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Detection of Hepatitis E Virus RNA in Chicken Droppings and Pig Feces in Ogun and Lagos States, South Western, Nigeria

E. E. Ogbolu[1] and R. K. Obi[1,2*]

[1]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria.
[2]Department of Microbiology, Federal University of Technology, Owerri, P.M.B. 1526, Owerri, Imo State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background/Aim: Hepatitis E virus (HEV) is a major cause of sporadic and epidemic cases of enteric non-A non-B hepatitis in humans. It estimated that 14 million symptomatic cases of HEV infection, with 300,000 deaths and 5,200 stillbirths occur annually worldwide, with developing countries in the Indian subcontinent, Southeast and Central Asia, the Middle East, and northern and western parts of Africa being the most affected. This study was carried out to detect the presence of HEV RNA in commercial chicken and pigs in some parts of Ogun and Lagos states, South Western, Nigeria.
Materials and Methods: A total of 550 fecal samples were collected from chicken and pigs in both states. HEV RNA was extracted from the fecal samples and amplified by nested-PCR. Gel electrophoresis was used to evaluate the nested-PCR products.
Results: HEV RNA was detected in 10(1.8%) of the 550 samples. This comprised of 5(6.7%) positive from chicken droppings and 5(1.7%) from pigs feces. The result also showed that 3(4.3%)

**Corresponding author: Email: obi.robert@ymail.com;*

of chicken droppings collected from Ogun state were positive while there was no positive cases recorded in pig feces. Similarly 2(40%) of chicken droppings collected in Lagos state were positive while 5(1.7%) of pig feces were also positive.
Conclusion: The detection of HEV among commercially available chicken and pigs poses a great economic danger to poultry farmers and a tremendous public health risk to consumers of pork meat in Nigeria.

Keywords: Poultry farm; pig farm; virus; stool sample; Lagos State; Ogun State.

1. INTRODUCTION

Hepatitis E virus (HEV) is a non-enveloped, single-stranded positive sense RNA virus in the family *Hepesviridae* and the sole member of the genus *Hepesvirus* [1,2]. HEV is composed of three strains namely mammalian HEV, avian HEV and cut-throat trout virus. Avian HEV and cut-throat virus represent a potentially separate genus to mammalian HEV strains [3] while, although, avian HEV strains cause big liver and spleen disease in chickens [4,3], they have neither been recovered from mammals nor associated with human cases of hepatitis [5]. Mammalian HEV causes acute hepatitis in human beings and has a reservoir in pigs and possibly a range of other mammals [6].

Mammalian HEV isolates can be separated into at least 4 phylogenetically related genotypes [7,8], which differ with respect to geographic distribution, host range, and pattern of infection [9,10] and as such might be cross-protective. They include genotype 1 or Asian, North African and South American strains; genotype 2 or Mexican and West African strains; and genotype 4 made up of other Asian strains responsible for large epidemics and sporadic cases of hepatitis E in areas of endemicity; while genotype 3 causes isolated clinical cases in a sizeable group of mostly asymptomatic seropositive residents in developed countries [7]. HEV genotypes 1 and 2 infect only humans while genotypes 3 and 4 infect humans, pigs and other mammals in both developing and developed countries and at the same time causes autochthonous infection in humans [11,3].

HEV is a major cause of sporadic and epidemic cases of enteric non-A non-B hepatitis in humans [8,12]. It estimated that 14 million symptomatic cases of HEV infection, with 300,000 deaths and 5,200 stillbirths occur annually worldwide, with developing countries in the Indian subcontinent, Southeast and Central Asia, the Middle East, and northern and western parts of Africa being the most affected [13,14]. Though information about HEV infection is sketchy in Nigeria, cases have been reported among healthcare workers [15], animal handlers, rural dwellers, pregnant women [16], and as a coinfection in HIV positive patients [17].

