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Research

Seroprevalence of Hepatitis E virus in slaughter pigs and practices of abattoir workers in Kampala, Uganda

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Abstract

Introduction: Hepatitis E Virus (HEV) is the most common cause of acute viral hepatitis in humans globally. Pigs are considered the main natural reservoir of zoonotic Hepatitis E virus. With the recent increasing cases of zoonotic Hepatitis E virus, there are limited reports on its seroprevalence in domestic pigs, and awareness among occupationally exposed individuals in Uganda. Therefore, this study aimed at determining the seroprevalence of Hepatitis E virus antibodies in slaughtered pigs and to assess the knowledge and practices of abattoir workers regarding zoonotic Hepatitis E virus.
Methods: a total of 182 blood samples were collected randomly from slaughtered pigs. The collected serum was processed and analysed using a commercial indirect enzyme linked immunosorbent assay (ELISA) kit to detect anti- Hepatitis E virus antibodies. Eighty (80) abattoir workers in Wambizzi were interviewed using a structured questionnaire to assess their level of knowledge and occupational practices that may expose to zoonotic Hepatitis E virus.
Results: our study reveals Hepatitis E virus antibody seroprevalence of 23.6% in slaughtered pigs. In addition, 95% (76/80) of the interviewed abattoir workers were not aware of the existence of zoonotic Hepatitis E virus. The study documented no usage of personal protective gear and that of direct contact with biological fluids and wastes from slaughtered pigs were potential risk practices for Hepatitis E virus transmission in pig abattoirs.
Conclusion: this study reports high seroprevalence of Hepatitis E virus antibodies in slaughter pigs, limited knowledge on the existence of zoonotic Hepatitis E virus among abattoir workers and potential risk practices for zoonotic transmission of Hepatitis E virus. Therefore, we recommend further studies with wider coverage to establish the dynamics of zoonotic Hepatitis E virus and characterise the genotypes circulating in pigs and humans. Pig abattoir workers and other occupationally exposed individuals should be sensitized on the existence of zoonotic Hepatitis E virus and equipped with public health safety measures to protect acquiring the virus.

Introduction

Hepatitis E Virus (HEV) is a major public health concern in developing countries with sub-standard sanitation conditions [1, 2]. HEV has also been reported as an endemic in many industrialized and developed countries [3]. In humans, HEV is the primary cause of acute hepatitis [4] with mortality rates up to 4% in immune-competent individuals. Higher mortality rate of up to 20% have been reported among pregnant women [5], while immune-compromised individuals develop chronic HEV infection [1, 6]. HEV is classified into five genotypes [7] and of the 5 genotypes, HEV genotypes 3 and 4 are zoonotic and are particularly harboured by the domestic pigs, wild boars and other animal species [8-11], while genotype 5 is exclusively known to infect chickens [12]. The risk for human infection with HEV genotypes 3 and 4 is associated with exposure to infectious body fluids of infected animals and consumption of contaminated undercooked pork, and other associated infected animal products [13]. In developed countries, HEV genotypes 1 and 2 infections have been diagnosed in travellers returning from endemic countries[14]. However, sporadic autochthonous cases of genotypes 3 and 4 have also been reported in individuals without any travel history to endemic regions [15-17]. Besides humans, infections of pig herds with HEVgenotype 3 and 4 have been reported in developed countries [18, 19]. In developing countries, several HEV outbreaks have been reported [20-22], and in 2007, outbreaks of HEV in humans in Uganda caused 160 deaths and over 10,196 illnesses [23]. The human population at risk of acquiring the zoonotic HEV that has been reported include; pig farmers, pork consumers, veterinarians and workers at pig abattoirs [24-26]. Uganda has the largest pig population in Eastern Africa, approximately 3.3million pigs (UBOS, 2008); and pig farming has greatly improved food security and livelihoods. Consequently, the ready pork market in Kampala attracts pig traders from different districts of Uganda. Hence, the objectives of this study were to determine the seroprevalence of HEV in slaughtered pigs; and to assess abattoir workers' knowledge and practices toward HEV in Wambizzi pig abattoir -Kampala, Uganda.

