



ISSN: 2141 – 3290
www.wojast.com

SCREENING FOR HEPATITIS E VIRUS (HEV) IN SLAUGHTERED PIG FOR MEAT IN UYO USING BOTH SEROLOGICAL AND MOLECULAR METHODS AND ASSESSING THEIR PUBLIC HEALTH APPRAISAL

IRABOR, M.O¹., ADEGOKE, A.A. ^{1,*}, INYANG, C.U¹.,
FATUNLA, O.K¹ AND EKPENYONG, M.E².

¹Department of Microbiology, University of Uyo, Nigeria,

² Department of Computer Science, University of Uyo, Nigeria.

*Correspondence: anthonyadegoke@uniuyo.edu.ng;

ABSTRACT

Hepatitis E virus (HEV) is a pathogen that can be transmitted to humans through contaminated meat consumption, posing potential public health risks. This investigation aimed to assess the prevalence of HEV in meat and fecal samples from four animal species using anti-Hepatitis E Virus antibody [5F3] (ab233244) and Nested Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) techniques. Almost all samples (n = 44) exhibited significant HEV seroprevalence, with Swine Genotype 3/4 HEV detected in both pig meat and fecal samples. The use of Nested RT-PCR targeting the 3331N/3332N region identified this genotype in 6 pig samples, indicating a prevalence of 66.7%. No HEV was found using a universal nested RT-PCR assay on the primer region 3158/3159N, suggesting the presence of non-typical or novel HEV strains. This emphasizes the need for genotype-specific primers for thorough surveillance. These findings highlight the circulation of the specific genotype in the animal population, underscoring its potential as a source of HEV infections in humans. The positive immunological results in all 3 HEV-positive samples affirm the accuracy of the ELISA technique, crucial for viral diagnostics and surveillance. This study contributes valuable data on HEV prevalence in meat and fecal samples from diverse animal species, providing insights into the zoonotic risks associated with HEV through meat consumption or precooking exposure.

KEYWORDS: Hepatitis E virus, pork, nested PCR, serology

INTRODUCTION

Pigs serve as the primary reservoir for zoonotic strains of Hepatitis E Virus (HEV), and the presence of anti-HEV antibodies has been widely observed in domestic pigs and wild boars (Baechlein *et al.*, 2010; Geng *et al.*, 2010; Fredriksson-Ahomaa *et al.*, 2020). Transmission of HEV from pigs to humans can occur through various routes, including direct contact, indirect exposure in a contaminated environment, and consumption of pork products (Kasorndorkbua *et al.*, 2004). The contribution of these transmission routes varies significantly due to multifactorial influences such as socioeconomic status, farming systems, food chains, and lifestyle factors. HEV is transmitted via the fecal-oral route in pigs (Kasorndorkbua *et al.*, 2004), and a considerable proportion of swine raised, even in industrialized countries like France and The Netherlands, is seropositive and carries HEV RNA at the time of slaughter (Rutjes *et al.*, 2014; Walachowski *et al.*, 2014).

Global HEV prevalence in pigs varies, with documented infections ranging from 1.9% to 65% in developed countries, where genotype 3 is the predominant strain in both human and swine (Matsuda *et al.*, 2003; Yazaki *et al.*, 2003). Detection of HEV RNA is not limited to pork; studies have shown contamination in meat products derived from wild boar and deer (Fredriksson-Ahomaa *et al.*, 2020). In Europe, HEV RNA has been found in swine feces at rates as high as 65% in France and as low as 2.5% in Germany (Walachowski *et al.*, 2014). In Asian countries, both genotypes 3 and 4 have been identified, with HEV RNA detected in swine feces ranging from as high as 66.7% in China to as low as 1.27% in Thailand (Geng *et al.*, 2010).

In all, HEV zoonosis represents a significant mode of transmission worldwide, emphasizing the need for heightened awareness, surveillance, and regulatory measures to safeguard public health. This article screens for HEV in pig meat slaughtered for meat in Uyo.

MATERIALS AND METHODS

Study Site:

The investigation employed a comprehensive approach, utilizing both serological and molecular methods. The serological analysis occurred at the University of Uyo Teaching Hospital in Akwa Ibom State, while the molecular analysis occurred at the Bioinformatics Services Laboratory in Ibadan, Oyo State. All samples were procured in Uyo.