Transmission is through the fecal-oral route involving ingestion of fecally-contaminated water and food is the most effective means of transmission of the virus [18,19]. This mode of transmission is usually associated with large waterborne epidemics in developing countries where poor sanitary conditions, scarcity of clean water, overcrowding and flooding are risk factors [12]. Zoonotic transmission of HEV has been documented in some parts of Asia and Europe [20,7,21]. Consumption of uncooked or insufficiently heat-treated meat or offal originating from domestic pigs, wild boar or deer as well as consumption of contaminated shellfish and direct contact with infected animals are other risk factors [22,23]. Direct transmission from human to human is rare but is common during an epidemic outbreak [24].

HEV infection might be benign or fulminant with death rates of ≤4% in the general population, and as high as 20-25% in pregnant women during their third trimester period [24,12]. Symptomatic disease is observed most frequently in young people and adults with age range of 14-40 years [25]. Clinically, hepatitis E manifests as icterus, malaise, anorexia, fever, hepatomegaly, and pruritus. These symptoms are accompanied by laboratory findings of elevated serum bilirubin levels, markedly elevated levels of liver enzymes, and mild increase in alkaline phosphatase activity [24].

No specific treatment exists for HEV. However the disease is self-limiting and normally does not lead to chronic liver disease and cirrhosis [26] except in the case of patients with immunodeficiency diseases such as HIV, those undergoing solid-organ transplant, and those with hematology disorders [27,28].

In view of the few recorded cases of HEV activity in the human population in Nigeria this study was designed to determine the prevalence of the virus in swine and bird populations in two states in South-Western, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 550 fecal samples comprising 475 stool samples from pigs and 75 sample of chicken droppings were collected from four poultry and piggery farms in Lagos (Ijeododo, Ojo, Apa, Okearo) and three in Ogun (Alagbado , Abule-Oko, Abeokuta) States, South-Western, Nigeria between June, 2013 and January, 2014. The samples were transported to the Virology and Immunology Laboratory, Central Research Lab, Department of Medical Microbiology and Parasitology, College of Medicine University of Lagos for processing and analysis.

2.2 Sample Preparation

Two grams of chicken and pig fecal samples were dissolved separately in 2 ml of phosphate buffered saline (PBS) and vortexed to ensure complete dissolution. The fecal suspensions were then stored at -50℃ till further usage.

2.3 RT-PCR

RNA extraction was carried out by diacetomaceous-earth extraction method using QIAGEN-QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Reverse Transcription (RT) and Nested polymerase chain reactions (PCRs) were performed using primers designed by Johne et al. [29].These degenerated primer pair were constructed from the alignment of 22 full-length genome sequences of HEV derived from humans, pigs, wild boar and chicken. The binding sites are within the open reading frame (ORF) 1 region of the primer. HEV forward primer for RT-PCR is TCGCGCATCACMTTYTTCCARAA while the reverse primer is GCCATGTTCCAGACDGTRTTCCA and for Nested the respective forward and reverse primers are TCTGCTCTGTTTGGCCCNTGGTTYCG and CCAGGCTCACCRGARTGYTTCTTCCA.

The PCR tubes containing the master mix and template were then transferred to the thermocycler (Eppendorf Master Cycler) for DNA amplification using the manufacturer's recommended cycling conditions which included initial denaturation for 2 m at 94℃, denaturation for 15s at 94℃, annealing for 30s at 40℃, extension for 30s at 72℃. The final extension was done for 5 m at 72℃. The total number of cycles was 40.

The cycling condition for Nested was slightly different. Initial denaturation was 5 m at 95℃, denaturation for 30s at 95℃, annealing for 30s at 57℃, extension for 1 m at 72℃. The final extension was done for 4 m at 72℃ and the total number of cycles was 38.