Methods

The cross-sectional study was carried out at Wambizzi pig abattoir in Nalukolongo in Kampala, Uganda. The study population consisted of Wambizzi abattoir workers, as well as pigs brought for slaughter. Blood samples were collected from 182 randomly slaughtered pigs between June and December, 2015. Samples were transported in cool boxes to Mulago Hospital Immunology Laboratory and serum was extracted, stored at -80 ºC until it was analyzed. Eighty (80) abattoir workers in Wambizzi present and consented at the time of study were interviewed using a structured questionnaire to assess their level of knowledge and occupational practices that might expose them to zoonotic HEV. Written consent was obtained from individual participants prior to being interviewed.

ELISA Assays for detection of anti-HEV antibodies: Pig collected from the blood samples, were screened for the presence of HEV-IgG antibodies using a commercially available ID Screen® Hepatitis E Indirect ELISA kit (IDVet Innovative Diagnostics, France) following manufacturer's instructions. Briefly, 190µl of dilution buffer was added to each micro well; then 10µl of the negative and positive controls were added to wells A1, A2, B1, B2 and C1, C2, D1, D2 respectively. For the remaining wells, 10µl of each respective pig serum was added, and incubated at 21°C for 45minutes. The microplate was washed three times with 300µl of a wash solution. Then, 100µl of the diluted conjugate was added to each well, and incubated at 21°C for 30 min. The microplate was then washed three times with 300µl of the wash solution. Then, 100µl of the substrate solution was added to each well, and incubated in the dark for 15minutes at 21°C. The colour-developing reaction was stopped by adding 100µl of the stop solution to each well. Reading of plates was done at 450nm with a microplate ELISA reader (Results were interpreted according to manufacturer's instructions.

Data analysis: data was entered into Microsoft Excel 2010 and analysed using SPSS (Statistical software version 20) computer programs. Chi-square test was performed to establish the relationship between different variables. 95% CI were computed, and test results were considered significant for $p< 0.05$.

Ethical consideration: this study was under a large study titled Capacity building for Integrated Management of Transboundary Animal Diseases and Zoonosis (CIMTRADZ) project at COVAB that was peer reviewed and approved by the College of Health Sciences -Institutional Review Board (CHS-IRB), College of Veterinary Medicine, Animal resources and Biosecurity Institutional Review Board (COVAB-IRB), and Uganda National Council of Science and Technology.

Results

Seroprevalence of anti-HEVantibodies among slaughter pigs by Indirect ELISA: one hundred and eighty two (182) serum samples from slaughtered pigs at Wambizzi abattoir were analysed for anti-HEV antibodies. Of the total number of pigs sampled, 23.6% or 43/182 (95% CI 18.1-30.2) were seropositive for anti-HEV. The analysis revealed higher seroprevalence rates of 14/38 (36.8%), 6/19(31.6%) and 5/14 (35.7%) of HEV antibodies in slaughtered pigs from Kampala, Masaka, and Wakiso districts respectively (Figure 1). In addition, the seroprevalence of HEV antibodies was higher in slaughter pigs (27.2%) older than 6 months.

Socio-demographic characteristics of study respondents: a total of 80 human abattoir workers were interviewed during the study period and was composed of 50% males and 50% females, of which only 5% had attained tertiary education as summarised in Table 1.

Knowledge about zoonotic HEV among Wambizzi abattoir attendants: most of the demographic social factors in the study population were not associated with the level of awareness/knowledge on the zoonotic HEV except for the education that was significantly associated ($p < 0.027$). The majority of the respondents 76 (95%) were not aware of HEV as a zoonotic disease, pigs as reservoir for the virus and risk factors for disease transmission (Table 2).

Practices that could predispose abattoir workers to HEV: fifty eight percent (58%) of the respondents in the study reported to have eaten under cooked pork, (80%-90%) as well as not wearing personal protective gear and most of the times in direct contact with blood, saliva and/or faeces from slaughtered pigs.

