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Hepatitis E Viral Infection in Pigs from North-Eastern Uganda: A Case Study of Amuria and Napak Districts

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Abstract: Hepatitis E is a zoonotic viral disease and swine are regarded as the major reservoirs. The aim of this study was to find out the sero-prevalence and risk factors of HEV in pigs from Napak District in Uganda where an outbreak of human HEV was reported in 2013/2014 and in the neighboring district of Amuria. Additionally, farmers' level of awareness and knowledge about livestock hepatitis was investigated. A total of 352 sera samples were tested; 302 from Amuria and 50 from Napak using a commercial indirect ELISA kit (ID vet, France). A pretested structured questionnaire was administered to a total of 139 respondents at every site of sample collection. The sero-prevalence was 84% in Napak and 14.6% in Amuria. Overall prevalence was 24%. The sero-prevalence was significantly higher in Napak as compared to Amuria District ($p<0.05$, $\chi^2 = 112.0056$). Therefore, pigs more likely to be seropositive were from Napak District ($p = 0.000$, OR = 30.334, CI = 11.847-77.672) and those from households without latrines ($p = 0.02$, OR = 3.482, CI = 1.568-7.733). In addition, 8.6% of the respondents knew of hepatitis in livestock and 7.9% knew that it spreads to humans. Meanwhile, 1.4% were aware of its possible control in humans by vaccination. This study reported a high sero-prevalence of HEV than previously reported in Uganda and a very low level of awareness among respondents about hepatitis in livestock. The study recommends other studies to identify more risk factors of infection, genotyping of the HEV and sensitizing communities about viral hepatitis in livestock.

INTRODUCTION

Hepatitis E Virus (HEV) is an RNA virus that exists in both enveloped and non-enveloped forms and its genome contains three non-overlapping Open Reading Frames (ORF 1-3). The HEV has one serotype with at least 4 genotypes and at least 24 subtypes[1]. Genotypes 1 and 2 are currently known to infect only humans and genotypes 3 and 4 are extremely diverse and infect majorly humans and pigs[1]. However, anti-HEV antibodies have also been detected in other animal species including rodents, dogs, cattle, sheep and goats[2] possibly due to infection with genotypes 3 and 4. In pigs, infection with genotypes 3 and 4 in most cases, results into unapparent infection with mild hepatitis hence pigs are considered the major reservoirs[3, 4] while in humans, the infection can become clinical, especially in children, pregnant women and immunocompromised individuals[5,6]. Therefore, HEV infection is considered an emerging viral zoonosis[7] with potentially high impact in large pig producing but financially constrained countries like Uganda. Transmission of HEV is largely to be through the feco-oral route following consumption of contaminated water or food with human or animal fecal material and consumption of contaminated meat[8]. Disease outbreaks or sporadic infections in humans due to zoonotic genotypes have been reported in Europe[9] and Asia[10]. Zoonotic HEV infections are also possible in Uganda because of the presence of a large pig population, close contact between humans and pigs, poor sanitary measures in some areas rearing pigs and high number of immunocompromised individuals due to HIV/AIDS.

The HEV infections have been reported in pigs in the developed and developing countries with varying prevalence[11-13]. Currently, there are no specific antiviral drugs to treat HEV infection. In addition, the first HEV vaccine registered in China in 2011 is not available globally and other candidate vaccines do not have complete data on their effectiveness for control of infection in humans and livestock[14]. Therefore, prevention and control of zoonotic hepatitis E will largely depend on identifying and minimizing exposure to the risks of infection. In 2008, during HEV outbreak investigation in humans from Kitgum District, Northern Uganda, the zoonotic HEV genotype 3 was reported to be circulating in pigs, in addition to the non-zoonotic genotype 1 that circulated in humans. However, only 8 pigs were tested. Another outbreak of HEV in humans in Napak District, North-Eastern Uganda in 2013/2014 was reported[15]. However, the genotypes in the Napak outbreak in North-Eastern Uganda were not characterized. Additionally, there is paucity of information on the epidemiology of HEV in pigs from North-Eastern Uganda. The objectives of this study were to assess community knowledge and awareness on hepatitis in livestock and to determine the sero-prevalence and risk factors of HEV in pigs from two districts in North Eastern Uganda.