2.4 Agarose Gel Electophoresis

After amplification, detection of amplified viral DNA was done using agarose gel electrophoresis. Briefly, 1.5 g of Agarose gel powder was weighed out and mixed with 100 ml of TAE Buffer and shaken vigorously to form a colloidal solution. The mixture was then heated gently in a microwave oven for 5 m at 80℃. The solution was continuously rocked until it cooled to about 45℃ and then it was dispensed into the gel tank with the comb properly positioned. 1.5 µl of SYBR Safe was then added to the gel. This setup was allowed to stand for 20 m until the agarose solidified to a gel form. The gel tank was placed in the trough that has been filled with TAE Buffer, and the comb was carefully removed thereby creating wells in the gel. Each of the amplicons, including the positive and negative controls were mixed with the 6x loading buffer (5 ml:1 ml) and loaded in the wells of the gel. The trough was then covered and plugged to the BIO-RAD PowerPac which was connected to the electricity. The gel was run at 120 volts for 45 m.

At the end of electrophoresis, the gel was viewed using ultra violet Invitrogen Safe Imager. The expected base pair of 331-334 bp was then compared to the ladder to check for positive cases.

3. RESULTS

Out of the 550 samples 75 of them were from chicken while 475 were from pigsfeces.70 of the chicken droppings were collected from Ogun state while 5 were collected from Lagos state. 175 pig feces were collected from Ogun state and 300 from Lagos state.

The result as revealed in Table 1 and Fig. 1 shows that a total of 10(1.8%) out of the 550

samples were positive for HEV RNA. The 10 positive samples consisted of 5(6.7%) from chicken droppings and 5(1.2%) from pig feces. The result further revealed that 3(4.3%) of chicken droppings collected in Ogun state were positive while there was no positive cases recorded in pig feces. Similarly 2(40%) of chicken droppings collected in Lagos state were positive while 5(1.7%) of pig feces were also positive.

4. DISCUSSION

Hepatitis E virus is a major cause of hepatitis and it is estimated that 2.3 billion people are infected with the virus globally [30]. The fecal-oral route is the commonest route of transmission of the virus in endemic regions of Asia and Africa [12]. However, in the developed countries HEV related hepatitis has been regarded as zoonotic [31-34]. In Africa the circulating strains of HEV either

Table 1. Results of the screening of chicken droppings and pig feces for HEV in Lagos and Ogun States

Farm location		Sample	No of samples	No of +ve samples
Lagos	Ogun			
	Abeokuta	Pig stool	100	0
	Abule-oko	Chicken droppings	45	3
	Alagbado	Chicken droppings	25	0
		Pig stool	75	0
Apa		Pig stool	100	4
Ijeododo		Chicken droppings	5	2
Ojo		Pig stool	100	0
Okearo		Pig stool	100	1
Total			**550**	**10**

