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Serological and Molecular Evidence of Hepatitis E Infection among Patients with Suspected Viral Hepatitis Seen at Korle-Bu Teaching Hospital, Accra

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LHOA and JKO contributed equally by designing the study, writing the protocol and writing the first draft of the manuscript. Author JHKB managed the literature searches and analyses of the study performed. Author JB managed the experimental process and author TA edited and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Ghana is not clearly demarcated in the endemic region for hepatitis E virus but serological studies have shown a high anti-HEV seroprevalence among pregnant women in Accra, Ghana. In this study, we investigated to assess the HEV seroprevalence in patients presenting with symptoms of hepatotropic virus at the Korle-Bu Teaching Hospital.
Study Design: Structured questionnaire was administered to each consenting patient with suspected viral hepatitis to document their demographic characteristics as well as diagnosis.
Place and Duration of Study: The investigations were done between between April 2010 and March 2011 at the Korle-Bu Teaching Hospital in Accra, Ghana.

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Methods: We obtained 17 patients blood samples and 86 archival blood samples that had previously tested negative for HBV and HCV to determine the seropositivity and seroprevalence of HEV (IgG and IgM). Information was obtained from subjects using structured questionnaire. We measured anti-HEV using enzyme-linked immunosorbent assay (ELISA) kit. HEV antigen from positive samples was measured by reverse transcription polymerase chain reaction (RT-PCR).
Results: The overall serological prevalence of anti-HEV specific antibodies was low. Of the 103 serum samples tested, only 6 were positive for anti-HEV specific antibodies which gave a seroprevalence of 5.8%. Fifty percent of the ELISA seropositive samples were confirmed to be positive for HEV RNA by real time RT-PCR. All the confirmed positives were from females, 1 of whom was pregnant.
Conclusions: Evidence from this study points to hepatitis E virus as one of the causes of viral hepatitis among patients seen at the Korle-Bu Teaching Hospital in Accra, Ghana suggesting that HEV remains an under-recognized and significant public health problem that needs further attention and research.

Keywords: Hepatitis E virus; seroprevalence; Korle-Bu; Ghana.

1. INTRODUCTION

Hepatitis E virus (HEV) is a nonenveloped virus with a single-stranded and positive-sense RNA genome of an approximately 7.2 kb length [1]. HEV is classified in the Hepeviridae family as a separate Orthohepevirus genus Orthohepevirus A species [2]. It is a major pathogen causing acute hepatitis in young people in developing countries where there is poor sanitation and a high population density. HEV infection is often asymptomatic but can induce a self-limited acute hepatitis [3]. According to infection of host, HEV is divided into the mammalian and avian HEV [4]. The mammalian HEV mainly infects human and other mammals. Research has revealed the existence of a zoonotic reservoir, as the virus has been isolated from wild and domestic animals including swine, cattle, chickens, sheep, goats, and rodents [5,6]. Four distinct genotypes have been identified in HEV isolates differing in geographical distribution and infection modalities, and further dividing 4 genotypes into 24 subtypes has been proposed [7]. Genotypes 1 and 2 are exclusively found in humans and in tropical areas in Africa, Asia and South America, and transmission usually occur via contaminated drinking water and often in large outbreaks [8]. Recent research has shown that genotypes 1 and 2 caused more than 2.8 million HEV infections in sub-Saharan Africa in 2005 [9]. Genotypes 3 and 4 have been identified in humans and several animal species in both developing and industrialized countries and exhibit the characteristics of zoonosis [10,11], but genotype 3 has a worldwide distribution and infections are increasingly reported from industrialized countries.

Extrahepatic complications including neurological disorders [12-16] myasthenia gravis [17], thrombocytopenia [18], aplastic anemia [19], glomerulonephritis [20] and acute pancreatitis [21] have been described in association with HEV infection in European and Asian countries. Among them, neurological complications are more frequently reported, including Guillain-Barré syndrome, brachial neuritis, transverse myelitis, cranial nerve palsy and peripheral neuropathy [12,13,15,16].