MATERIALS AND METHODS

Study design and study area: This was a cross-sectional study carried out in Napak District of Uganda where outbreak of human HEV infection and sporadic cases were reported in 2013/2014[15]. In addition, one district (Amuria District) with the highest pig population and neighboring Napak was selected. The study was carried out from 2017-2018.

Sample size: For this study, the minimum sample size was calculated using the prevalence from the previous study involving samples from central, Western and Northern Uganda[11] and therefore, the prevalence of 23.6% was considered in the formula by Dohoo *et al*.[16].

$$n = \frac{Z\alpha^2 pq}{L^2}$$

where, n = sample size, Zα = 1.96, p = 0.357, q (1-p) = 0.643 and L = 0.05, minimum sample size, n = 277 sera samples. However, 352 sera samples were analyzed in this study in order to increase precision.

Sampling of pigs and data capture: The total number of pigs in Amuria District from previous estimates was 41,320 pigs[17]. During the previous estimates[17], Napak was part of Moroto District with a population of 5,530 pigs. This gave approximate pig ratio of about 7.5:1. For lack of recent numbers for Napak District, this ratio was used to approximate the number of pigs to be sampled. Therefore, in this study, 50 samples were from Napak and the rest were from Amuria District. In Amuria District, sampled pigs were from three sub-counties (Acowa, Akoromit and Wera) with the highest number of pigs based on the previous census[17]. In Napak District, the pigs were from Matany town council with the highest concentration of people in this district. The pig herds in each district were identified by snowball method as previously done[18]. In each pig herd, all adult and growing pigs and a half of the piglets were sampled. In addition, a pre-tested questionnaire was administered to collect information on disease awareness to assess knowledge on hepatitis in livestock and management of pigs to identify possible risk factors of HEV infection. Where necessary during data collection, the information in the questionnaire was translated into the local languages (Ateso and Ngakarimojong) spoken in the two districts. Consent from the head of the household was sought after explaining the objective of the research.

Blood collection from pigs: Blood was collected from each pig into labeled uncoated vacutainer tubes (AV consumables, India) by jugular venipuncture using 21G needle (BD, USA). The blood was kept on ice in a cool box and transported to the district Veterinary laboratory where the blood was allowed to clot overnight. Serum was collected from each tube, aliquoted and transported on ice to the laboratory at the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University for storage at -20°C until analysis.

Analysis of serum for exposure to HEV to determine sero-prevalence: Serum was analysed for antibodies (IgG) against HEV using Enzyme-Linked Immunosorbent Assay (ELISA)[19, 20] following the manufacturer's recommendations (ID Vet. France). Frozen samples (-20°C) were placed on the bench for 10 minutes to thaw at room temperature. All reagents in the ELISA kit were warmed to room temperature and homogenized by inversion before use. To each of the 96 microwells, 190 µL of dilution buffer-2 were added and then 10 µL of negative control were added to four control wells (A1, A2, B1, B2) and 10 µL of positive control to four wells (C1, C2, D1, D2). Then 10 µL of each sample were added in a bi-well format to the remaining wells (each sample into adjacent odd and even wells) and incubated for 45 min at room temperature. The wells were emptied and washed three times with 300 µL of the wash solution. Then 100 µL of the multispecies Horseradish Peroxidase (HRP) conjugate was added to the wells and incubated for 30 min at room temperature. The working conjugate was prepared by diluting the concentrated conjugate in dilution buffer-3 in a ratio of 1:10. The wells were emptied and washed again three times with 300 µL of wash solution. Then 100 µL of the substrate (TMB) were added to each well and incubated for 15 min in the dark and at room temperature. Exactly 100 µL of the stop solution (Sulphuric acid) were added to each well to stop the reaction. The Optical Density (OD) in each well was read using ELISA plate reader at 450 nm.

Interpretation of the ELISA OD values: The net OD for each sample was obtained ($OD_{Even\ well}$-$OD_{odd\ well}$). The net mean OD of the positive control was also calculated. The test was valid if the net mean OD for positive control was >0.350 or the ratio of net mean OD positive control to net mean OD negative control was >3.

The Sample Positive (S/P) percentage for each sample was calculated using the formula:

$$S/P = \frac{\text{Net OD sample}}{\text{Net OD positive}} \times 100$$

Any sample with S/P≥70% was considered positive for exposure to HEV.

