First Report of Hepatitis E Virus Circulation in Domestic Pigs in Nigeria

Olajide A. Owolodun,[†] Priscilla F. Gerber,[†] Luis G. Giménez-Lirola, Jacob K. P. Kwaga, and Tanja Opriessnig*

Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa; Applied Biotechnology Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria; Department Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria; The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom

Abstract. Hepatitis E virus (HEV) is an important cause of acute hepatitis in humans. Zoonotic transmission between pigs and humans has been confirmed. Human HEV infection is common in Nigeria; however, characterization of HEV infection in other species was lacking. The objective of this study was to investigate HEV infection in Nigerian pigs. A total of 286 serum samples from six states in Nigeria were tested for presence of anti-HEV IgG. Ninety fecal samples from one of these states (Plateau State) were tested for presence of HEV RNA. The overall prevalence of anti-HEV IgG-positive or suspect-positive pigs was 55.6% (159 of 286) with regional prevalence rates ranging from 36% (9 of 25; Delta State) to 88% (22 of 25; Taraba State). The overall HEV RNA prevalence rate was 76.7% (69 of 90). All polymerase chain reaction-positive samples belonged to HEV genotype 3 based on sequencing. The results indicate that HEV genotype 3 infection is widespread in Nigerian pigs.

INTRODUCTION

Hepatitis E, a usually self-limiting but occasionally fulminant hepatitis is an important public health concern in low- and middle-income countries worldwide.¹ Hepatitis E is the single most important cause of acute clinical hepatitis among adults throughout Central and Southeast Asia and the second most important cause throughout the Middle East and North Africa following hepatitis B.² Models derived from epidemiologic and clinical studies project a conservative estimate of the annual impact of hepatitis E to include ~3.4 million symptomatic cases, 70,000 deaths, and 3,000 stillbirths.³ The etiologic agent of hepatitis E is hepatitis E virus (HEV),⁴ classified as a member of the genus *Hepevirus*, family *Hepeviridae*.⁵ The virus is a non-enveloped, single-stranded positive-sense RNA virus that contains three open reading frames (ORF); ORF1 encodes for non-structural proteins, ORF2 encodes for the capsid protein, and ORF3 encodes for a multi-functional small protein.⁵

Currently, HEV has at least four recognized genotypes capable of infecting humans.⁵ The HEV genotypes 1 and 2 are restricted to humans and related to waterborne large outbreaks of hepatitis E in developing countries with poor sanitation conditions.⁶ Genotype 1 is primarily found in North Africa and Central and South Asia.⁷ Genotype 2 circulates in Mexico and West Africa.⁷ Genotype 3 circulates worldwide and contains strains from sporadic, cluster, and chronic cases of hepatitis E in humans⁶ and strains from pigs and wild boars, which are considered the most important reservoirs for HEV.⁸ Genotype 4 circulates mainly in Asia; however, it has been reported recently in Europe^{9–11} and includes strains from sporadic and cluster cases of hepatitis E in humans and strains from pigs.

In Africa, HEV is still an emerging pathogen. The very low rate of reporting is the result of suboptimal investigations and neglect of the disease and its public health problem. The majority of available reports describe human HEV outbreaks in North Africa and there are few reports from Uganda, the

Central African Republic, the Democratic Republic of the Congo, Sudan, South Sudan and Kenya.¹ Available information on HEV infection in animals in Africa is very limited. Two reports describe detection of HEV in pigs in the Democratic Republic of the Congo and in Cameroon,^{12,13} indicating increased awareness, surveillance, and widespread nature of the virus in the environment. The only available characterized isolate of a human HEV case in Nigeria belongs to genotype 2.¹⁴ This particular genotype is commonly linked to human outbreaks. As in other African countries, the HEV infectious status of pigs in Nigeria is largely unknown. The objective of this study was to characterize HEV infection in pigs of various ages from the main pig farming regions in Nigeria.