In Ghana, anti-HEV has been detected in children living in rural areas [22]. Serological studies have shown a high anti-HEV seroprevalence among pregnant women in Accra, Ghana [23] and fatal cases of HEV in pregnant women have also been documented in southern Ghana [24]. However, patients who visit the hospital with signs and symptoms consistent with viral hepatitis are normally investigated for hepatitis B and C viruses but not HEV. As a result a lot of samples that tested negative for HBV and HCV were not investigated further. Manifestation of clinical or subclinical HEV infection and the circulating genotypes have not yet been reported in the literature in Ghana, even though HEV presents a major public health problem with epidemic potential in most developing countries. We investigated to assess the HEV seroprevalence in patients presenting with symptoms of hepatotropic virus at the Korle-Bu Teaching Hospital.

2. MATERIALS AND METHODS

This study was carried out between April 2010 and March 2011 at the Korle-Bu Teaching Hospital in Accra, Ghana.

2.1 Study Site, Participants and Sample Collection

The Korle-Bu Teaching Hospital is the leading tertiary referral hospital in Ghana. The hospital serves the city of Accra (the capital city of Ghana with a population of about four million) and its surrounding environs and also receives referred cases from the rest of the country (Fig. 1). Structured questionnaire was administered to each consenting patient with suspected viral hepatitis to document their demographic characteristics as well as diagnosis. Blood samples (5 ml) were collected from 17 patients by venepuncture from all the enrolled subjects into sterile serum separator tubes (Table 1). In addition, another 86 archived samples from the Central Laboratory that had tested negative for HBsAg and anti-HCV were recovered and tested for antibodies to HEV by Enzyme-Linked Immunosorbent Assay (ELISA).

2.2 Serologic Testing for HEV Infection

ELISA for the qualitative determination of antibodies to hepatitis E virus was performed using the DRG HEV Ab ELISA as described by the manufacturer for samples that were negative for immunoglobulin (Ig) M class antibodies against hepatitis A virus (HAV), hepatitis B virus (HBV) markers (anti-HBV core IgM and hepatitis B surface antigen (HBsAg)), anti-hepatitis C virus (anti-HCV).

Microwells coated with HEV specific synthetic antigens derived from oral reading frame 2 and 3 (ORF2 and ORF3) regions of the HEV genome from genotypes 1 and 2 HEV strains were used (DRG Instruments GmbH, Germany). The ORF2 gene encodes the major capsid protein whiles the ORF3 gene is suggested to regulate host cell environment via its interaction with various intracellular pathways [25].