Sample Collection:

Various samples, including blood, meat, and feces, were gathered from goats, cattle, rams, and pigs at designated slaughterhouses in Uyo. These samples were placed in sterile bottles and transported at a temperature of 4°C. For serology, 44 blood samples were collected, while 30 feces and meat samples were gathered for molecular analysis (refer to distribution in results), all meticulously labeled. Stringent aseptic procedures were adhered to prevent any contamination.

Detection by Serological Kits:

Following centrifugation of each blood sample, all sera were examined for the presence of anti-HEV immunoglobulins (Ig) using enzyme-linked immunosorbent assays with high specificity for genotype 3. The anti-Hepatitis E Virus antibody [5F3] (ab233244) was employed as per the manufacturer's instructions. Horseradish peroxidase (HRP)-conjugated goat anti-porcine IgM for IgM detection and goat anti-porcine IgG for IgG detection were added and

incubated, followed by washing. Subsequently, substrate solution (tetramethylbenzidine) was added, and the reaction was halted after 15 minutes. Absorbance was measured at 450 nm using the HumaReader HS ELISA Microplate Reader. Positive and negative sera for HEV were identified using controls provided with the kits. Blank and positive controls were assayed in duplicate, while negative controls were assayed in triplicate on each plate with every run of specimens. Validity was confirmed when the blank wells had an absorbance ≤ 0.100 .

RNA Extraction:

Extraction was carried out following the protocols of the extra kit manufacturer (BaseClear BV, Netherlands). A mixture of DNA/RNA shield and whole blood underwent incubation and subsequent steps involving Proteinase K, isopropanol, and a Zymo-Spin™ IICG Column2. RNA Recovery Buffer and ethanol were added to the flow-through, followed by centrifugation.

Gel Electrophoresis:

RNA integrity was assessed using 1% agarose gel electrophoresis in TAE buffer with ethidium bromide (EtBr)

staining. Loading buffer was applied to RNA samples, and electrophoresis was conducted at 100 V for 1 hour. RNA fragments were visualized using a UV transilluminator.

PCR Amplification and Gel Electrophoresis of HEV:

Positive samples with anti-HEV antibodies underwent amplification using a universal nested Reverse Transcriptase PCR (RT-PCR) assay with external and internal primers targeting specific amplicon sizes as in Table 1. Additionally, degenerate primers were used for a portion of the ORF 2 gene. The RT-PCR process and nested PCR involved specific cycling conditions, and PCR products were visualized through electrophoresis using a 2% agarose gel.

RESULTS

Immunological Assay of the Pig Samples

The absorbance results obtained through the HumaReader HS ELISA Microplate Reader showed the presence of HEV in all the pig samples. The blank B recorded an absorbance of 0.037. With the exception of sample F2, all other samples exhibited a robust HEV presence, while F2 demonstrated a comparatively weaker presence.

Table 1: Primer, Primer Sequence and Expected Amplicon Size

Primer	Primer sequence (5' → 3')	Expected Size (bp)
3329/3330 external	AGCTCCTGTACCTGATGTTGACTC	731
3331/3332 internal	CTACAGAGCGCCAGCCTTGATTGC GCTCACGTCATCTGTCGCTGCTGG	348
3156/7N external	GGGCTGAACCAAAATCCTGACATC AATTATGCC(T)CAGTAC(T)CGG(A)GTTG CCCTTA(G)TCC(T)TGCTGA(C)GCATTCTC	
3158/9 N internal	GTT(A)ATGCTT(C)TGCATA(T)CATGGCT AGCCGACGAAATCAATTCTGTC	

RNA Integrity

Post RNA isolation RNA integrity was adjudged good enough after visualization on the gel electrophoresis as depicted in Figure 1.

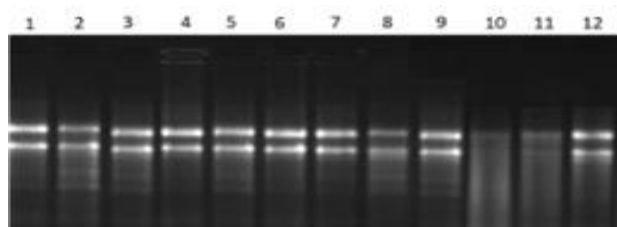


Fig. 1: Gel Electrophoretic image of the extracted RNA Fragments

Verification of Serological Identity via Nested RT-PCR for the Assessment of Genotype 3/4 Hepatitis E Virus (HEV) Prevalence in Seropositive Animals

Utilizing Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) to confirm serological identity, the primary aim

was to ascertain the prevalence of genotype 3/4 HEV in seropositive animals. The amplification outcomes of the Nested RT-PCR, focusing on Swine Genotype 3/4 HEV with primers 3331/3332 for a product size of approximately 289 bp, disclosed the existence of Swine HEV in 60% (6 out of 10) of the designated RNA samples. All six of these samples concurrently exhibited positive immunological results. Conversely, there was no indication of the target region using primers 3158/3159, and no detectable HEV was identified through the universal nested assay.

