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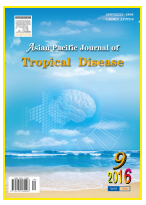

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Occurrence of human hepatitis E virus in Norway rats: A zoonotic potential with great public health implications

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ABSTRACT

Objective: To investigate the occurrence of human hepatitis E virus genotype I among sheep and rats as well as seroprevalence of hepatitis E virus immunoglobulin G (IgG) antibodies among people live in rural settings.
Methods: Fecal samples were collected from 43 Norway rats and 30 sheep. All fecal samples were examined for the presence of human hepatitis E virus genotype I through direct detection using RT-PCR. In addition, serum samples collected from 90 apparently healthy persons live in rural settings were examined for the presence of hepatitis E virus IgG antibodies by using enzyme linked immunosorbent assay.
Results: Out of 73 examined animals, human hepatitis E virus genotype I was detected in five animals giving an overall prevalence 6.8% while only rats given positive results 11.6%. Furthermore, the overall seroprevalence of hepatitis E virus IgG antibodies among the examined individuals was 63.3% while the seroprevalence in adults (75.0%) was higher than that in children (34.6%).
Conclusions: The detection of human hepatitis E virus genotype I in the feces of Norway rats in such high prevalence highlights the possible role which may be played by such animal in the epidemiology of hepatitis E virus infections in rural settings where the virus is more prevalent among human populations.

1. Introduction

Hepatitis E virus was firstly recognized in 1978 after large epidemic of hepatitis in Kashmir Valley while the virus was sequenced in 1990 to be genetically recognized[1]. Nowadays, hepatitis E is considered one of the most important enterically transmitted viral hepatitis affecting humans throughout the world. The virus is endemic in many developing countries where outbreaks usually occurred whereas sporadic form of the disease is more common in the developed ones[2]. Hepatitis E virus is a single stranded positive sense RNA virus belonged to genus *Hepevirus*, family Hepeviridae. To date, hepatitis E virus can be classified in to four genotypes I-IV with characteristic geographical distribution, genotype I in Asia and North African countries but Central Africa and Mexico for genotype II; America, Europe and Japan for genotype III while genotype IV was exclusively present in Asia[3]. The global burden of hepatitis E was estimated to be 2 billon out of total human population (6 billion) and most of them were concentrated in countries of poor resources[4]. In addition, according to World Health Organization records, annually there are more than 20 million hepatitis E virus infections with 3 million acute cases and about 56 000 related to deaths[5]. Hepatitis E infections usually manifested as acute viral hepatitis including fever, malaise, icterus, hepatomegaly and elevated liver enzymes with low mortality rate (less than 1%) while the disease is extremely serious in pregnant women with high case fatality rate which may reach 25%[6]. The epidemiology of hepatitis E virus

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is not well-known. Hepatitis E virus genotype I and genotype II were strictly infect humans whereas genotypes III and IV infect both humans and animals[7]. The main mode of transmission is thought to be fecal-oral route especially via contaminated water as many large water-borne outbreaks were reported meanwhile the zoonotic route of transmission was proposed after the detection of hepatitis E virus in pigs[8]. Recently, the virus has been detected in a wide range of animal species rather than swine including boars, deer, sheep and rabbits to expand the zoonotic spectrum of the virus[9]. Consequently, the zoonotic transmission of hepatitis E to humans has gained ground and it was assumed to be taken place either through direct contact with infected animals or ingestion of contaminated food of animal origin[10]. Curiously, undercooked meat from infected animal reservoirs such as pigs, deer and wild boars were incriminated to be sources of hepatitis E infections to underline the importance of the zoonotic route in the epidemiology of hepatitis E virus among humans[11]. Moreover, in recent study that was conducted in Egypt, the seroprevalence rates of the hepatitis E antibodies among farm animals were 21.6%, 14%, 4.4% and 9.4% for cows, buffaloes, sheep and goats respectively which may point out to the potential role of animals in the epidemiology of hepatitis E in Egypt[12]. In contrast, the recent identification of hepatitis E virus variant in Norway rats in Germany[13] which then become specific genotype of hepatitis E virus for rats may fade the contention of zoonotic potential of hepatitis E virus from some animals as the cross antigenic reactivity between human and non-human hepatitis E virus variants cannot be ruled out. Hence, the accurate data about the zoonotic burden of hepatitis E virus is still sparse and requires the direct detection of human hepatitis E virus in the feces of such animals to prove the zoonotic link. Therefore, the current study was conducted to investigate the occurrence of human hepatitis E virus genotype I in sheep and Norway rats as well as the seroprevalence of hepatitis E virus antibodies among humans resided in rural settings to improve the knowledge about the actual role of such animals in the epidemiology of hepatitis E virus.

