Seroprevalence of hepatitis E in swine abattoir workers.

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Abstract

Background: Hepatitis E (HE) caused by Hepatitis E virus (HEV) is an emerging global public health threat. It has been identified as potentially zoonotic and swine act as main reservoirs.

Objectives: The objective of this study was to determine the seroprevalence and risk factors associated with HEV in swine abattoir workers.

Methods: This was a cross sectional study where 45 workers were sampled N=50, serum collected and tested for presence of anti HEV IgM using ELISA.

Results: A seroprevalence of 13.3% was obtained with the highest 50% among slaughterers and the lowest amongst cleaner, dobi and inspector. Those in direct contact with live pigs, their carcasses and tissues were at a high risk compared to those in indirect contact. Seroprevalence was seen to increase with age with the highest rate among those above 24 years.

Conclusion: There is silent HE virus infection in abattoir workers at Wambizi as reflected by presence anti HEV IgM in 13% of the tested serum. However, no single case of HE has ever been reported in swine abattoir workers or general population in Kampala city. This silent maintenance of HEV infection amongst swine abattoir workers is an occupational risk that could challenge public health systems.

Keywords: Hepatitis E; Seroprevalence; Swine abattoir workers.

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Introduction

Background

Hepatitis due to HEV is an emerging global public health problem with 20 million cases reported annually of which 3 million are acute resulting in 57,000 deaths worldwide and is the second most important cause of acute clinical hepatitis in adults in Asia and Africa. The disease initially considered a sanitation problem in resource limited countries has emerged as a zoonotic breaking boundary in the original outbreaks in developing countries to find its way into industrialized countries with high sero-prevalence. Hepatitis E was first documented in Uganda in 2007 as a sporadic outbreak in Kitgum where it caused over 10,196 illnesses and 160 deaths and the primary exposure has never been documented. The disease has since remained endemic in Karamoja sub region with high morbidity. Between July 2013 and February 2014, close to 200 people were reported to have died of HE in Napak district, but the source/point of entry remained elusive.

HEV is mainly transmitted through the oral-fecal route, and consumption of fecal contaminated water and food has been implicated in major outbreaks. Evidence has shown that HEV is zoonotically transmitted mainly through consumption of meat from infected animals. Swine have been singled out as the most important source of zoonotic infection and HEV seroprevalence of 51% has been reported in pig handlers and 14% in wild boar handlers. The zoonotic transmission of HEV pres-
ents a high risk to a larger population especially for individuals that work in game parks, abattoirs, butcherries and consumers of animal products such as pork, pig liver and undercooked game meat\textsuperscript{15,22}, and given that many people in Uganda depend on pigs and their products, HEV is of great public health concern.

Hepatitis E virus HEV is a positive sense; non enveloped single stranded RNA virus of the family Hepeviridae.\textsuperscript{23-25}. It causes viral hepatitis in humans that has been reported worldwide since it was first reported in Burma, India in 1955\textsuperscript{26}; and confirmed in 1983\textsuperscript{27}. The disease affects mainly persons aged between 15 and 40 years which is the most productive age bracket, and with mortality reported to be as high as 25% in pregnant women. The disease poses great economic challenges not only as loss of lives and huge medical bills but also millions of man hours are lost due to direct or indirect disease devastation. Although the zoonotic form has been suggested as the cause of the disease in the developed world mainly through consumption of undercooked pork products\textsuperscript{4,8,17,28-30}, zoonotic transmission possibility has not been documented in Uganda.

Based on the available data, five HEV genotypes have been identified; HEV I, II, III, IV and V\textsuperscript{31}, and all these genotypes have been documented in swine\textsuperscript{32}. This underscores the role of swine and swine handlers in the epidemiology of HEV that has unfortunately not been studied in Uganda. Swine HEV shares similar antigenic epitopes with the capsid protein of the human HEV, with the accompanying antibody cross reactivity and 90-100% sequence homology\textsuperscript{17,22,33-37}, a clear indication of a common ancestor. These findings point to the role of pigs which are widely used as a source food in many regions in Uganda could play in the epidemiology of HEV. Bearing in mind that abattoir workers are in constant interaction with pigs and their products, the possibility of introducing the virus in their communities cannot be underestimated. Furthermore, research has shown that HEV can also be transmitted through blood transfusion\textsuperscript{38} but screening for HEV infection is not on blood transfusion guidelines in Uganda. This study thought to understand the seroprevalence of HE and the risk factors that influence its transmission in swine abattoir workers in Kampala. The findings will offer evidence based guidelines to public health experts and epidemiologists in understanding HEV dynamics.