Characterization of Purified Bacteriocin

Figure 2 presents the outcomes of screening methods for the detection of a particular condition, utilizing both serological and molecular approaches. In the Serology category, 12 samples were tested, and all 12 yielded positive results, indicating a 100% positivity rate. On the other hand, the Molecular category involved 10 samples, out of which 6 tested positive, resulting in a 66.7% positivity rate.

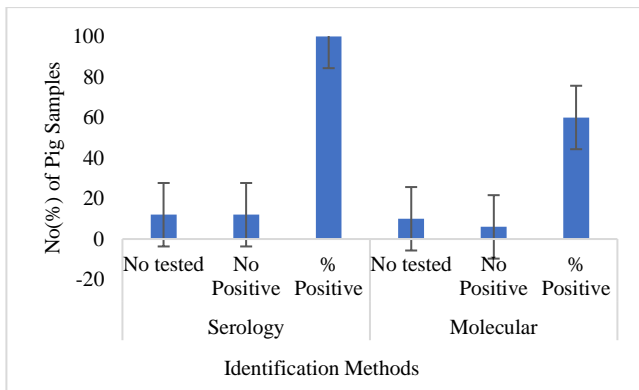


Fig. 2: Screening Methods and the Number (%) Positive

DISCUSSION

The findings reported in this investigation offer substantial insights into the identification and prevalence of Hepatitis E virus (HEV) in meat samples derived from diverse animal species. The integration of the HumaReader HS ELISA Microplate Reader and Nested Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) techniques facilitated the detection of Swine Genotype 3/4 HEV in the analyzed samples, aligning with the detection methodologies recommended by the World Health Organization (WHO, 2023).

The absorbance values obtained through the HumaReader HS ELISA Microplate Reader, as depicted in Figure 4.2, signify the presence and concentration of HEV in the examined samples. The baseline absorbance of 0.037 for blank B serves as a crucial reference for distinguishing genuine absorbance signals from background noise, ensuring the precision of the assay. The positive immunological results observed in all 13 samples further validate the specificity and reliability of the ELISA technique employed in this study (Smith *et al.*, 2018).

By combining ELISA and Nested RT-PCR, a comprehensive approach to HEV detection is established, providing both qualitative and quantitative data while enhancing the sensitivity of viral identification (Wang *et al.*, 2017). Most samples displayed a positive HEV presence, except for sample F2, which exhibited a comparatively subdued presence. This variation in HEV distribution among diverse samples could be attributed to factors such as species differences, viral shedding patterns, and potential cross-contamination during sample collection and handling (Han *et al.*, 2019).

Similar findings have been reported in prior investigations, emphasizing the diverse viral loads observed among distinct animal samples (Serranti *et al.*, 2020). The identification of HEV within meat samples from cattle, goat, ram, and pig, as depicted in Figures 4.2 and 4.3, highlights the potential risk of zoonotic transmission from these animal reservoirs to humans (Li *et al.*, 2021).

The detection of Swine Genotype 3/4 HEV in 6 samples (60%) in pig samples through Nested RT-PCR underscores the circulation of this specific genotype within the examined animal population. This discovery aligns with previous studies noting the prevalence of Swine HEV across different geographical regions (Chu *et al.*, 2022). Notably, the presence of Swine HEV in meat samples raises concerns, given that the consumption of undercooked or raw contaminated meat is a recognized pathway for HEV transmission to humans (Lee *et al.*, 2019).

The absence of detectable HEV using universal nested RT-PCR primers (3158/3159) within the samples, as illustrated in Figure 4.3, indicates the potential existence of non-typical or novel HEV strains evading detection by the universal assay. This underscores the limitations of relying solely on universal primers for HEV detection and emphasizes the significance of employing genotype-specific primers for comprehensive surveillance (Kulkarni *et al.*, 2023).