Data management and analysis: The captured data were entered into Microsoft excel package office 2010 and analyzed. The Chi-square test was used to analyze the difference in the sero-prevalence between the two districts. The coded questionnaire data were transferred to IBM SPSS Statistics 25 (IBM, USA) and analyzed at univariate level for potential risk factors. All factors with a $p<0.25$ were cross-tabulated to test for confounding. For confounding factors, one of them was retained for multivariate analysis based on biological plausibility.

Ethical approval: The study was approved by the Research and Ethics Review Board at COVAB under the number SBLS.KI.2017.

RESULTS AND DISCUSSION

Sero-prevalence of HEV infection in pigs from North-Eastern Uganda: In total, 352 samples were tested for exposure to swine HEV. Of the 352, 50 were from Napak District and 302 were from Amuria District. In total, 86 of the 352 samples were sero-positive, hence overall sero-prevalence was 24% in the two districts (Fig. 1). In Amuria District, 44 samples (14.6%) were sero-positive while in Napak District, 42 samples (84%) were sero-positive. Using the Chi-square test, the sero-prevalence was significantly higher in Napak than in Amuria ($p<0.05$, $\chi^2 = 112.0056$).

Management of the pigs from the two districts: Data from 310 pigs was analyzed for the management system. In Napak District, most of the pigs (n = 41.93%) were kept in semi-permanent enclosures or kraals inside the "Manyattas" (Fig. 2). This method of management was categorized as semi-intensive. A "Manyatta" is a fenced hamlet enclosing several homesteads in one village. There was accumulation of pig dung in the enclosures or kraals, a sign of poor fecal disposal. Human fecal material was also commonly observed within the Manyattas. In Amuria District, most of the pigs (n = 167.63%) were tethered and 15 pigs (5.6%) were in semi-intensive system (Fig. 3).

Knowledge and awareness on hepatitis in livestock among the pig farmers: A total of 139 individuals were recruited using a questionnaire; 5 (3.6%) were from Matany sub-county in Napak District and 134 (96.4%) from Amuria District. In Amuria district, 40 (28.8%) were from Acowa sub-county, 46 (33.1%) from Akoromit sub-county and 48 (34.5%) from Wera sub-county (Table 1). Most participants (64%) were male and aged between 41-60 years (43.2%). The highest number (87.8%) were peasants and a majority (44.6%) had only attained primary (basic) education (Table 1).

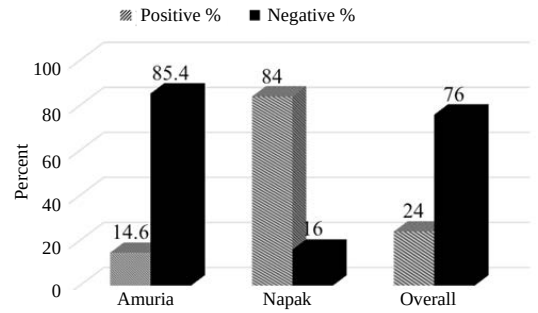

Fig. 1: Prevalence of exposure to hepatitis E virus among pigs from North-Eastern Uganda

Fig. 2: Pigs kept in a semi-permanent enclosure in Napak (A) and pigs tethered (Red Arrow) in Amuria (B)

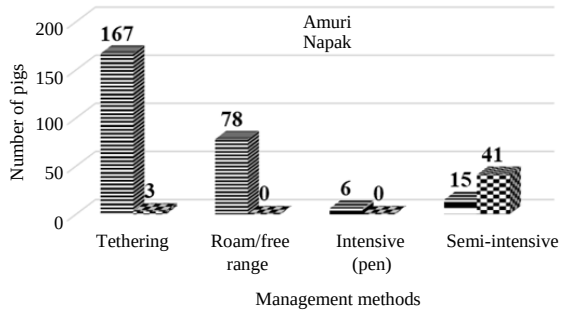

Fig. 3: Number of pigs under different management systems in the two districts

Out of the 139 respondents, 127 (91.4%) of them did not know about hepatitis in livestock but a few (n = 12; 8.6%) were aware of the existence of hepatitis in livestock. Similarly, 128 (92%) individuals did not know that hepatitis in livestock can spread to humans but 11 (8%) knew that it can affect humans too. Two of the people (1.4%) knew that hepatitis in humans can be prevented and treated by vaccination but the majority (n = 137; 98.6%) had no idea about the treatment or prevention of hepatitis in humans (Table 2).