\textbf{Methodology}

A cross-sectional study was carried out in Wambizi pig Abattoir located in Nalukolongo in Lubaga Division, in Kampala, Central Uganda. The abattoir has 50 workers and supplies pork to pork joints in and around Kampala. Blood 2mls was collected from 45 abattoir workers 90% by venous puncture into plain vacutainer tubes and was labeled with the subjects’ unique identification number which was given to them at the site. Quantitative data was collected using a structured questionnaire to assess the risk factors for transmission of HEV.

In the Laboratory, the samples were centrifuged at 3000 rpm for 10 minutes and the supernatant serum was recovered and stored at -20°C until analysed. Analysis was done using DS-EIA-Anti HEV-M ELISA IgM assay kit for Hepatitis E virus IgM antibodies according to the manufacturer’s instructions. Results from the structured questionnaire were analysed using STATA statistical package version 12 to determine the degree of association between risk factors and the results.

\textbf{Results}

Six out of forty five samples collected tested positive for anti HEV IgM, giving a general seroprevalence of 13.3% at a confidence interval of 95%. Specifically seroprevalence varied with the activity someone was involved in identified as risk factors as summarised in table 1.

\textbf{HE Seroprevalence in different occupations}

The results showed 50% \(\frac{3}{6}\) of the positives to be slaughterers, 16.67% \(\frac{1}{6}\) for traders, supplier and for the cooks.
Table 1: Analysis of factors associated with HEV in workers at Wambizi pig abattoir

| Variable            | Hepatitis E N=45 | N=45 | cPR   | p-value |
|---------------------|------------------|------|-------|---------|
| Occupation          |                  |      |       |         |
| Dobi                | 1100% 00%       | 1    | 1.0   | 1.0     |
| Cleaner             | 1100% 00%       | 1    | 1.0   | 1.0     |
| Supplier            | 150% 150%       | 2    | 5.0x10^6 | <0.001 |
| Cook                | 375% 125%       | 4    | 2.5x10^6 | <0.001 |
| Trader              | 888.9% 111.1%   | 9    | 1.1x10^6 | <0.001 |
| Slaughter           | 2488.9% 311.1%  | 27   | 1.1x10^6 | <0.001 |
| Inspector reference | 1100% 00%       | 1    | 1.0   |         |
| Work duration       |                  |      |       |         |
| <4yrs               | 2395.8% 14.2%   | 24   | 5.71  | 1.102   |
| >4yrs reference     | 1676.2 523.8    | 21   | 1.0   |         |
| Sex                 |                  |      |       |         |
| Females             | 1285.7% 214.2%  | 14   | 1.107 | 0.9     |
| Males reference     | 2787.1% 412.9%  | 31   | 1.0   |         |
| Age                 |                  |      |       |         |
| <24yrs              | 4100% 00%       | 4    |       |         |
| 24-35yrs            | 2385.2% 414.8%  | 27   |       |         |
| >35yrs              | 1285.7% 214.3   | 14   |       |         |
| Eat pork            |                  |      |       |         |
| Yes                 | 3788.1% 218.2%  | 11   | 1.55  | 0.588   |
| No                  | 266.7 411.8%    | 34   | 1.0   |         |

*cPR= Crude Prevalence ratio

Discussion
This study was done in a population of swine abattoir workers where he infection has not been reported either in workers or in pigs. A seroprevalence of 13.3% n=45 was observed. The general seroprevalence range reported in developing countries is 3-80%39-40. Studies in healthy individuals in the United States where HEV is not endemic indicated a significant proportion of anti HEV antibodies of up to 22%41-42. This increased seroprevalence in a developed country has been associated with consumption of swine products such as pork and pig liver and travelling to areas where HEV is endemic. Other studies done in Africa indicated a seroprevalence ranging from 6-80%39. In Egypt a country where pork consumption is uncommon due to the dominant Islamic religion, HEV seroprevalence was reported to be 80%39. This can be attributed to other routes of transmission other than pork consumption probably the oral faecal route or other zoonotic sources. Hepatitis E virus RNA has been detected in faecal wastes and has been seen to persist in the environment43-44. In developing countries where farming methods and sewage treatment are poor, introduction of faecal wastes in the water system is likely. This compromises water quality and increases chances of disease outbreak. Exposure to animal wastes and fluids has been reported as another important source of infection45.