MATERIALS AND METHODS

Samples. Samples used in this study included 176 blood samples from apparently healthy pigs collected at slaughter at the Jos main abattoir, located in the Plateau State, from January 2011 to January 2012. The samples were obtained from pigs that were 4 months to 3 years of age. The pigs came from different farming systems including intensively raised pigs and alternative free ranging pigs. All blood samples were stored on ice before being transported to the laboratory. Serum was decanted after centrifugation and stored at –80°C until use. In addition, archived serum samples ($N = 112$) collected in 2009 for an African swine fever virus survey were also used. The archived serum samples were from 6 months to 3-year-old pigs from the following five states: Oyo ($N = 24$), Ogun ($N = 19$), Lagos ($N = 17$), Delta ($N = 25$), and Taraba ($N = 25$) (Figure 1A). Serum samples were unrelated to fecal samples.

Nine fecal samples from each of 10 different regions within the Plateau State including Barkin Ladi, Bassa, Bokkos, Jos North, Jos South, Langtag North, Langtang South, Mangu, Pankshin, and Shendam were also included (Figure 1B) resulting in a total number of 90 samples tested. Sampled pigs ranged from 1 to 6 months of age (average $\pm$ SD, 3.35 ± 1.25) (Table 1). Each sample within a given area corresponded to a different farm.

Anti-HEV immunoglobulin G (IgG) enzyme-linked immunosorbent assay (ELISA). All serum samples were tested for anti-HEV IgG using an ELISA protocol previously described.^{15,16} In brief, a commercially available *Escherichia coli*-derived

* Address correspondence to Tanja Opriessnig, The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, United Kingdom. E-mail: Tanja.Opriessnig@roslin.ed.ac.uk

[†] These authors contributed equally to this work.

FIGURE 1. Geographic origin of the samples tested in this study. (A) Map of Nigeria indicating the states where samples were obtained for serology. (B) Map of the Plateau State indicating the regions where samples were obtained for detection of HEV RNA.

TABLE 1

Prevalence of HEV RNA positive samples in fecal samples collected in different regions of the Plateau State. Each sample in a given region was collected from different farms within the specified region. Data presented as percentage (total number of positive samples)

Region	Number tested	Age (months)	Positive
Barkin Ladi	9	2–4	88.9% (8)
Bassa	9	1–6	88.9% (8)
Bokkos	9	2–6	88.9% (8)
Jos North	9	2–4	77.8% (7)
Jos South	9	2–4	66.7% (6)
Langtang North	9	2–4	77.8% (7)
Langtang South	9	2–5	77.8% (7)*
Mangu	9	2–6	66.7% (6)*
Pankshin	9	2–5	77.8% (7)
Shendam	9	2–3	55.5% (5)*
TOTAL	90		76.7% (69)

* The presence of HEV was confirmed by sequencing of one reverse transcriptase-polymerase chain reaction (RT-PCR) positive sample from the Langtang South region, three from the Mangu region, and one from the Shendam region.

recombinant protein of the HEV ORF2 (Genway, San Diego, CA) was used to coat the plates. Each well of a microtiter plate (Nunc, Thermo Fischer Scientific, Inc., Rochester, NY) was coated with 100 µL of the antigen at a concentration of 0.6 µg/mL in phosphate buffered saline (PBS). The plates were incubated at room temperature overnight. The microtiter plate was washed three consecutive times with PBS containing 0.05% Tween-20 and subsequently blocked with 1% fetal bovine serum (FBS) (Gibco, Grand Island, NY). Serum samples at a dilution of 1:100 with PBS containing FBS were added into each well and incubated for 30 min at 37°C. The plates were washed three times and 100 µL of a 1:25,000 dilution of horse radish peroxidase goat anti-swine IgG conjugate (Jackson ImmunoResearch, Inc., Groove, PA) was added into each well and incubated at 37°C for 30 min. Plates were again washed and the reaction was visualized by using tetramethylbenzidine-hydrogen peroxidase as substrate (KPL, Gaithersburg, MD). The reaction was terminated after 15 min by adding 2.5 M sulphuric acid to each well and the plates were read at 450 nm using a spectrophotometer. Three internal controls, a positive control, a negative control, and a cut-off control, originating from pigs negative for HEV and pigs infected experimentally with HEV genotype 3 as described⁸ were included on each plate. Cut-off controls were determined by means of a detailed receiver operating characteristic (ROC) curve analysis as described elsewhere.⁸ When the internal cut-off control value was within the expected range (0.50–0.54), the run was considered valid and the sample results were used. The results were calculated as “antibody index” (sample optical density [OD]/cut-off serum mean OD) with results < 0.9, 0.9–1.0, and ≥ 1.1 defined as negative, inconclusive, and positive, respectively.