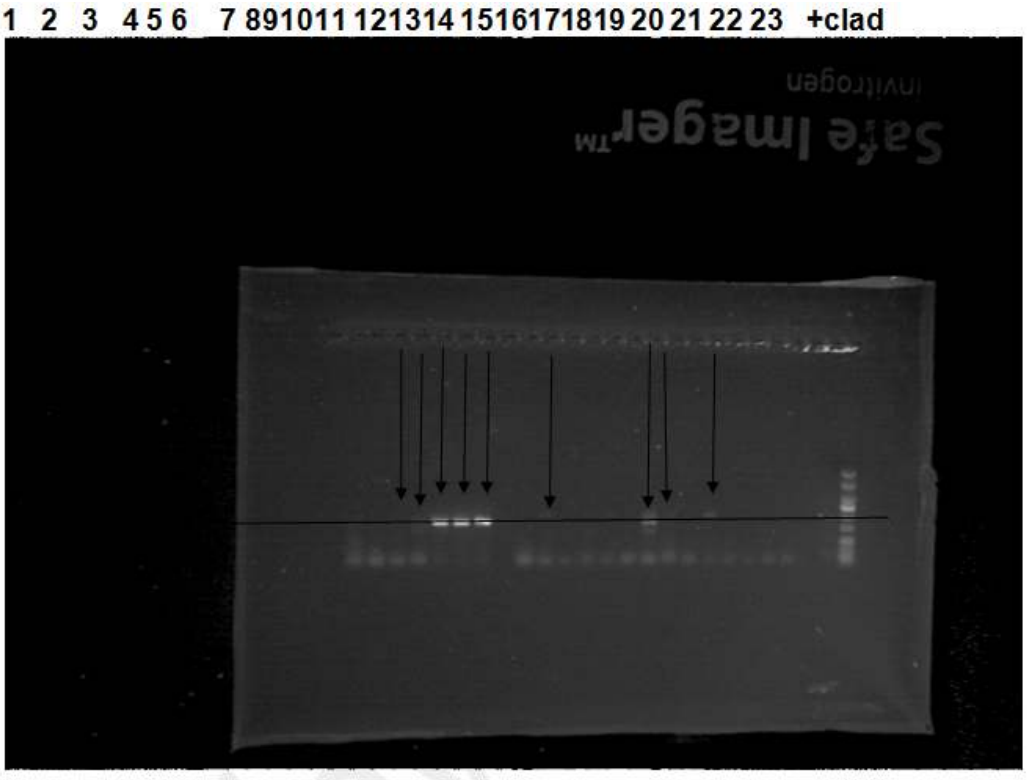

Fig. 1. HEV RNA detection by nested PCR. Lanes 4-8 are positive for HEV RNA from chicken droppings while 11, 16, 17&19 are positive for HEV RNA from swine feces

Key:+C = positive control; Lad = ladder

belong to genotypes 1 or 2 [7,8] but these do not infect pigs [3]. It is known that avian strains of HEV do not infect humans and pigs but could kill a reasonable number of chicken leading to commercial losses to the poultry farmer [3].

As this study in Lagos and Ogun States, South Western, Nigeria has revealed, HEV RNA was detected in 5(6.7%) of chicken droppings and 5(1.2%) of pigs stools confirming that this virus is prevalent among the animal population in the country. While Lagos recorded 1.7% and 40% among the pig and chicken populations respectively, Ogun had only 4.3% among the chicks and none in pigs. The pig population (31.8%) studied is large enough for there to be a positive result in Ogun and if none was recorded, it could be assumed that HEV is absent among the pigs in that State. Studies on HEV among pigs in other states in Nigeria revealed that there was higher prevalence of the virus in this animal population in Delta State (36%), Taraba State (88%), and Plateau State (76.7%) [35]. In another study in Plateau State, Junaid et al. [36] reported prevalence of the virus in pigs (32.8%) and other domestic animals including goats (37.2%) and sheep (10.5%).

The reason for the differences observed among the pigs and chicks in this study may not be unconnected with the hygienic condition under which the animals were kept, with pigs known for their filthy habits having more cases in Lagos than chicken. Similarly the hygienic status of the states may also have accounted for the differences in the level of prevalence of the virus in each of the states, with Lagos, with its high human population density, a predisposing factor to poor environmental sanitary conditions, recording cases in both domestic animals, while Ogun recorded only one.

The results of this study, and confirmed by previous workers has therefore revealed that HEV is indeed an under-recognized and significant public health issue prevalent among domestic animals in Nigeria. Seroepidemiological data has also shown that HEV is endemic in Nigeria among the human population [15-17]. In one study, Adesina et al. [37] conducted a survey on sick and healthy individuals in Ekiti state for the presence of antibodies to HEV. The workers obtained a prevalence rate of 13.4% indicating that the HEV had been in the community before the survey.

Sero-epidemiological studies have revealed that anti-HEV antibodies are present in numerous animal species including pigs, rodents, chicken, dogs, cows, sheep and goats from developing and industrialized countries [38]. HEV RNA has been detected in pig feces in Spain, China and Cameroon [39-41]. There have been evidences showing that pigs are reservoirs for HEV [3] and humans get infected when they consume infected pig liver [23]. Residents and indigenes of Lagos are therefore at risk of contracting this virus through consumption of pork meat as this study, which recorded a prevalence rate of 1.7% among the pig population in the state, has shown.