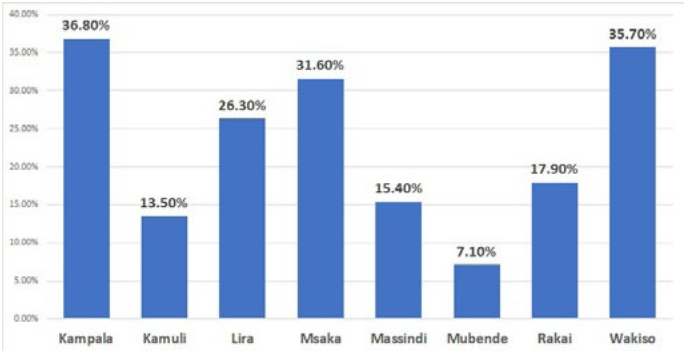

Figure 3
higher education resource services, East Africa, organizational and management structure

Table 1: socio-demographic characteristics of respondents

Items	Freq	%	95% CI
Sex			
Male	40	50	38.8 - 61.3
Female	40	50	38.8-61.3
Marital status			
Married	48	60	48.8-70.0
Single	21	26.3	17.5-36.3
Divorced	9	11.3	5.0-18.8
Widowed	2	2.5	0.0-6.3
Education			
Primary	42	52.5	41.3-63.8
Secondary	28	35	25.0-45.0
Tertiary	4	5	1.3-10
others	6	7.5	2.5-13.8

Table 2: knowledge of abattoir workers toward Zoonotic HEV, Wambizzi slaughter house

Variable for Knowledge	Response	Frequency (n=80)	Percentage (%)	95% CI
Heard about HEV	Yes	4	5	1.3-10
	No	76	95	90.0-98.8
Pig zoonosis				
	Agree	4	5	1.3-10.1
	Neither agree or disagree	56	70	59.5-79.7
	Disagree	15	18.8	10.1-26.6
	Strongly disagree	5	6.3	1.3-12.7
Pigs as major reservoir				
	True	2	2.5	0.0-6.3
	False	4	5	1.3-11.4
	Don't Know	74	92.5	86.1-97.5
Infection due to sausages				
	True	2	2.5	0.0-6.3
	False	4	5	1.3-10.1
Due to undercooked pork	Don't Know	74	92.5	86.1-97.5
	True	4	5.1	1.3-11.4
	False	6	7.6	2.5-13.9
	Don't Know	70	87.3	79.7-93.7
Due to direct contact-pigs				
	True	2	2.5	0.0-6.3
	False	8	10.1	3.8-17.7
	Don't Know	70	87.4	79.7-93.7
Due occupational exposure				
	True	2	2.5	0.0-6.3
	False	10	12.7	6.3-20.3
	Don't Know	68	85	74.7-91.1

Discussion

In the present study, the overall seroprevalence of Hepatitis E virus (HEV) antibodies among slaughtered pigs was 23.6% (95% CI 18.1-30.2). This is the first known study to document anti-HEV antibodies in pigs from Uganda. This provides evidence that pigs are exposed to HEV and could be a potential reservoir for disease transmission to humans. HEV in slaughter pigs has been previously reported elsewhere including the United Kingdom [27]. Our report of HEV antibodies in slaughtered pigs at slaughter houses raises potential public health concerns for zoonotic transmission of HEVgenotypes 3 and 4 strains from pigs to humans. However, this study did not characterise the circulating genotypes in pigs. The seroprevalence of HEV antibodies in pigs observed in this study is comparable to 24.4% reported in Zaria state in Nigeria [28]. Higher HEV seroprevalence (55.6% and 97.0%) in pigs have been previously reported in Nigeria [29, 30]. The difference in the seroprevalence could be attributed to various factors, including; farming systems, geographical location as well as methodological differences [31, 32]. Findings from this study also demonstrate a variation in the seroprevalence of HEV antibodies in pigs from different districts with the highest being documented in urban districts. Similarly, a study done in Canada showed significant variations of HEV seroprevalence between the different geographical regions [33]. In this study, the observed differences could be due to the dynamics of transmission of HEV in pigs and husbandry practices in different farming systems from which pigs are selected and brought for slaughter at Wambizzi in Kampala. The high anti-HEV antibodies from urban districts could probably be due to the fact that pigs reared within these areas are kept in confinement buildings/ pens which hastens the spread of the virus amongst pigs within the same pen environment. Pigs housed in the same pen are routinely exposed to saliva, nasal secretions and urine of multiple sty-mates daily [34], and this results in rapid spread of the virus in a very short time. Additionally, besides greater contact frequency, pigs in the same enclosure experience more exposure to pig manure which increases the transmission rate [31]. Within-pen environmental transmission of HEV occurs between pigs in the same enclosure [35]. On the other hand, pigs reared in rural districts like Kamuli and Mubende are left to freely move and not always kept under confinement thus the low rate of spread of the virus due to less contact frequency between pigs, minimal exposure to infectious wastes, faeces, and urine and nasal secretions [31]. It should be noted, however, that the free ranging pigs could be exposed to infectious wastes of infected wild animals, rodents and other primate [25, 36, 37] which can be encountered in rural districts. This study reports a higher seroprevalence of HEV in pigs of six months and above than in pigs below 6 months as previously reported in other studies [19, 38, 39], and this could be due to long-term exposure of pigs to the virus [40].