RNA extraction. Total nucleic acid extraction was carried out on 50 µL fecal supernatant using the MagMAX 96 Viral Isolation kit (Ambion, Foster City, CA) according to the manufacturer's instructions on an automated extraction platform (KingFisher Flex; Thermo Scientific, Waltman, MA). A negative control (nuclease-free water) and a positive control (fecal suspension from pigs experimentally infected with HEV genotype 3, GenBank accession no. AF082843) kindly provided by Dr. Xiang-Jin Meng, Virginia Polytechnic Institute and State University, Blacksburg, VA were added to each extraction plate. The extracted RNA was stored at –80°C until use.

Real-time reverse transcriptase (RT)-PCR assay. The real-time RT-PCR targeting the HEV ORF3 region was carried

out in a 96-well plate using the TaqMan One-Step RT-PCR Master Mix Reagent (Applied Biosystems, Foster City, CA) in a 25 µL volume comprising 5 µL of extracted RNA and 20 µL of master mix according to the manufacturers' recommendation using the following primers and probe: JVHEV-F (5'-GGTGGTTTCTGGGGTGAC-3'); JVHEV-R (5'-AGG GGTGGTTGGATGAA-3'); JVHEV-P (5'-FAM-TGATT CTCAGCCCTTCGC-BHQ-3').¹⁷ One-step RT-PCR amplification was performed on an ABI 7500 real-time PCR instrument (Applied Biosystems) under the following conditions: 30 min at 50°C for the RT reaction, 15 min at 95°C followed by 45 cycles at 95°C for 15 s for denaturation and 60°C for 45 s for annealing and extension. A sample was considered negative if the cycle threshold (*Ct*) was ≥ 41 amplification cycles. A no template control (nuclease-free water) and a positive HEV RNA control were added to each PCR plate. The positive control had an expected *Ct* value of 28.

Conventional nested RT-PCR assay. At least one sample per geographic region and all samples with real-time RT-PCR *Ct* values lower than 35 were selected for amplification by a conventional nested RT-PCR using primers targeting an ORF2 fragment as described.¹⁸ Briefly, 6 µM each of primers 3156N (5'-AATTATGCYCAGTAYCGRGTTG-3') and 3157N (5'-CCCTTRTCYTGTCTGMGCATTCTC-3') and OneStep RT-PCR kit (Qiagen, Valencia, CA) were used. The thermal cycling conditions for the first reaction were as follows: 50°C for 30 min, 95°C for 15 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and a final elongation step at 72°C for 10 min. The second reaction was performed with 0.2 µM each of primers 3158N (5'-GTWATGCTYTGCA TWCATGGCT-3') and 3159N (5'-AGCCGACGAAATCA ATTCTGTCT-3') and ReadyMix Taq PCR Reaction Mix (Sigma-Aldrich, St. Louis, MO). The thermal cycling conditions for the second PCR reaction were as follows: 95°C for 5 min, 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final elongation step at 72°C for 7 min. The 348 nucleotides (nt) second round PCR products were visualized after electrophoresis on a 1% agarose gel. To avoid contamination, the following precautions were taken: each step of the experimental procedure, reaction mixture preparation, RNA preparation and extraction from the samples, first reaction of the RT-PCR amplification, pipetting of the first reaction template to the second PCR reaction, and analysis of the PCR products, was performed in separate rooms. Separate disposable filter tips, which were changed for each step and sample, were used throughout the entire experimental procedure. In addition, all procedures with the exception of the gel analysis were performed inside a biosafety cabinet level 2. To exclude carryover contamination, at least three negative controls, including a no RNA template control, were tested together with the actual samples in each RT-PCR run.