Risk factors among individual pigs: Fourteen independent variables were analyzed as potential risk factors for hepatitis E seropositivity among 310 pigs at

Table 1: Sociodemographic characteristics of the 139 participants recruited for the study in Amuria and Napak districts, North-Eastern Uganda

Variables	Number (%)
Districts	
Amuria	134 (96.4)
Napak	5 (3.6)
Sub-county	
Acowa	40 (28.8)
Akoromit	46 (33.1)
Wera	48 (34.5)
Matany	5 (3.6)
Gender	
Male	89 (64)
Female	50 (36)
Age group (years)	
17 and below	1 (0.7)
18-30	29 (20.9)
31-40	37 (26.6)
41-60	60 (43.2)
61 and above	12 (8.6)
Education level	
None	26 (18.7)
Primary	62 (44.6)
Secondary	39 (28.1)
Tertiary	12 (8.6)
Occupation	
Civil servant	11 (7.9)
Peasant	122 (87.8)
Others	6 (4.3)

univariate analysis using Chi-square or Fisher's Exact test. These variables with $p \leq 0.25$ were tested for confounding by cross-tabulation and two variables i.e., sharing boars for breeding by the households and sighting of wild swine near homesteads did not confound with the district where samples were collected ($p > 0.05$). However, sharing boars and sighting of wild swine near homesteads were confounding ($p = 0.021$). Therefore, two models, each with two factors were tested for model fitness at multivariate analysis. For the better fitting model, presence/absence of latrine in the homestead was forced into, based on biological plausibility (Table 3) and the fitness of the model was assessed.

At multivariate analysis, two factors were significantly associated with sero-positivity (Table 4). The pigs in Napak District were more likely to be sero-positive than those in Amuria ($p = 0.000$, OR = 30.334, CI = 11.847-77.672) and pigs from households without latrines were also more likely to be sero-positive than those from households with latrines ($p = 0.002$, OR = 3.482, CI = 1.568-7.733) (Table 4).

The current study is arguably the first study to document sero-prevalence of HEV in domestic pigs from Uganda at farm/homestead level while the other study of a kind was done at the slaughterhouse. The overall sero-prevalence of 24% from the two districts in North-Eastern Uganda in the current study was the same as that reported by Katagwa *et al.*[11] in a similar study in a Ugandan abattoir where pigs from several districts in central, Eastern, Western and Northern Uganda were sampled. However, in the current study, higher sero-prevalence of

Table 2: Knowledge of hepatitis in livestock, spread to humans and treatment

Questions	Response by Famers	Number (%)
Are you aware of hepatitis in livestock?	No	127 (91.4)
	Yes	12 (8.6)
Does hepatitis in livestock spread to humans?	Don't know	128 (92)
	Yes	11 (7.9)
What is the treatment in humans?	Don't know	137 (98.6)
	Vaccination	2 (1.4)

Table 3: Variables chosen for multivariate analysis

Variables	Level	Number of pigs seropositive (%)	Number of pigs seronegative (%)	p-value at univariate analysis
District	Amuria	40 (15)	226 (85)	
	Napak	38 (86.4)	6 (13.6)	0.000
Latrine	Present	50 (19.2)	210 (80.8)	
	Absent	28 (56)	22 (44)	0.000
Boars for mating	Share	43 (19.8)	174 (80.2)	
	Not share	35 (37.6)	58 (62.4)	0.001

Table 4: Risk factors at multivariate analysis

Variables		Coefficient	SE	p-values	OR	CI OR
District						
Napak	Amuria	3.412	0.480	0.000	30.334	11.847-77.672
Boars						
Not share	Share	0.609	0.357	0.088	1.838	0.914-3.697
Latrine						
Absent	Present	1.248	0.407	0.002	3.482	1.568-7.733

