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Case Report

High seroprevalence of hepatitis E among pigs suggests an animal reservoir in Cameroon

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Abstract

Introduction: Hepatitis E virus (HEV) is one of the most prevalent cause of acute hepatitis in humans worldwide. The risk of HEV transmission is not limited only to spread from human to human but the infection can also spread from animals to humans, especially from the domestic pigs. Despite mounting evidence regarding the zoonotic potential of porcine HEV infection, there are limited data on its prevalence in pigs in the sub-Sahara Africa region. Therefore, the present study aimed to determine the seroprevalence of HEV antibodies among pigs in two Cameroonian regions.

Methodology: A total of 162 sera were collected from slaughtered-age pigs from January to March 2012. To determine whether pigs might represent a HEV reservoir in the Northern and Western region in Cameroon, anti-HEV IgG and IgM were tested by ELISA using commercials available kits.

Results: Overall, 70 of the 162 samples (43.2%, 95% CI: 35.5% - 51.2%) were positive for at least one of the serological markers of HEV infection (IgM and / or IgG).We observed a significant seroprevalence of HEV antibodies between the northern and western regions (60% (42/70) and 40% (28/70), p = 0.01796) respectively.

Conclusion: Overall, this study reports a high seroprevalence of Hepatitis E virus antibodies in slaughter pigs in Cameroon. Our findings suggest that pigs might be a cause of zoonotic HEV transmission in Cameroon. Therefore, further studies are warranted to establish the dynamics of zoonotic HEV and characterize the different genotypes circulating in humans and pigs.

Key words: Hepatitis E virus; seroprevalence; Cameroon.

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Introduction

Hepatitis E virus (HEV) is one of the most prevalent cause of acute hepatitis in humans worldwide. One-third of the world's population is at risk of becoming infected with HEV [1]. HEV is responsible for over 50% of cases of acute viral hepatitis in endemic countries. A case-fatality rate (CFR) of 1-4% was observed in the general population and might reach 30% in the pregnant women especially during the third trimester of pregnancy.In patients with chronic livere diseases, CFR may be even higher [2].

Four HEV genotypes cause human infections (genotypes 1, 2, 3 and 4). Genotypes 1 and 2 are predominantly found in human populations in developing countries. However, the risk of HEV transmission is not limited only to spread from human to human but the infection can also spread from animals to humans, especially from the domestic pigs and wild boars, suggesting the zoonotic potencial of the virus [3]. Genotypes 3 and 4 are globally distributed in humans and animals [4]. Genotypes 5 and 6 are found in wild boars [5]. Genotypes 7 and 8 were recently identified in dromedary and Bactrian camels, respectively [5,6].

Several transmission routes of hepatitis E have been identified, and include: transmission by contaminated drinking water [7]; food-borne transmission by ingestion of uncooked meat from infected animals [8], transmission through camel milk consumption [9] and transfusion of infected blood products [10]. Also, HEV can be transmitted via the transplantation of solid organs [11]. In developed countries, HEV is predominantly transmitted by the ingestion of pork and wild boar meat. Evidence for transmission of HEV-3 and HEV-4 by direct contact of humans with animals has been repeatedly described. Several studies have shown that persons with occupational contact to

domestic pigs such as slaughterers, pig farmers or veterinarians exhibit significant higher anti-HEV antibody prevalence than the general population [1]. In Africa, contaminated water causes serious epidemic outbreaks [1]. Other sources of infection such as animal transmission cannot be excluded since genotype 3 responsible for the zoonotic transmission of HEV has already been reported in some African countries [12]. The information on HEV infection in animals in this continent remains underreported [12]. In Cameroon, minimal attention has been paid to HEV epidemiology in humans and animals. Therefore, in this study, we evaluate the seroprevalence of hepatitis E in slaughter-age pigs in two regions of Cameroon.

Methodology

From January to March 2012, a total of 162 sera samples were randomly collected from slaughter-age pigs in a slaughterhouse in two Cameroonian regions (84 from the North region and 78 from the West region) (Figure 1). Blood samples were collected during the pigs slaughtering at the bleeding post as previously described [13]. All blood samples were transported in a cool box at 4°C, and then frozen and stored at -80°C until analysis.