Sequencing and phylogenetic analysis. Sequencing of HEV RNA positive samples was performed directly on both strands at the Iowa State University DNA Facility, Ames, IA. Sequences were aligned with published data using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/>). Sequences were compiled using Lasergene software and the Clustal W alignment algorithm (DNASTar, Madison, WI). For sequence analysis, the 310 nt sequences of the 5' end of the ORF2 gene were compared with each other and to sequences of genotype 3 HEV isolates from Cameroon

TABLE 2
Prevalence of anti-HEV IgG antibodies in pigs located in different states in Nigeria*

State	Number tested	Sample source	Age (months)	Positive	Suspect	Negative
Plateau	176	Abattoir	< 5	66.7% (10)	0	33.5% (5)
			5–8	37.5% (18)	8.3% (4)	54.2 (26)
			8–12	41.2% (21)	3.9% (2)	54.9% (28)
			< 12	56.5% (35)	4.8% (3)	38.7% (24)
			Total	50.6% (89)	4% (7)	45.5% (80)
Delta	25	Farms	Unknown	20% (5)	16% (4)	64% (16)
Lagos	17	Farms	Unknown	29.4% (5)	23.5% (4)	47.1% (8)
Ogun	19	Farms	Unknown	63.2% (12)	10.5% (2)	26.3% (5)
Oyo	24	Farms	Unknown	20.8% (5)	16.7% (4)	62.5% (15)
Taraba	25	Farms	Unknown	76% (19)	12% (3)	12% (3)
TOTAL	286			47.2% (135)	8.4% (24)	44.4% (127)

*Data presented as percentage (total number of positive samples).

(GenBank accession no. KC012634), the Democratic Republic of the Congo (GenBank accession no. FJ600536), the United States (GenBank accession no. AF060668), Japan (GenBank accession no. AB094231), the Netherlands (GenBank accession no. AF332620), and Kyrgyzstan (GenBank accession no. AF455784). Additionally, a genotype 2 isolate (GenBank accession no. M74506) and a genotype 1 isolate (GenBank accession no. M80581) were also included. The nucleotide distance of the sequences was evaluated by neighbor-joining using Lasergene MegAlign. Confidence in the neighbor-joining tree was estimated by 1,000 bootstrap replicates. Sequences reported in this work have been deposited in the GenBank database under the accession nos. KJ451629 to KJ451633.

RESULTS

Evidence of seroconversion to HEV in Nigerian pigs. The HEV ELISA results are summarized in Table 2. The overall prevalence of anti-HEV IgG was 47.2% (135 of 286) among the samples. Although anti-HEV antibodies were detected in pigs in all states tested, there were regional differences. Particularly in the Ogun and Taraba States the prevalence of HEV positive pigs was 73.7% (14 of 19) and 88% (22 of 25), although it was only 36% (9 of 25) in the Delta State. An association between the type of husbandry and HEV infection detection could not be established (data not shown).