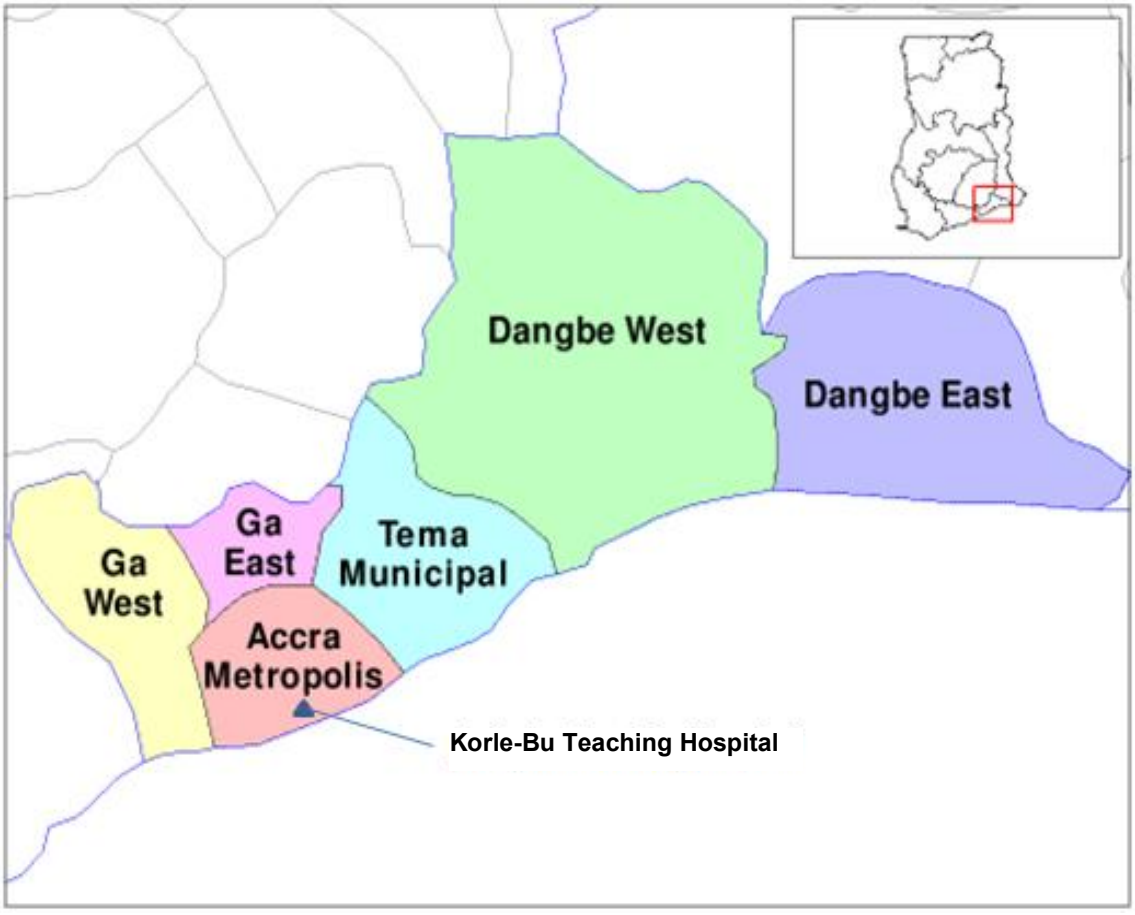

Fig. 1. District map greater Accra region showing the location of Korle-Bu Teaching Hospital and surrounding environs

Table 1. History of samples screened for Hepatitis E

Sample ID	Age	Sex	Date of collection	Location
001	28	Male	12-05-2010	Medical Ward
002	67	Male	24-06-2010	Medical Ward
003	49	Female	24-06-2010	Medical Ward
023	27	Female	22-07-2010	Medical Ward
024	44	Female	22-07-2010	Medical Ward
025	Adult	Female	22-07-2010	Medical Ward
026	29	Male	22-07-2010	Medical Ward
027	25	Female	22-07-2010	Medical Ward
034	43	Male	11-08-2010	Medical Ward
035	56	Male	11-08-2010	Medical Ward
036	28	Female	16-08-2010	Medical Ward
054	43	Female	17-09-2010	Medical Ward
055	41	Female	22-09-2010	Medical Ward
068	38	Male	25-10-2010	Medical Ward
079	31	Female	18-11-2010	Medical Ward
080	47	Female	18-11-2010	Medical Ward
081	19	Female	18-11-2010	Medical Ward

2.3 RNA Extraction and Real Time RT-PCR for HEV Infection

RNA extraction and real-time PCR were as described before [26]. Briefly, viral RNA was extracted from 140 µl of samples using the QIAmp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was eluted in 60 µl of elution buffer and 8µl used as template for real time Reverse Transcription-Polymerase Chain Reaction (rRT-PCR) for the detection of HEV RNA. The total RNAs were subjected to real time RT-PCR using reverse (5'-AGG GGT TGG TTG GAT GAA-3') and forward (5'-GGT GGT TTC TGG GGTGAC-3') primers and a probe consisting of an oligonucleotide with a 5'-reporter dye (FAM) and a 3'-quencher dye (TAMRA) (5'-FAM-TGA TTC TCA GCC CTT CGC-TAMRA-3'). The primers targeted the ORF2/ORF3 overlapping region of the HEV genome and the assay had the capability of amplifying all four known HEV genotypes.

RNA was reverse transcribed for 30 minutes at 50°C, followed by one cycle of denaturation at 95°C for 15 minutes. This was followed by 50 amplification cycles at 94°C for 15 seconds, 56°C for 30 seconds and 76°C for 30 seconds. Denaturation was performed at 94°C for 15 min, an annealing step at 56°C for 30 sec 50 cycles and an extension step at 76°C for 30 sec. Data acquisition and data analysis were carried out on a 7500 Fast Real Time PCR System from Applied Biosystems. Negative and non-template controls were included to rule out non-specific amplification.

