isolated and cultivated in an *in vitro* cell culture using continuous cell lines derived from the lung, kidney, or liver. Recently, a Chinese HEV isolate was successfully cultivated in an A549 cells (human lung carcinoma cells) under the conditions of a relatively high concentration of MgCl₂ (30 mM), a pH of 7.2, and a short (< 6 months) preservation time of propagated strains. Cytopathic effects (cell rounding and mono layer destruction) were visible at day 2 post-inoculation and could be neutralized by specific acute phase antibodies to HEV [14].

Xenotransplantation with swine organs and tissues can potentially offer an alternate solution to the shortage of transplantable human organs. However, zoonosis, the inadvertent transmission of swine pathogens from pigs to human xenograft recipients, is of major concern [15]. Previous studies have shown that swine HEV appears to be ubiquitous in pigs, and can infect across species barriers. Thus, transmission of swine HEV from pig tissues/organs to human xenograft recipients and the potential subsequent transmission of the virus to others such as family members and healthcare workers are possible since hepatitis E virus. Is most likely transmitted fecal-orally, there is also growing concern about the possibility of contracting the disease by consuming undercooked pork meat or by drinking swine feces and contaminated water [16]. Therefore, the objectives of this study were to assess the risks of transmitting swine HEV from infected pigs to naïve pigs and to evaluate the usefulness of an *in vitro* RT-PCR assay in detecting swine HEV as compared to an *in vivo* swine bioassay.

The routes of inoculation varied in order to mimic the procedures in xenotransplantation (intravenous), meat consumption (oral), and natural transmission (presumably fecal-oral route). Intravenous inoculation (with feces and liver tissue) was successful, but oral inoculation (with feces and muscle tissue) was not successful in transmission of HEV in this study. Even our standard infectious pool (swine feces) of HEV with a titer of 10⁶ GE/ml was not adequate to induce infection orally [17]. This observation is consistent with earlier studies with hepatitis A and E viruses in non-human primates the results suggest that HEV transmission via fecal-oral route requires a much higher dose compared to the intravenous route of transmission. It also suggests that the likelihood of transmitting HEV via consumption of pork from an HEV infected pig is minimal. Many factors may influence the infectivity of HEV infection such as the virus titer, the ratio of infectious to defective viral particles, the inoculation routes, and host factors such as immune status and age at exposure [18]. In the developed world the rate is significantly low. A study in Spain by Lindemann *et al.*; on 1040 pregnant women reported the rate of anti-HEV IgG was 3.6%. Prevalence of HEV IgG was found to be 7.7% and 10% in pregnant women in France and China, respectively which is much lower than our finding (68-70). Regarding anti-HEV IgM antibodies, 0.5% (2/386) of pregnant women were positive. Other have reported prevalence rate of 0% in France, 0.64% in Spain and 10% in Ghana [19]. Reason for these differences could be due to difference in level of hygiene, educational status, social status, and endemicity of virus, different lifetime exposures of the participants to HEV and use of different test systems with varying sensitivity. For example in Egypt a study found higher HEV seroprevalence among pregnant women (84.3%). Their result confirmed that Egypt's high HEV endemicity and show that almost all women of child bearing age in the community had prior HEV exposures without a history of liver disease [20]. They suggested that reasons for high HEV sero prevalence and lack of clinical hepatitis could be the result of early childhood HEV exposures, producing long-lasting immunity and/or modify subsequent responses to exposure. High rates of HEV infection are usually seen in areas with low standard of living where major contamination of water supply is likely to occur. These conditions include, low standard of hygiene, lack of proper disposal system plus unsafe water supply [21].

CONCLUSIONS:

Extensive data suggest that hepatitis E is a major contributor to disease and mortality across much of the Indian continent, with country-level variability, as expected. Still, it is challenging to make comparisons across these populations, given differing methodologies and assays used to determine HEV etiology [23]. Despite its substantial impact on human health, HEV

has, even in hyper-endemic South Asia, been neglected in recognition as a major public health problem since its identification. Given the emerging evidence that HEV could be vaccine preventable, we hope this review will shed light on a pathogen of significance across the Asian continent [24].