HEV RNA was identified in Nigerian pigs and belongs to genotype 3. The PCR results are summarized in Table 1. Samples positive for HEV RNA were identified in all regions and pig populations tested. A total of 15 samples were selected for follow-up sequencing. Among these, eight samples could be amplified using the nested RT-PCR and five yielded a sequence. Sequencing confirmed that all of the positive samples belonged to HEV genotype 3 (Figure 2). Sequencing results on one sample from the Langtang South region, three from the Mangu region, and one from the Shendam region indicated that the strains in this study had 99.3% to 92.5% nucleotide identities for the 310 nt fragment of the 5' end ORF2. Specifically, the MG25 and MG18 samples shared 99.3% of nucleotide identity with each other, 97.2% with LS13, 94.8% with SHD50, and 92.5% with the MG7 sample. Between sequences described here and other available African pig HEV sequences, there was 85.2–89.6% nucleotide identity (GenBank accession nos. KC012634 and FJ600536). The HEV sequences reported herein were closely related to strains from Japan (GenBank accession no. AB094231) and from the Netherlands (GenBank accession no. AF332620) with 90.5–94% and 88–92.2% nucleotide identity, respectively.

DISCUSSION

In Nigeria, although hepatitis of other viral origin is widely recognized,^{19–21} the HEV situation is still largely unknown

FIGURE 2. Phylogenetic tree based on a 310 nucleotide region in the 5' end ORF2 of the HEV genome. Sequences that were obtained in this study are underlined. The phylogenetic tree was constructed by the NJ method. Significant bootstrap *P* values are indicated as a percentage for 1,000 replicates.

because incidence of the disease and its etiologic agent are likely underreported. Human HEV genotype 2 infection in Nigeria has been documented¹⁴ and the prevalence of anti-HEV antibodies in humans is ~13.4%.²² The HEV transmission occurs mainly through the fecal–oral route, and pigs have been established to be reservoirs. The objective of this study was to characterize HEV infection by determining the presence of anti-HEV antibodies and RNA in Nigerian pigs.

Pig husbandry systems in Nigeria are similar to what is currently used in Asia and South American countries where HEV has been identified in pig populations.^{1,23} In Nigeria, the husbandry systems practiced include intensive, semi-intensive, and extensive or free range farming²⁴ where pigs are either housed or allowed to roam freely to scavenge for food. In the free range system, pigs travel far in search of food and water. They visit waste heaps and stagnant or flowing water bodies thereby contaminating such areas with feces and urine. These husbandry systems predispose pigs to diverse infections including HEV and thus facilitate subsequent transmission to humans, especially in environments where there is close association of pigs and people. However, an association between the type of husbandry and HEV infection detection could not be established herein.

Presence of HEV antibodies and HEV RNA was demonstrated in varying proportions of pigs in several important pig rearing regions in Nigeria. There was a high detection rate of HEV RNA in fecal samples from 2 to 6 months old pigs; however, pigs in Nigeria are usually slaughtered at 9 months of age. Further studies testing samples of pigs at different ages need to be done to verify the shedding of HEV RNA, especially in pigs at slaughter age.

Similar to other reports in Africa, HEV genotype 3 was identified.^{12,13} Interestingly, strains characterized in this study presented a higher nucleotide identity with Japanese and European HEV strains than with published African strains. Indeed, HEV transmission from one country to another through international trading has been previously discussed in Asia, South America, and Africa.^{12,25–27} The majority of the pigs raised in Nigeria belong to the pure Nigerian local pig breed, although crossbreeds between the local breed and Large White, Hampshire, or Landrace²⁸ imported from North America and Europe are common. The HEV genotype 3 recovered in this study could potentially be traced back to European pigs but more information is needed to confirm this.

Both HEV genotype 3 and 4 circulate in pigs and are also associated with sporadic HEV infections in humans, whereas genotypes 1 and 2 are mainly linked to human epidemics.^{6,8} In addition to reports of large hepatitis E outbreaks in Africa, sporadic hepatitis E in Africa has been reported in Nigeria, Kenya, and Egypt. In these cases, routes of HEV transmission were not documented, and the extent of sporadic hepatitis E in Africa remains largely unknown. Food-borne transmission of HEV through consumption of raw or undercooked pork meat and entrails in high-income countries has been extensively documented.⁸ In contrast to countries in which pork is traditionally consumed raw, in Nigeria meat products are usually well-cooked and served in the form of stew and very often are conserved in salt for preservation. Cooking of the meat thoroughly is the most efficient method to inactivate HEV and to prevent food-borne HEV infection in humans.⁸ However, foodborne transmission cannot be ruled out as a possible transmission route in Nigeria.