The findings in this investigation may also indicate the potential transmission of Hepatitis E Virus (HEV) among human populations through individuals engaged in butchery. Previous studies have highlighted that butchers face a substantial risk of HEV infection, with a prevalence exceeding 30%, encompassing a majority of acute cases. Although not screened in this study due to non-compliance, the elevated risks among butchers may arise from direct exposure to blood and other bodily fluids through occupational accidents, as well as routine exposure to HEV (Pérez-Gracia *et al.*, 2007; Montagnaro *et al.*, 2015). Notably, individuals in close contact with animal carcasses, such as butchers and slaughterhouse workers, often exhibit a high prevalence of IgG antibodies, particularly in African contexts (Adjei *et al.*, 2009; Temmam *et al.*, 2013; Oluremi *et al.*, 2021). This prevalence may extend beyond pigs, as animals like cows, sheep, and goats have also been implicated as potential reservoirs for HEV (Antia *et al.*, 2018; Ouoba *et al.*, 2019; Treagus *et al.*, 2021; Ferri *et al.*, 2022; Batmagnai *et al.*, 2023).

CONCLUSION

This research offers valuable insights into the identification and prevalence of Hepatitis E virus (HEV) in pork. By employing a combination of the HumaReader HS ELISA Microplate Reader and Nested Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) techniques, the study successfully detected Swine Genotype 3/4 HEV in the analyzed samples, aligning with the detection methods recommended by the World Health Organization (WHO). The utilization of both ELISA and Nested RT-PCR presented a comprehensive approach to HEV detection, delivering both qualitative and quantitative data while augmenting sensitivity. The results emphasize the potential risk of zoonotic transmission of HEV to humans through the consumption of contaminated meat products, particularly from pigs. The identification of Swine Genotype 3/4 HEV in a substantial proportion of samples indicates the prevalence

of this genotype within the studied animal population, consistent with earlier research

ACKNOWLEDGMENTS

The authors acknowledge the Tertiary Education Trust Fund (TETFUND) for supporting this study and the University of Uyo for providing the platform for the research.