Source of support – nil

Conflict of interest none

REFERENCES

1. Balayan MS. Epidemiology of hepatitis E virus infection. *Journal of viral hepatitis*. 1997 May 1; 4(3):155-66.
2. Nanda SK, Yalcinkaya K, Panigrahi AK, Acharya SK, Jameel S, Panda SK. Etiological role of hepatitis E virus in sporadic fulminant hepatitis. *Journal of medical virology*. 1994 Feb 1; 42(2):133-7.
3. Hussaini SH, Skidmore SJ, Richardson P, Sherratt LM, Cooper BT, O'Grady JG. Severe hepatitis E infection during pregnancy. *Journal of viral hepatitis*. 1997 Jan 1; 4(1):51-4.
4. Khuroo MS, Kamali S, Jameel SH. Vertical transmission of hepatitis E virus. *The Lancet*. 1995 Apr 22; 345(8956):1025-6.
5. Krawczynski K, Kamili S, Aggarwal R. Global epidemiology and medical aspects of hepatitis E. *InForum (Genoa, Italy)* 2000 Dec (Vol. 11, No. 2, pp. 166-179).
6. Panda SK, Thakral D, Rehman S. Hepatitis E virus. *Reviews in medical virology*. 2007 May 1; 17(3):151-80.
7. Smith JL. A review of hepatitis E virus. *Journal of Food Protection®*. 2001 Apr 1; 64(4):572-86.
8. Choi IS, Kwon HJ, Shin NR, Yoo HS. Identification of swine hepatitis E virus (HEV) and prevalence of anti-HEV antibodies in swine and human populations in Korea. *Journal of clinical microbiology*. 2003 Aug 1;41(8):3602-8.
9. Yoo D, Willson P, Pei Y, Hayes MA, Deckert A, Dewey CE, Friendship RM, Yoon Y, Gottschalk M, Yason C, Giulivi A. Prevalence of hepatitis E virus antibodies in Canadian swine herds and identification of a novel variant of swine hepatitis E virus. *Clinical and Diagnostic Laboratory Immunology*. 2001 Nov 1; 8(6):1213-9.
10. Boutrouille A, Bakkali-Kassimi L, Crucière C, Pavio N. Prevalence of anti-hepatitis E virus antibodies in French blood donors. *Journal of clinical microbiology*. 2007 Jun 1; 45(6):2009-10.
11. Fukuda S, Sunaga J, Saito N, Fujimura K, Itoh Y, Sasaki M, Tsuda F, Takahashi M, Nishizawa T, Okamoto H. Prevalence of antibodies to hepatitis E virus among Japanese blood donors: identification of three blood donors infected with a genotype 3 hepatitis E virus. *Journal of medical virology*. 2004 Aug 1; 73(4):554-61.
12. Labrique AB, Thomas DL, Stoszek SK, Nelson KE. Hepatitis E: an emerging infectious disease. *Epidemiologic reviews*. 1999 Jan 1; 21(2):162-79.
13. Emerson SU, Purcell RH. Hepatitis E virus. *Reviews in medical virology*. 2003 May 1; 13(3):145-54.
14. Ticehurst JR. Hepatitis E virus. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover FC, eds. *Manual of Clinical Microbiology*. Washington, DC: ASM Press. 1995. pp. 1056–1067.
15. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Reviews in medical virology*. 2006 Jan 1; 16(1):5-36.
16. Meng XJ. Novel strains of hepatitis E virus identified from humans and other animal species: is hepatitis E a zoonosis?. *Journal of hepatology*. 2000 Nov 1; 33(5):842-5.
17. Bradley DW. Hepatitis E virus: a brief review of the biology, molecular virology, and immunology of a novel virus. *Journal of hepatology*. 1994 Dec; 22(1 Suppl):140-5.
18. Clayson ET, Shrestha MP, Vaughn DW, Snitbhan R, Shrestha KB, Longer CF, Innis BL. Rates of hepatitis E virus infection and disease among adolescents and adults in Kathmandu, Nepal. *Journal of Infectious Diseases*. 1997 Sep 1; 176(3):763-6.
19. Khuroo MS, Teli MR, Skidmore S, Sofi MA, Khuroo MI. Incidence and severity of viral hepatitis in pregnancy. *The American journal of medicine*. 1981 Feb 28; 70(2):252-5.