2.4 Data Management and Analysis

Data was double entered and cleaned in Excel and the analyses done with SPSS software package version 19. Appropriate measures of central Tendency for mean, median frequency distributions, percentages, standard deviation and a student T-test for 95% confidence interval were calculated.

2.5 Ethical Considerations

The study protocol was approved by the Ethical Review Committee of the University of Ghana Medical School. Protocol identification number MS-Et/M.6-P.4.2/2009-10. The purpose and procedures of the surveys were explained to all participants, and a written informed consent was obtained from all of them. We protected the confidentiality of participants through use of codes.

3. RESULTS

A total of 103 serum samples collected from 17 individual patients and 86 archival samples from the Central Laboratory with suspected viral hepatitis were obtained and tested for anti-HEV. The samples tested came from patients whose age ranged from two weeks to seventy-five years old. The mean age was 33, with a standard deviation of 14.5. Sixty-five per cent of the

population was female with female:male ratio of 1.9:1.

Majority (37.86%) of the study participants fell within the 16-30 year group, followed by the 31-45 year group with a percentage of 34.95 (Fig. 2). Six samples tested positive for anti-HEV specific antibodies by ELISA; 5 had been clinically diagnosed as viral hepatitis and 1 as enteric sepsis. Of the six, 5 were females, 4 of which were in the child-bearing age of between 15 years to 45 years, and 3 out of this 4 were pregnant. (Table 2) Only 1 (2.8%) out of the 36 men tested for anti-HEV was positive by ELISA while 35 (97.2%) were negative. Five (7.5%) out of the 67 women tested for anti-HEV showed positive reactivity while 62 (92.5%) were negative by ELISA. An overall seroprevalence rate of 5.8% was observed (Table 3).

A significant proportion of the patient samples tested (5.8%, 95% CI = 0.0245-0.1238) were anti-HEV seropositive. Three (50%) were from pregnant women while 2 (33.3%) were from non-pregnant women. (Table 4). Majority (83.3%) of the anti-HEV seropositive patients fell in the young to middle aged adult group (16-45 years) (Fig. 3).

Out of the 6 ELISA positive samples, 3 were positive for HEV RNA by real time RT-PCR (Fig. 4). All 3 PCR positives were from females aged 27, 35 and 70 years (Mean Age = 44) (Table 5). The mean copy number for the three positive samples was 36.19 (Range: 34.92-37.80, S.E. = 0.85, S.D. =1.47) (Table 4) with a significance level P=0.001 and 95% C.I. =32.54-39.84.