2. Materials and methods

2.1. Animal samples

Fecal samples were collected from the recta of 43 Norway rats [*Rattus norvegicus* (*R. norvegicus*)] after they were anesthetized and humanely killed via cervical dislocation[14]. All rats were trapped from rural districts of Giza and Fayium governorates, Egypt. Moreover, fecal samples were gathered from 30 apparently healthy sheep. All animal samples were inserted in sterile tubes and kept at –80 ˚C till processed.

2.2. Human samples

Blood samples were collected from 90 apparently healthy individuals in such contact with farm animals and resided in rural districts of Giza and Fayium governorates, Egypt. All blood samples were received in sterile tubes and transported in icebox to the laboratory. Upon arrival, blood samples were centrifuged to yield sera which stored at –20 ˚C until used. Human samples were collected after the participants gave their consents while sampling was done in private medical laboratories via experts and under complete medical supervision.

2.3. Molecular detection of human hepatitis E virus genotype I in the feces of examined animals

2.3.1. RNA extraction

RNA was extracted from fecal samples of the examined animals using GeneJET viral DNA and RNA purification kit (Thermo Scientific, Waltham, USA) and the procedure was done according to the manufacturer's instructions. The obtained RNAs were stored at –80 ˚C till used.

2.3.2. RT-PCR step

The extracted RNAs were entered RT-PCR step to amplify ORF1 conserved region of human hepatitis E virus genotype I using the following set of primers: forward 5' CAT GGT CGA GAA GGG CCA GG 3'; reverse: 5' GCG GAA GTC ATA ACA GTG GG 3'. Primers were synthesized by Metabion (Munich, Germany) according to Zhao *et al.*[15]. The primers were checked through BLAST analysis for its specificity to human hepatitis E virus genotype I and did not match with hepatitis E virus variants such as rat specific hepatitis E virus genotype and avian specific hepatitis E virus genotype. RT-PCR was carried out as one step reaction using PrimeScript™ one step RT-PCR kit ver.2 (Takara, Japan) with the following thermal profile: 50 ˚C for 30 min; 95 ˚C for 15 min followed by 40 cycles of 95 ˚C for 55 s; 53 ˚C for 55 s; 72 ˚C for 90 s; then final extension at 72 ˚C for 10 min. After RT-PCR step, amplicons were electrophoresized to visualize specific bands at 562 bp.

2.3.3. Nested PCR step

Nested PCR was done using the DNA products of the former RT-PCR as DNA templates to confirm hepatitis E virus genotype I using the following set of primers: forward one: 5'-ATG ACT TTG CTG AGT TTG ACT-3'; reverse one: 5'-CAT ATT CCA GAC AGT ATT CC-3'. The reaction was done using EmeraldAmp® GT PCR master mix (Takara, Japan) and the procedure was done according to Zhao *et al.*[15] while specific bands for hepatitis E virus genotype I were noted at 218 bp after electrophoresis step.

2.4. Detection of immunoglobulin G (IgG) antibodies against hepatitis E virus among the examined persons

Serum samples from the examined persons were tested for the presence of IgG antibodies against hepatitis E virus using ELISA kit (Wantai, Beijing, China), the protocol of the test was performed according to the instructions of the kit.

3. Results

Out of 73 examined animals, 5 yielded human hepatitis E virus genotype I in their feces giving an overall prevalence 6.8%. Only rats yielded positive results with prevalence 11.6% whereas none of sheep samples was positive (Table 1). On the other hand, the overall seroprevalence of hepatitis E virus antibodies among the examined individuals was 63.3% while the seroprevalence in adults (75.0%) was higher than that in children (34.6%) (Table 2).

Table 1
Occurrence of human hepatitis E genotype I among examined animals.

Animal species	Number examined	Number of positive	Percentage (%)
Norway rats	43	5	11.6
Sheep	30	0	0.0
Total	73	5	6.8

Table 2
seroprevalence of hepatitis E IgG antibodies among the examined individuals in rural settings.