In similar studies Haqshenas et al. [42] identified nucleic acid and viral particles of HEV in bile samples from chicken affected by hepatitis-splenomegaly (HS) syndrome in the USA while Vasickova et al. [43] reported that virus mainly affects commercial breeds of hens in Australia. In addition post mortem examination of broiler flocks in Hungary revealed that 19% of birds with symptoms of enlarged liver had HEV RNA detected in their fecal samples. Fecal contamination of food and drinking water are likely sources of infection [44]. Avian HEV could result in commercial chicken farmers incurring loss in their businesses. This could also occur in Ogun and Lagos states which recorded prevalence figures of 4.3% and 40% respectively and by extension other states in Nigeria.

Poultry, swine farmers and veterinarians are occupational risk groups to HEV [45] and could transmit the virus to other members of the population. Good personal hygiene practices which include regular hand washing, proper cooking of pork meat before consumption, proper disposal of animal wastes and avoiding fecal contamination of drinking water sources will go a long way in reducing the risk of transmission of HEV. In addition, health education and public enlightenment in the print and electronic media will, also, indeed assist in preventing epidemic of HEV in Nigeria. Finally it is absolutely necessary to conduct a study to characterize the detected strains so as to determine the circulating strains of HEV in humans, pigs and commercial birds in Nigeria.

5. CONCLUSION

The detection of HEV among commercially available chicken and pigs poses a great economic danger to poultry farmers and a tremendous public health risk to consumers of pork meat in Nigeria.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chandra V, Kar-Roy A, Mayor S, Jameel S. The hepatitis E virus ORF3 protein modulates epidermal growth factor receptor trafficking, STAT3 translocation, and the acute-phase response. Journal of Virology. 2008;82(14):7100-7110.
2. Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU. A novel virus in swine is closely related to the human hepatitis E virus. Proceedings of the National Academy of Sciences. 1997;94: 9860–9865.
3. Khuroo MS. Discovery of hepatitis E: The epidemic non-A, non-B hepatitis 30 years down the memory lane. Virus Research. 2011;161:3–14.
4. Panda SK, Ansari IH, Durgapal H, Agrawal S, Jameel S. The *in vitro*-synthesized RNA from a cDNA clone of hepatitis E virus is infectious. Journal of Virology. 2000;74: 2430–2437.
5. Buisson Y, Grandadam M, Nicand E, Cheval P, van Cuyck-Gandre H, Innis B, Rehel P, Coursaget P, Teyssou R, Tsarev S. Identification of a novel hepatitis E virus in Nigeria. Journal of General Virology. 2000;81:903–909.
6. Pavio N, Meng XJ, Renou C. Zoonotic hepatitis E: Animal reservoirs and emerging risks. Vetenary Research. 2010; 41(6):46-60.
7. Hughes J, Wilson M, Teshale E, Hu D, Holmberg S. The two faces of hepatitis E virus. Clinical Infectious Diseases. 2010; 51(3):328-334.
8. Zhu Y, Si F, Cao D, Yu X, Yu R, Dong S, Huang F, Zhang Y, Li Z. Molecular epidemiology of hepatitis E virus infections in Shanghai, China. Virology Journal. 2011;8:541.
9. Zheng Y, Ge S, Zhang J, Guo Q, Mun Hon N, Wang F, Xia N, Jiang Q. Swine as a principal reservoir of hepatitis E virus that infects humans in Eastern China. Journal of Infectious Disease. 2006;193(12):1643-1649.
10. Zhao Q, Zhang J, Wu T, Li S, Mun-Hon N, Xia N, Shih J. Antigenic determin–induced immunogenicity and efficacy. Journal of Gastroenterology. 2012;65:293–295.
11. Dalton HR, Bendall R, Keane F, Tedder R, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. New England Journal of Medicine. 2009; 361(10):1025–1027.
12. Goumba AI, Konamna X, Komas NP. Clinical and epidemiological aspect of a hepatitis E outbreak in Bangui, Central African Republic. BMC Infectious Disease. 2011;11:93.
13. Aggarwal R. The global prevalence of hepatitis E virus infection and susceptibility: Systematic review. WHO/IVB/10.14. Viral hepatitis in the WHO South-East Asiaregion; 2010. SEA-CD-232.
14. Ahmed JA, Moturi E, Spiegel P, Schilperoord M, Burton W, Kassim NH, Mohamed A, Ochieng M, NderituL, Navarro-Colorado C, Burke H, Cookson S, Handzel T, Walboc LW, Montgomery JW, Teshale E, Marano N. Hepatitis E Outbreak, Dadaab Refugee Camp, Kenya, 2012. Emerging Infectious Disease. 2013; 19(6):1010-1011.
15. Ola SO, Odaibo GN, Olaleye OD, Ayoola EA. Hepatitis B and E viral infections among Nigerian healthcare workers. Afr J Med Med Sci. 2012;41(4):387-91.
16. Junaid SA, Agina SE, Khadijah A Abubakar. Epidemiology and associated risk factors of hepatitis E virus infection in Plateau State, Nigeria. Virology: Research and Treatment. 2014;5:15-26.
17. Odaibo GN, Olaleye DO. Hepatitis E virus infection in HIV positive ART naïve and experienced individuals in Nigeria. World Journal of AIDS. 2013;3:216-220.
18. Kosinova E, Bendova J, Vasickova P, Smitalova R, Prodelalova J. The prevalence of hepatitis E virus in piglets on Czech pig production farms and phylogenetic analysis of recovered isolates. Veterinarni Medicina. 2012;57(3): 115–120.