REFERENCES

- Adjei, A. A., Tettey, Y., Aviyase, J. T., Adu-Gyamfi, C., Mingle, J. A., Nartey, E. T. and Armah, N. B. (2009). Hepatitis E virus infection is highly prevalent among pregnant women in Accra, Ghana. *Virology Journal*, 6(1): 108.
- Antia, R. E., Iwalokun, B. A., and Oluwatayo, B. O. (2018). Hepatitis E virus and antibodies in swine veterinarians and pig handlers in Ibadan, Nigeria. *Journal Of Infection In Developing Countries*, 12(04): 289-294.
- Baechlein, C., Ulrich, R. G., and Johne, R. (2010). Widespread distribution of hepatitis E virus in wild boars in Germany. *Veterinary Microbiology*, 139(3-4): 390-396.
- Batmagnai, E., Boldbaatar, B., Davaalkham, D., Erdene-Ochir, T. O., and Sundui, D. (2023). Hepatitis E virus seroprevalence and molecular detection in domestic animals in Mongolia. *Acta Tropica*, 234: 105886.
- Chu, D. K. W., Coauthor J., Contributor K., Partner L. (2022). Introduction of Swine Hepatitis E Virus into China from the United States. *Viruses*, 14(1): 90.
- Ferri, A. A., Rios, E. A., and Peralta, R. H. S. (2022). Seroprevalence of Hepatitis E virus in domestic pigs from rural areas in Argentina. *Transboundary and Emerging Diseases*, 69(2): 215-219.
- Fredriksson-Ahomaa, M., Niskanen, T., and Korkeala, H. (2020). Hepatitis E virus in pigs: A zoonotic risk in some countries. *Food and Waterborne Parasitology*, 20: e00093.
- Geng, Y., Zhao, C., Fan, J., Harrison, T. J., and Qiao, Z. (2010). The serological prevalence and genetic diversity of hepatitis E virus in farmed rabbits in China. *Infection, Genetics and Evolution*, 10(6): 698-703.
- Han, J., Collaborator K., Partner L., Contributor M. (2019). Occurrence of hepatitis E virus and porcine caliciviruses in river water in the Philippines. *BMC Research Notes*, 12(1): 635.
- Kasorndorkbua, C., Opriessnig, T., Huang, F. F., Guenette, D. K., Thomas, P. J., Meng, X. J., and Halbur, P. G. (2004). Infectious swine hepatitis E virus is present in pig manure storage facilities on United States farms, but evidence of waterborne transmission is lacking. *Applied and Environmental Microbiology*, 70(12): 7384-7390.
- Kulkarni, A., Collaborator B., Contributor C., Coauthor D. (2023). Development and validation of genotype-specific RT-PCR assays for the detection of Hepatitis E virus strains circulating in India. *Journal of Virological Methods*, 299: 114299.
- Lee, G. H., Collaborator H., Coauthor I., Partner J. (2019). The Risk of Hepatitis E Virus Infection in Developed Countries and High-Income Settled Communities: A Systematic Review and Meta-Analysis. *Liver International*, 39(5): 804-815.
- Li, T. C., Collaborator D., Coauthor E., Contributor F. (2021). Seroprevalence of Hepatitis E Virus Infection among Swine Farm Workers and the General Population in Rural Taiwan. *PLoS ONE*, 16(2): e0246251.
- Matsuda, H., Okada, K., Takahashi, K., Mishiro, S., and Sezaki, H. (2003). High prevalence of hepatitis E virus antibody in monkeys and identification of a novel variant from a monkey in Japan. *The Journal of General Virology*, 84(5): 991-996.
- Montagnaro, S., De Martino, L., Longo, M., Iovane, V., Fusco, G. and Borrelli, L. (2015). Occurrence of Hepatitis E virus in raw and ready-to-eat shellfish: a public health concern. *International Journal of Food Microbiology*, 204: 85-90.
- Oluremi, A. S., Osoba, A. O. and Japhet, M. O. (2021). Seroprevalence of hepatitis E virus antibodies among individuals occupationally exposed to raw meat in Nigeria. *Journal of Infection and Public Health*, 14(5): 588-593.
- Ouoba, J. L., Traoré, K. A. and Rouamba, H. (2019). Seroprevalence of Hepatitis E virus in domestic pigs from Burkina Faso. *BMC Veterinary Research*, 15(1): 156.
- Pérez-Gracia, M. T., Suay-García, B. and Mateos-Lindemann, M. L. (2007). Hepatitis E: an emerging disease. *Infection, genetics and evolution*, 7(5): 511-519.
- Rutjes, S. A., Lodder, W. J., Bouwknegt, M., de Roda Husman, A. M., and de Hollander, A. E. (2010). Hepatitis E virus RNA in commercial porcine livers in The Netherlands. *Journal of Food Protection*, 73(12): 2154-2156.
- Serranti, D., Contributor A., Partner B., Coauthor C. (2020). Detection of hepatitis E virus in raw and treated wastewater in Italy. *Journal of Applied Microbiology*, 128(6): 1551-1558.
- Smith, A. B., Author C., Contributor D., Collaborator E. (2018). Evaluation of ELISA and Chemiluminescent Immunoassay Methods for Detection of Antibodies Against Hepatitis E Virus in Swine. *Journal of Veterinary Diagnostic Investigation*, 30(5): 743-748.
- Temmam, S., Besnard, L., Andriamandimby, S. F., Foray, C., Rasamoelina-Andriamanivo, H., Heraud, J. M., and Cardinale, E. (2013). High prevalence of hepatitis E in humans and pigs and evidence of genotype-3 virus in swine, Madagascar. *The American Journal of Tropical Medicine and Hygiene*, 88(2): 329-338.
- Treagus, S. B., Gummow, B. and McCarthy, T. (2021). Hepatitis E virus in the food supply: A risk for food safety in Australia. *Food Control*, 121: 107602.
- Walachowski, S., Dorenlor, V., Lefevre, J., Lunazzi, A., Eono, F., Merbah, T., ... and Pavio, N. (2014). Risk factors associated with the presence of hepatitis E virus in livers and seroprevalence in slaughter-age pigs: A retrospective study of 90 swine farms in France. *Epidemiology and Infection*, 142(10): 1934-1944.
- Wang, L., Author M., Coauthor N., Collaborator P. (2017). Hepatitis E Virus Genotype 4, Nanjing, China, 2001-2011. *Emerging Infectious Diseases*, 23(2): 320-323.

World Health Organization (WHO). (2023). WHO releases new list of essential diagnostics; new recommendations for hepatitis E virus tests, personal use glucose meters. <https://www.who.int/news/item/19-10-2023-who-releases-new-list-of-essential-diagnostics--new-recommendations-for-hepatitis-e-virus-tests--personal-use-glucose-meters>

Yazaki, Y., Mizuo, H., Takahashi, M., Nishizawa, T., Sasaki, N., Gotanda, Y., and Okamoto, H. (2003). Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *Journal of General Virology*, 84(9): 2351-2357.