Age	Number examined	Number of positive	Percentage (%)
Children < 12 years	26	9	34.6
Adults	64	48	75.0
Total	90	57	63.3

4. Discussion

In the last few years, hepatitis E virus has emerged to be one of the leading causes of acute viral hepatitis with a global impact nevertheless knowledge about its epidemiology is still limited. The zoonotic potential of hepatitis E virus is strongly proposed especially in rural communities where animals and humans usually share the same habitat. In Egypt, hepatitis E virus genotype I is the circulated hepatitis E virus genotype and has been detected among human patients[16,17]. The results of the current study revealed the occurrence of human hepatitis E virus genotype I among the examined Norway rats in a prevalence 11.6%, such high surprising result was augmented by that obtained by Maneerat *et al*. who experimentally infect *R. norvegicus* with hepatitis E virus obtained from infected human and they detected the virus in the serum and feces of infected rats for more than one month but unfortunately, this virus was not genotyped[18]. Moreover, Lack *et al.* in United States detected hepatitis E virus genotype III among the examined wild rats (*R. norvegicus*) and pointed out the possibility of rats to be potential reservoirs for such zoonotic hepatitis E virus genotype[19]. Therefore, the results of the current study highlighted the potential role which may be played by rats in the epidemiology of hepatitis E virus, a matter which has great public health implications. Noteworthy, Norway rat is a common inhabitant in rural settings in many countries and has the ability to access surface water to contaminate it through shedding of hepatitis E virus bearing in mind that hepatitis E virus is a water-borne virus and contaminated water stands behind many human infections[9]. Accordingly, infected Norway rats may contaminate irrigated water and consequently the virus may contaminate fresh produce and thereby reach human gut[20]. Furthermore, hepatitis E virus was previously detected in sewage[21] and whenever sewage is a common habitat for Norway rats (also called sewer rats) so that they may easily contract the infection from sewage and disseminate hepatitis E virus elsewhere during their daily activities considering hepatitis E virus is stable in soil for several weeks at environmental temperature[22]. Importantly, Norway rats may get entered human houses in rural settings and whereby contaminate house garden, households and human foodstuffs a matter which is of special concern for children in such settings regarding food-borne transmission of hepatitis E virus cannot be neglected as the virus may tolerate the cooking temperatures of some cooking procedures[23]. On the other hand, none of the examined sheep yielded hepatitis E virus genotype I in their feces however Wu *et al.* detected hepatitis E virus in sheep liver but it was mostly related to hepatitis E virus genotype IV[24].

In Egypt, despite the high seroprevalence of hepatitis E virus antibodies among humans in rural settings, hepatitis E virus outbreaks were not recorded and clinical cases of hepatitis E virus seem to be uncommon a matter which may be owed to that Egyptian hepatitis E virus strains may be of low virulence[16]. The results of the current study revealed that the seroprevalence of hepatitis E virus IgG antibodies among the examined individuals was 63.3%. This result is matched with previously obtained one by Fix *et al.* who recorded a seroprevalence exceeded 60% among examined rural inhabitants in Egypt with peak 76% in young adults[25]. Lower result was obtained by El-Tras *et al.* (38.1%)[12] whereas higher one (84.3%) was recorded by Stoszek *et al.*[26]. Such high seroprevalence of hepatitis E virus IgG antibodies obtained in the current study may be attributed to all examined individuals were resided in rural communities and mostly in intimate contact with animals while both factors were previously identified to be risk factors for hepatitis E virus infections[27]. Furthermore, the seroprevalence of hepatitis E virus IgG among adults (75.0%) was higher than that in children 34.3% a result which is lower than that of Fix *et al*. (2000) who recorded that the seroprevalence of hepatitis E virus antibodies among children reach 60%[25]. Our results indicated that children in rural settings may be exposed to hepatitis E virus in

their early life due to poor sanitation or contaminated households. These results were agreed with that obtained by Aboulata *et al.* (2005) who concluded that in Egypt children was early exposed to hepatitis E virus and the seroprevalence of hepatitis E virus IgG increases with age[28]. In conclusion, the results of the current study provided concrete evidence on the ability of Norway rat to acquire and disseminate human hepatitis E virus genotype I under natural conditions. Therefore, Norway rat should be considered as a potential reservoir for such hepatitis virus especially in rural settings where the disease is more prevalent.

Conflict of interest statement

We declare that we have no conflict of interest.

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