19. Lee J, Shao P, Chang L, Xia N, Chen P, Lu C, Huang L. Seroprevalence of hepatitis E virus infection among swine farmers and the general population in rural Taiwan. PLoS ONE. 2013;8(6):76-80.
20. Teo CG. The two clinico-epidemiological forms of hepatitis E. Journal of Viral Hepatology. 2007;14:295-297.
21. Mushahwar IK. Hepatitis E virus: Molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. Journal of Medical Virology. 2008;80(4):646-658.
22. Meng XJ. Hepatitis E virus: Animal reservoirs and zoonotic risk. Veterinary Microbiology. 2010;140:256–265.
23. Wenzel JJ, Prei J, Schemmerer M, Huber B, Plentz, A, Jilg W. Detection of hepatitis E virus (HEV) from porcine livers in Southeastern Germany and high sequence homology to human HEV isolates. Journal of Clinical Virology. 2011;52(1):50-54.
24. Teshale EH, Howard CM, Grytdal SP, Handzel TR, Barry V, Kamili S, Drobeniuc J, Okware S, Downing R, Tappero JW. Hepatitis E epidemic. Uganda Emerging Infectious Disease. 2010a;16:126-129.
25. Purcell RH, Emerson SU. Hepatitis E: An emerging awareness of an old disease. Journal of Hepatology. 2008;48:494–503.
26. Krawczynski K, Aggarwa lR, Kamili S. Epidemiology, clinical and pathologic features, diagnosis, and experimental models. In: Thomas HC, Lemon S, Zuckerman AJ, eds. Viral hepatitis. Malden, MA: Blackwell Publishing. 2005;624-634.
27. Gerolami R, Moal V, Picard C, Colson P. Hepatitis E virus as an emerging cause of chronic liver disease in organ transplant recipients. Journal of Hepatology. 2009; 50(3):622–624.
28. Caron M, Bouscaillou J, Kazanji M. Acute risk for hepatitis E virus infection among HIV-1-positive pregnant women in central Africa. Virology Journal. 2012;9:254.
29. Johne R, Plenge-Bonig A, Hess M, Ulrich RG, Reetz J, Schielke A. Detection of a novel hepatitis E-like virus in feces of wild rats using a nested broad-spectrum RT-PCR. Journal of General Virology. 2010; 91:750-758.
30. Teshale EH, Hu DJ. Hepatitis E: Epidemiology and prevention. World Journal of Hepatology. 2011;3(12):285-291.
31. Sato Y, Sato H, Naka K, Furuya S, Tsukiji H, Kitagawa K, Sonoda Y, Usui T, Sakamoto H, Yoshino S, Shimizu Y, Takahashi M, Nagashima S, Nishizawa T, Okamoto H. A nationwide survey of hepatitis E virus (HEV) infection in wild boars in Japan: identification of boar HEV strains of genotypes 3 and 4 and unrecognized genotypes. Archives of Virology. 2011;156(8):1345-1358.
32. Tam AW, Smith MM, Guerra ME, Huang CC, Bradley DW, Fry KE, Reyes GR. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology. 1991;185:120–131.
33. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Review of Medical Virology. 2006;16:5–36.
34. Wedemever H, Pischke S, Manns MP. Pathogenesis and treatment of hepatitis E virus. Infectious Gastroenterology. 2012; 142(6):1388-1397.
35. Owolodun OA, Gerber PF, Giménez-Lirola LG, Kwaga JK, Opriessnig T. First report of hepatitis E virus circulation in domestic pigs in Nigeria. Am J Trop Med Hyg. 2014;91(4):699-704.
36. Junaid SA, Agina SE, Jaiye K. Seroprevalence of hepatitis E virus among domestic animals in Plateau State–Nigeria. British Microbiology Research Journal. 2014;4(8):924-934
37. Adesina OA, Japhet MO, Donbraye E, Kunapayi TE and Kudoro A. Anti-hepatitis E virus antibodies in sick and healthy Individuals in Ekiti State, Nigeria. African Journal of Microbiology Research, 2009; 3(9):533-536.
38. Arankalle VA, Joshi MV, Kulkarni AM, Gandhe SS, Chobe LP, Rautmare SS. Prevalence of anti-hepatitis E virus antibodies in different Indian animal species. Journal of Viral Hepatolog. 2001;8:223–227.
39. Emerson SU, Nguyen HT, Torian U, Burke D, Engle R, Purcell RH. Release of genotype 1 hepatitis E virus from cultured hepatoma and polarized intestinal cells depends on open reading frame 3 protein and requires an intact PXXP motif. Journal of Virology. 2010;84:9059–9069.
40. Xiao P, Li R, She R, Yin J, Li W, Mao J, Sun Q. Prevalence of hepatitis E virus in swine fed on kitchen residue. PLoS ONE. 2012;7(3):73-79.

41. Paula V, Wiele M, Mbunkah AH, Daniel AM, Manchang T, Kingsley MT, Schmidt-Chanasi J. Hepatitis E virus genotype 3 strains in domestic pigs, Cameroon. Emerging Infectious Disease. 2013;19(4): 14-20.
42. Haqshenas G, Shivaprasad HL, Woolcock PR, Read DH, Meng XJ. Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. Journal of General Virology. 2001; 82:2449–2462.
43. Vasickova P, Dvorska L, Lorencova A, PavlikI. Viruses as a cause of foodborne diseases: A review of the literature. Veterinarni Medicina. 2005;50:89–104.
44. Munne MS, Altabert NR, Vladimirsky SN, Moreiro R, Mares LO, Soto SS, Brajterman LS, Castro RE, Gonzalez JE. Identifications of polyphyletic variants in acute hepatitis suggest an underdiagnosed circulation of hepatitis E virus in Argentina. Journal of Clinical Virology. 2011;52(2): 138-141.
45. Lewis HC, Wichmann O, Duizer E. Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: A systematic review. Epidemiology of Infection. 2010;138:145-166.

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