An increased seroprevalence was observed in people who were in direct contact with pigs, pig wastes and carcasses such as slaughterers, traders and suppliers. This is comparable to previous studies which indicated a high seroprevalence as high as 66%18,45 among swine farmers. This may be due to the fact that these individuals are exposed to swine wastes and blood when cleaning and assisting in birth. Of the 6 positives, 50% was observed in slaughterers 3/6, 16.67% in cooks and 16.7% 1/6 was observed in Traders and suppliers. A significant association was observed between the type of work and HEV seropositivity p-value=0.001, Table 1 suggesting animal-to-human transmission probably through animal wastes and blood. In this study exposure could also be from water or hands to the food they eat since no relationship was found between pork consumption and HEV seropositivity. On the contrary, inspectors who are equally exposed did not show any sero-reactivity for IgM. This may be due to fact that inspectors have a higher level of education and practice good personal hygiene than other workers, as this has been documented to influence HEV exposure45. This however remains a speculation as the education level of the participants was never analysed.
None of the positive subjects was showing clinical signs but it has been reported that HEV infections are associated with HEV viremia and faecal excretion of the virus for a few weeks sometimes in absence of clinical symptoms. The detection of anti HEV IgM antibodies indicates a recent active infection which suggests that the positive subjects had probably acquired the infection recently. These findings therefore allude to the fact that HEV is being transmitted asymptomatically among swine abattoir workers but the implication of this is moot. HEV has been reported in several regions with pigs as the source, although there is no documentation to show this in Uganda. There were two suppliers in the tested population of which one was positive. Since he moves to several pig farms purchasing animals, it cannot be concluded whether he got infected in the abattoir or the farms where he buys animals. Studies have shown that persons engaged in occupations related to swine farming were found to have a higher risk of infection than those in other occupations.

The results show a high seroprevalence of 23.8% n=21 among those who have spent over four years as compared to the 4.2% n=24 among those who have spent 4 years and less in the abattoir. This compares to other studies where period of occupational exposure to swine was associated with HEV infection. IgM antibody levels decline rapidly in 6 weeks while IgG persists in the host for long period of time providing protection against subsequent infections. The duration of persistence of circulating IgG antibodies remains unclear as they have been found in healthy subjects living in all geographical areas, although the prevalence varies widely. Since the workers were not tested for IgG antibodies, it cannot be determined whether those who had spent four years and less in the abattoir had already been exposed and were immune-competent or those above four years in the abattoir their immunity had waned.

Seroprevalence of HE was seen to increase with age. The results indicate a seroprevalence of 18.8% and 18.3% among the middle aged workers between 24 and 35 years and above 35 years respectively as compared to none among those below 24 years. This trend has been observed in other studies where a prevalence of 3 to 30% was observed in young adults between ages of 20 and 40 years than in children 0.3 to 10%. This can be attributed to the fact that middle aged adults may be involved in activities such as tending to animals and casual labour in places where they eat and drink poor quality food and water. However in another study in Nigeria a high seroprevalence was observed among those below 10 and above 60 years and the lowest seroprevalence was seen among those between 20 and 40 years. In this study, the increased seroprevalence among the middle aged may be explained by the fact that most of them are energetic males who are involved in slaughtering animals in the abattoir which increases their degree of exposure.

Males had a high seroprevalence compared to females, due to the fact that males are more involved in activities of slaughtering and supplying animals thus were more exposed to animals and animal carcasses as compared to females.

The population of workers who eat pork is large and in previous studies, pork consumption has been documented as another source for infections. The results of this study however indicate no risk associated with consumption of pork P value= 0.267. This could be because workers eat well cooked pork and other vehicles of HEV transmission such as exposure to the animal wastes during slaughtering and handling come into play. Although the animals were not tested the results indicate that those in direct contact with animals have higher chances of acquiring the infection as compared to those who were in indirect contact.

**Conclusion**

There is silent HEV infection amongst abattoir workers at Wambizi, as reflected by the high seroprevalence and this presents a huge public health concern. This silent infection is maintained by other means other than pork consumption, of which being in direct contact with pigs and pig products increases risk of HEV infection. There is need for an epidemiological investigation involving pigs and more humans in various occupations to help understand the epidemiology of HEV and guide control measures.

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Conflict of interest
The authors declare that there is no conflict of interest.

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