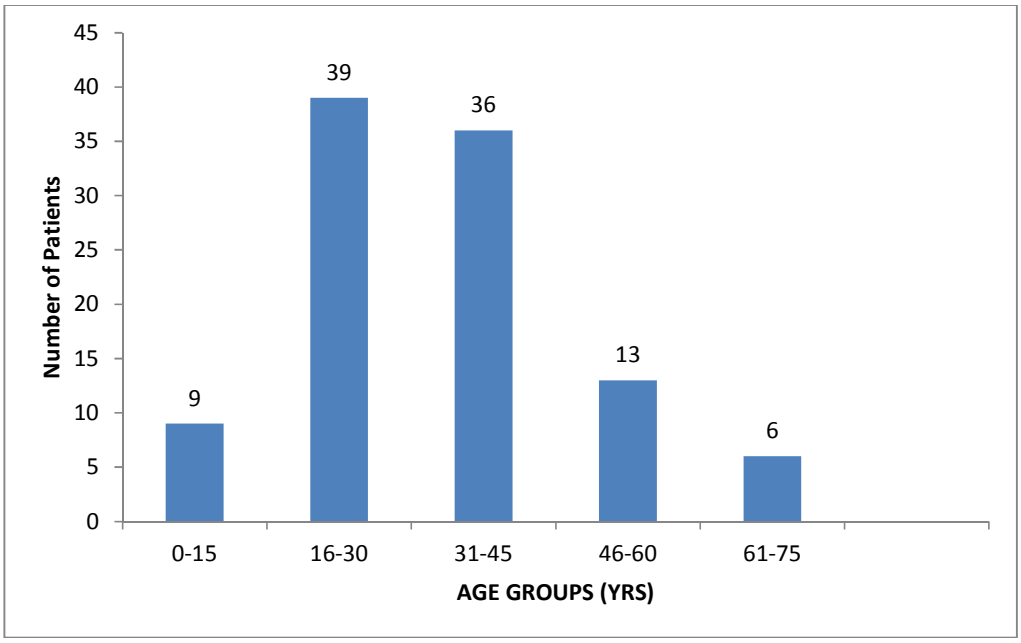

Fig. 2. Age distribution of study participants

Table 2. HEV Ab ELISA seropositive samples

Sample ID	Sex	Age	SOD/CO	Elisa result	Clinical diagnosis
058	F	70	8.034	POS.	Viral hepatitis
019	F	27	1.423	POS.	Viral hepatitis in cyesis
091	F	35	8.157	POS.	Viral hepatitis
006	M	43	5.980	POS.	Enteric sepsis
093	F	28	1.231	POS.	Viral hepatitis in cyesis
077	F	38	1.233	POS.	Viral hepatitis in cyesis

Cut off-values with optical density (OD) >1.1 are positive, between 0.9 and1.1 are equivocal and <0.9 are negative

Table 3. Prevalence of anti-HEV in males and females

Results	Male	Female	Total
Positive	1 (2.8%)	5 (7.5%)	6 (5.8%)
Negative	35 (97.2%)	62 (92.5%)	97 (94.2%)
Total	36	67	103

Table 4. Proportion of males, pregnant and non-pregnant women with anti-HEV seropositivity

Category	Ab Pos	Ab Neg	Total
Pregnant females	3	27	30
Non-pregnant females	2	35	37
Males	1	35	36
Total	6	97	103

4. DISCUSSION

Hepatitis E Virus (HEV), the causative agent of hepatitis E, poses a major public health problem in both the developing and advanced world. Ghana is not clearly demarcated within the endemic areas for hepatitis E virus infections although isolated cases in pregnant women have been documented. The present study was performed to investigate the presence of HEV infection in samples that have tested negative for HBV and HCV in patients at the Korle-Bu Teaching Hospital. We found that HEV infection exist in patients but in low prevalence rate of 5.8%. Studies by Martinson et al. [22] who observed similar low seroprevalence rate of 4.4% in children living in rural Ghana which is contrary to the high rate of 38.1% observed among pig handlers in Accra [27] and 28.36% among pregnant women at the Obstetrics and Gynecology Department of the Korle-Bu Teaching Hospital [28]. These discrepancies in prevalence rates are likely to be related to the rural urban differences in study areas. The observed high seroprevalence by Adjei et al. [27] in the two groups they studied gave the indication that HEV may be circulating in the general population. This might be due to the fact that hepatitis E is a self-limiting illness with variable severity depending on host and immune factors [28].

Table 5. Prevalence of HEV RNA in ELISA seropositive patients

Sex / Results	Male	Female	Total
Positive	0 (0%)	3 (60%)	3 (50%)
Negative	1 (100%)	2 (40%)	3 (50%)
Total	1 (100%)	5 (100%)	6 (100%)