Fit statistics: Hosmer and Lemeshow p = 0.940, Model = 0.000

84% was registered in Napak District which was more than double the highest prevalence of 36.8% recorded by Katagwa *et al*.[11] in pigs from Kampala City/District. In addition, the prevalence in pigs from Napak District was significantly higher than that recorded in pigs from Amuria District. This suggests that the prevalence of HEV in pigs from Uganda varies from district to district. It is therefore of interest to investigate the reasons for these differences. According to Walachowski *et al*.[21], mingling of pigs and hygienic conditions were the risk factors for high sero-prevalence of HEV in pigs from France. Mingling of pigs in Napak could be related to the very high sero-prevalence of HEV because pigs in Napak were kept in "Manyattas" where there is easy mingling of pigs from different homesteads. The pigs in a Manyatta were mostly kept in a kraal but sometimes left to roam. Free range/roaming pigs travel far in search for food and water (scavenge). They also visit heaps of refuse and stagnant water, places that can act as sources of infection[15]. On the other hand, the homesteads in Amuria were far apart, separate from each other, pigs were mostly tethered and therefore, there was less mingling of pigs from different homesteads. Additionally, hygiene in pig kraals is often poor with accumulation of dung and if contaminated, transmits infection to other pigs within the kraal. Napak is also known to have the lowest hygienic and sanitary conditions. According to Amanya *et al*.[15], atleast 76% of individuals in Napak did not have latrines and that 71% performed open defeacation habits with a low latrine coverage of 18.6% and safewater coverage of 62% and only 44% of the safe water sources were functional. Therefore, poor hygienic and sanitary conditions and semi-intensive management of pigs in Napak could be the real reasons for significantly higher sero-prevalence of HEV in this district than in Amuria District since the disease is majorly spread through the oro-fecal route. The possible role played by these factors in the spread of HEV among pigs should be investigated.

The ELISA kit used in this study was coated with HEV genotype 3 antigen. The HEV genotype 3 is one of the strains associated with sporadic/isolated cases of HEV in humans in the developed countries. Sporadic cases of human HEV have also been reported in North-Eastern Uganda[22]. Although, it is known that the large outbreaks of HEV in humans are caused by the non-zoonotic genotypes 1 and 2, the possible role played by HEV genotype 3 in sporadic cases in humans in this sub-region should be investigated. It is possible to have human cases due to genotype 3 in Napak because of the high sero-prevalence in pigs, close contact between livestock and humans and poor hygienic and sanitary conditions.

About 8.6% of the recruited individuals knew about livestock hepatitis and 7.9% knew that it spreads to humans. Only 1.4% knew that it is controlled by vaccination. These findings are similar to those by Chowdhury *et al*.[23] where they noted a very low level of knowledge toward zoonoses by farmers and the most known zoonoses being rabies and tetanus at 96.65%. In a similar study by Cruz *et al*.[24], they noted a very low level of knowledge and awareness toward viral hepatitis with over 83% of individuals not knowing the forms of viral hepatitis. They further identified that a large proportion of the population (over 50%) cited HEV as being non-existent. As opposed to this current study

where 1.4% of the recruited individuals knew that hepatitis is controlled by vaccination, Cruz *et al*.[24] found a high percentage of over 63% in Brazil. Similarly to the swine HEV study carried out in Uganda in slaughter houses by Katagwa *et al*.[11], >90% of the individuals did not know about zoonotic HEV, possible causes, transmission and wether it can affect them or not. The low level of knowledge and awareness in the current study coud be due to the low level of education and low income status. Most individuals had attained only primary (basic) level education, were peasant farmers with most of them in the 41-60 age bracket and this was similarly observed among the Brazilians by Cruz *et al*.[24].

CONCLUSION

The findings from the study showed a high proportion of pigs from the two districts sero-positive for HEV and this varied by district. This still conforms to the assumption that pigs are the prime reservoirs of HEV and hence a threat to public health if no prevention and control measures are put in place. Despite the high sero-prevalence of HEV in pigs, the level of awareness about zoonotic HEV among the pig farmers was very low. Lack of a latrine, a major sanitary facility in homesteads in Uganda was a risk factor for seropositivity.

The study recommends other studies in both urban and rural settings since pork consumption is taking a big trend, to find the epidemiology of HEV in Uganda. Genotyping of the HEV isolates from the two regions is necessary to know the various zoonotic genotypes and more risk factors of infection in pigs should be determined. Due to close resemblance with domestic swine, studies should also target wild swine (wild pigs and warthogs) to determine their sero-prevalence and role in disease reservation. The role of government and non-governmental organizations in creating awareness among farmers about viral hepatitis, livestock HEV and potential risk factors is essential in the control of outbreaks in very prone areas like Napak.

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