Strikingly, the study revealed that the majority of Wambizzi abattoir workers (95%) did not know the existence of the zoonotic HEV, the possible source of the virus and how the virus is transmitted. They did not even know that they are occupationally exposed to the risk of acquiring the zoonotic HEV. This raises a serious concern on the health and safety of the pig abattoir workers and undocumented exposure to the public of this zoonotic HEV through contact with pigs by products and wastes. The high risk exposure is further potentiated by documented behavioural practices in this study whereby 58% of the respondents were reported to have eaten under cooked pork. In addition, 80-90% of the respondents were not using personal protective gear regularly hence directly exposed to blood, saliva, and/or faeces from slaughtered pigs. Not surprisingly therefore, Uganda has recently reported several HEV outbreaks in several districts in Northern Uganda. The most recent report of HEV outbreak in Karamonja region in 2014 recorded 1,390 confirmed cases of HEV; whereby 15 individuals died, of which 50% were pregnant mothers [41]. Previously high seroprevalence of HEV (25.5%) and evidence of recent infection (37.3%) was documented in children aged 0-15 years during 2007 HEV outbreak in Uganda [41]. Between 2007 and 2009, Uganda had the largest HEV outbreak in the world, an epidemic that caused more than 10,196 cases and 196 deaths [23]. This provides clear evidence that Uganda is a hotspot for HEV epidemics and concerted efforts are required to identify the source/ reservoirs for infection to prevent the disease in human population. Previous studies have shown that pig handlers and abattoir attendants and, farmers are at great risk of infection with HEV [42-44] due to exposure with HEV containing infectious material and eating undercooked pork [45-48]. This study provides an insight on the serological existence of anti-HEV antibodies in slaughtered pigs, practices that predispose abattoir workers to HEV and potential risks of HEV transmission to public.

Conclusion

Findings from this study show that there is a considerable proportion of HEV seropositivity in slaughtered pigs at Wambizzi abattoir that originated from different parts of Uganda. This demonstrates that pigs are a potential reservoir for HEV transmission and more studies are needed to understand the epidemiology of the virus in pigs and characterise genotypes circulating in pigs. Other potential reservoir sources of the HEV should be explored and documented to facilitate interventions for control measures.

What is known about this topic

- Hepatitis E virus (HEV) is the causative agent of hepatitis E and is a public health concern in many developing countries like Uganda;
- HEV is a zoonotic disease, and those at risk of infection are; pig farmers, pig abattoir workers, veterinarians and pork consumers.

What this study adds

- Our study provides information on the seroprevalence of HEV in slaughter pigs from various districts of Uganda as well as the levels of knowledge among occupationally exposed individuals at the pig abattoir;
- It also indicates that there are potential risks of transmission of HEV from pigs to humans who work at the abattoir due to occupational exposure and consumption of undercooked pork.

Competing interests

The authors declare no competing interest.

Authors' contributions

Katagwa Moses, Mugisha Lawrence, Samuel Majalija and Marvin Ssemadaali designed the study. Katagwa Moses conducted the field and laboratory work. Mugisha Lawrence, Samuel Majalija supervised the data collection and analysis. Katagwa Moses, Samuel Majalija and Gabriel Tumwine did the data analysis. Katagwa Moses, Lawrence Mugisha wrote the manuscript. All authors read and approved the final version of the manuscript.

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