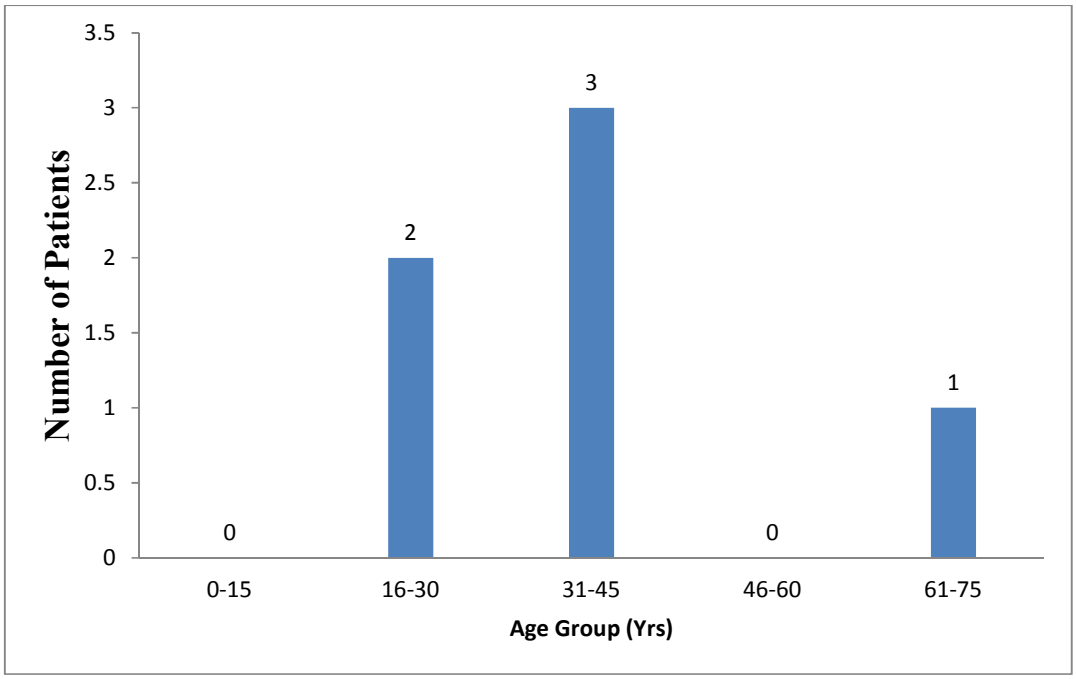

Fig. 3. Age distribution of patients with anti-HEV

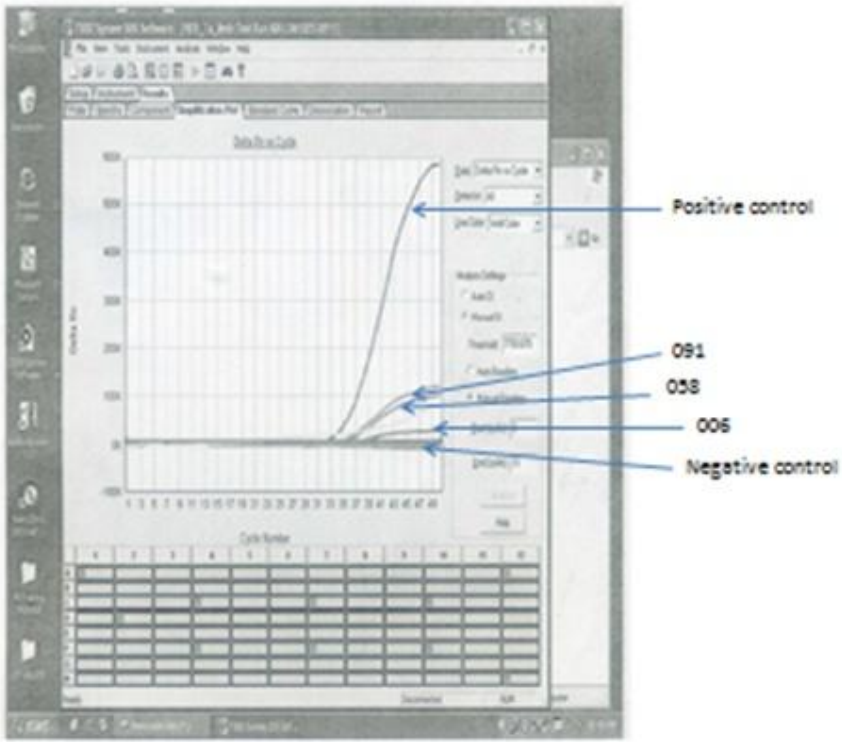

Fig. 4. Aplification plots/curves showing the Ct values of positive and negative controls and 3 positive samples

Hepatitis E infection is particularly life-threatening in pregnant women and several studies indicate HEV to be apparently the only hepatotropic virus with this virulent impact on pregnant women. [29]. Even though this study did not directly involve pregnant women it is worth noting that 5 of the ELISA positives were from females and 4 (66.7%) of them were of childbearing age. A very high proportion of 3 (50%) out of the 6 seropositive patients were actually pregnant women, one of whom was confirmed positive for HEV RNA and they constitute the highest at-risk group [30]. In addition all the 3 samples that were confirmed positive for HEV RNA by real time RT-PCR were from the female population.