To the authors' knowledge this is the first report of characterization of HEV infection in pigs in Nigeria.

Received March 10, 2014. Accepted for publication June 6, 2014.

Published online July 7, 2014.

Acknowledgments: The authors thank Hussaini Ullaramu and Agom Danmarwa for providing a subset of the serum samples used in this study.

Financial support: OAO received a Robert S. McNamara fellowship from the World Bank.

Authors' addresses: Olajide A. Owolodun, Priscilla F. Gerber, and Luis G. Giménez-Lirola, Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, E-mail: owolodun@iastate.edu, jidecoy@yahoo.com, pgerber@iastate.edu, and luisggl@iastate.edu. Jacob K. P. Kwaga, Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, E-mail: jacobkwaga@yahoo.com. Tanja Opriessnig, Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, E-mail: tanjaopr@iastate.edu, and The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom, E-mail: Tanja.Opriessnig@roslin.ed.ac.uk.

REFERENCES

1. Kmush B, Wierzbica T, Krain L, Nelson K, Labrique AB, 2013. Epidemiology of hepatitis E in low- and middle-income countries of Asia and Africa. *Semin Liver Dis* 33: 15–29.
2. Purcell RHEmerson SU, 2008. Hepatitis E: an emerging awareness of an old disease. *J Hepatol* 48: 494–503.
3. Rein DB, Stevens GA, Theaker J, Wittenborn JS, Wiersma ST, 2012. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology* 55: 988–997.
4. Emerson SUPurcell RH, 2003. Hepatitis E virus. *Rev Med Virol* 13: 145–154.
5. Meng XJ, Anderson D, Arankalle VA, Emerson SU, Harrison TJ, Jameel S, Okamoto H, 2012. Hepeviridae. King AM, Adams MJ, Carstens EB, Lefkowitz EJ, eds. *Virus taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. London, UK: Academic Press, 1021–1028.
6. Kamar N, Bendall R, Legrand-Abravanel F, Xia NS, Ijaz S, Izopet J, Dalton HR, 2012. Hepatitis E. *Lancet* 379: 2477–2488.
7. Lu L, Li C, Hagedorn CH, 2006. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol* 16: 5–36.
8. Meng XJ, 2013. Zoonotic and foodborne transmission of hepatitis E virus. *Semin Liver Dis* 33: 41–49.
9. Tesse S, Lioure B, Fornecker L, Wendling MJ, Stoll-Keller F, Bigaillon C, Nicand E, 2012. Circulation of genotype 4 hepatitis E virus in Europe: first autochthonous hepatitis E infection in France. *J Clin Virol* 54: 197–200.
10. Colson P, Romanet P, Moal V, Borentain P, Purgus R, Benezech A, Motte A, Gerolami R, 2012. Autochthonous infections with hepatitis E virus genotype 4, France. *Emerg Infect Dis* 18: 1361–1364.
11. Hakze-van der Honing R, van Coillie E, Antonis AF, van der Poel WH, 2011. First isolation of hepatitis E virus genotype 4 in Europe through swine surveillance in the Netherlands and Belgium. *PLoS ONE* 6: e22673.
12. Kaba M, Colson P, Musongela JP, Tshilolo L, Davoust B, 2010. Detection of hepatitis E virus of genotype 3 in a farm pig in Kinshasa (Democratic Republic of the Congo). *Infect Genet Evol* 10: 154–157.
13. de Paula VS, Wiele M, Mbunkah AH, Daniel AM, Kingsley MT, Schmidt-Chanasit J, 2013. Hepatitis E virus genotype 3 strains in domestic pigs, Cameroon. *Emerg Infect Dis* 19: 666–668.
14. Buisson Y, Grandadam M, Nicand E, Cheval P, van Cuyck-Gandre H, Innis B, Rehel P, Coursaget P, Teyssou R, Tsarev S, 2000. Identification of a novel hepatitis E virus in Nigeria. *J Gen Virol* 81: 903–909.

15. Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU, 1997. A novel virus in swine is closely related to the human hepatitis E virus. *Proc Natl Acad Sci USA* 94: 9860–9865.
16. Owolodun OA, Gimenez-Lirola LG, Gerber PF, Sanford BJ, Feagins AR, Meng XJ, Halbur PG, Opriessnig T, 2013. Development of a fluorescent microbead-based immunoassay for the detection of hepatitis E virus IgG antibodies in pigs and comparison to an enzyme-linked immunoassay. *J Virol Methods* 193: 278–283.
17. Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR, 2006. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods* 131: 65–71.
18. Huang FF, Haqshenas G, Guenette DK, Halbur PG, Schommer SK, Pierson FW, Toth TE, Meng XJ, 2002. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. *J Clin Microbiol* 40: 1326–1332.
19. Forbi JC, Iperepolu OH, Zungwe T, Agwale SM, 2012. Prevalence of hepatitis B antigen in chronic HBV carriers in North-central Nigeria. *J Health Popul Nutr* 30: 377–382.
20. Okusanya BO, Aigere EO, Eigbefoh JO, Ikheola J, 2013. Sero-prevalence and clinico-epidemiological correlates of hepatitis C viral antibodies at an antenatal booking clinic of a tertiary hospital in Nigeria. *Arch Gynecol Obstet* 288: 495–500.
21. Sule WF, Kajogbola AT, Adewumi MO, 2013. High prevalence of anti-hepatitis A virus immunoglobulin G antibody among healthcare facility attendees in Osogbo, Nigeria. *J Immunoassay Immunochem* 34: 75–82.
22. Adesina OA, Japhet MO, Donbraye E, Kumapayi TE, Kudoro A, 2009. Anti-hepatitis E virus antibodies in sick and healthy individuals in Ekiti state, Nigeria. *Afr J Microbiol Res* 3: 533–536.
23. Echevarria JM, Gonzalez JE, Lewis-Ximenez LL, dos Santos DR, Munne MS, Pinto MA, Pujol FH, Rodriguez-Lay LA, 2013. Hepatitis E virus infection in Latin America: a review. *J Med Virol* 85: 1037–1045.
24. Kofoworola AM, 2007. Socioeconomic characteristics of pig producers and their husbandry practices in the sub-humid zone of northern Guinea Savannah, Nigeria. *Agricultural Journal* 2: 306–311.
25. Wu JC, Chen CM, Chiang TY, Tsai WH, Jeng WJ, Sheen IJ, Lin CC, Meng XJ, 2002. Spread of hepatitis E virus among different-aged pigs: two-year survey in Taiwan. *J Med Virol* 66: 488–492.
26. Munne MS, Vladimirovsky S, Otegui L, Castro R, Brajterman L, Soto S, Guarnera E, Molina V, Monfollano M, Schlauder GG, Gonzalez JE, 2006. Identification of the first strain of swine hepatitis E virus in South America and prevalence of anti-HEV antibodies in swine in Argentina. *J Med Virol* 78: 1579–1583.
27. Tanaka Y, Takahashi K, Orito E, Karino Y, Kang JH, Suzuki K, Matsui A, Hori A, Matsuda H, Sakugawa H, Asahina Y, Kitamura T, Mizokami M, Mishiro S, 2006. Molecular tracing of Japan-indigenous hepatitis E viruses. *J Gen Virol* 87: 949–954.
28. Oseni S, 2005. Evaluation of the F1 and backcrosses of Nigerian local pigs and the large white for litter characteristics in south-west Nigeria. *Livest Res Rural Develop* 17: 44.