In this study, it was observed that more females than males were positive for HEV by ELISA. Our findings are in accordance with the work done by Martinson [22] but inconsistent with what was reported in other studies in Nigeria [30] and South Korea [31] where more males than females were affected. However, differences in other countries may be influenced by different test systems with varying sensitivities, effects of sample selection e.g. age, exposure to contaminated food sources (uncooked meat) and contact to certain animal species e.g. pigs, wild boar and deer [32]. In the studied population, anti-HEV was found to occur more in the 31-45 year age-group, followed by the 16-30 year age-group. This is consistent with what Irshad et al. [33] had reported earlier even though they found hepatitis E infections to be predominate in young male adults.

Inadequate treatment of drinking water, improper disposal of sewage and low standards of sanitation have been implicated in major outbreaks in developing countries [34]. Parenteral transmission by blood transfusion and zoonotic transmission in people with occupational contact with these animals have been documented [35,36]. It is not known if the HEV infection found in the study population was acquired via fecal contamination of drinking water, consumption of raw/undercooked meat or as a result of occupational contact with animals. This is because demographics showing sources of drinking water and possibility of contact with animals were not documented. Also patient characteristics such as history of blood transfusion and travel to known endemic countries were not taken. It is known that highest

rates of infection occur in regions where low standards of sanitation promote the transmission of the virus [30]. Epidemics of hepatitis E have been reported in Central and South-East Asia, North, East and West Africa, and in Mexico, especially where faecal contamination of drinking water is common. [34,37]. In Accra, slums are rapidly developing and in such areas, places of convenience are virtually non-existent. Sanitary conditions are also deplorable. There is no provision of clean, potable water and where available, a lot of illegal water connections can be found with some of the pipes broken and sewage seeping into them. It is also not uncommon to find the urban poor living in close association with the few animals, especially sheep and goats that they rear. These are all risk factors for contracting the hepatitis E virus infection.

At the Korle-Bu Teaching Hospital, there is no well-defined investigative protocol to detect hepatitis E in pregnancy or in the general sick population. Investigations of viral hepatitis only focus on hepatitis B and C viruses, because these cause frequent chronic infections and are perceived to present a more pressing public health problem. The research findings herein indicate that hepatitis E virus is one of the causes of hepatitis among patients with suspected viral hepatitis seen at the Korle-Bu Teaching Hospital in Accra, Ghana. Since the protocol of investigating viral hepatitis does not extend to HEV, a significant number of cases of hepatitis due to HEV are being missed. When differential diagnosis such as yellow fever and malaria turn out to be negative, samples from patients presenting with signs and symptoms of hepatitis are discarded. Our findings showed that HEV contributes to the hepatitis burden in patients with suspected viral hepatitis. This sense of complacency, particularly because mortality rates are approximately less than 1% in the general population has led to HEV being relegated to the background. From the research findings, it is prudent to stipulate that many cases of hepatitis due to HEV may have been missed over the years and these cases may have contributed to fatalities in the high-at risk group. It must now be recognized that where other causes of acute hepatitis have been excluded, HEV infection should be considered.

5. CONCLUSIONS

The observed seroprevalence rate of anti-HEV in patients with suspected viral hepatitis at KBTH indicates that HEV contributes to viral hepatitis cases seen in KBTH but because HEV is not investigated its involvement has been overlooked. Although the sample population was not large enough, this study shows that a significant proportion of pregnant women had the hepatitis E virus and this is the highest at-risk group. This observation suggests that HEV remains an under-recognized and significant public health problem that needs further attention and research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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