At Centre Pasteur of Cameroon (CPC), in the virology department, sera were separated, aliquoted and stored at -80 °C until testing. All these sera were later tested for the presence of anti-HEV immunoglobulins (Ig) with enzyme-linked immunosorbent assays: HEV IgG ELISA and HEV IgM ELISA 3.0 kits (MP Biomedicals Asia Pacific Pte Ltd, Singapore).The test was carried out according to the manufacturer's instructions. Briefly, the sera diluted in diluent buffer were placed in wells coated. After incubation of 30 min at 37 °C, followed by washing, the conjugate (horseradish peroxidases) was added and incubated for 30 min at 37 °C. Plates were washed, and 100 µL of substrate solution (tetramethylbenzidine) were added. The reaction was stopped after 15 min with 50 µL of stop solution (hydrochloric acid) and absorbance was measured at 450 nm using spectrophotometer. For each analysis, positive and negative controls, provided with the kit, were used. The cut-off value was set at 0.500 for IgG and 0.400 for IgM added to the mean absorbance of the non-reactive controls.

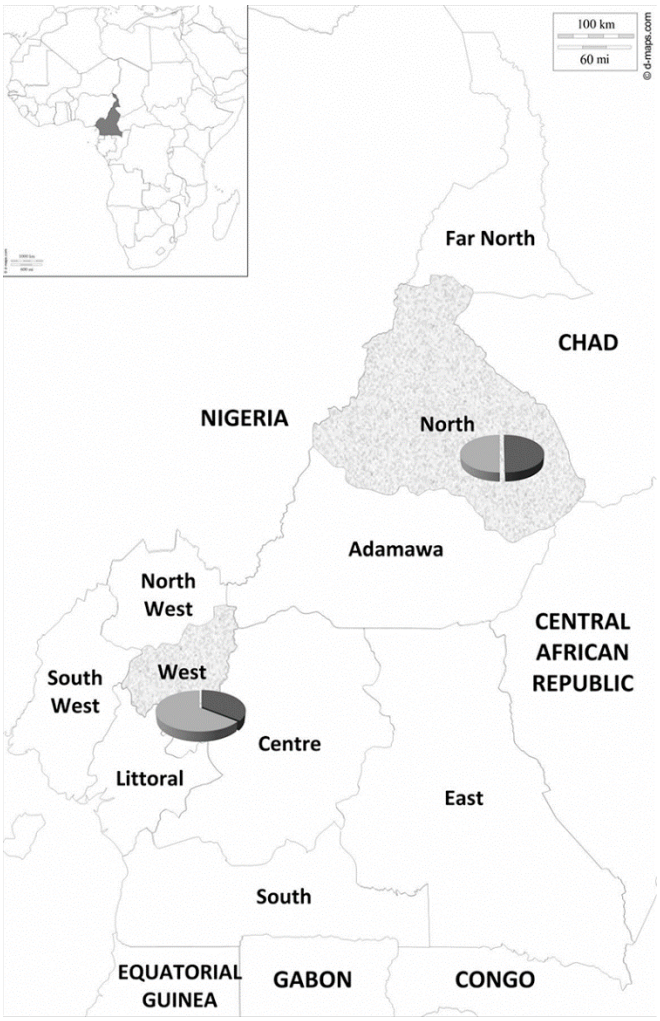
Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (IBM SPSS Statistics v22.0, USA). A p-value of less than 0.05 was deemed statistically significant. Comparison between serology of samples and regions was assessed by the chi- squared test.

Results

Seventy of the 162 samples (43.2%, 95% CI: 35.5% - 51.2%) were positive for at least one of the serological markers of HEV infection (IgM and / or IgG). The HEV ELISA results are summarized in Table 1. Higher seroprevalence of 50% (42/84) was noted in the North region of Cameroon as compared to the West region (28/78; 35.9%). There was established a significant difference in the seroprevalence between the two regions (p = 0.018). Seroprevalence of the recent HEV infection (presence of IgM alone), ongoing infection (simultaneous presence of IgM and IgG) and past infection (presence of IgG alone) in this population

Figure 1. Map of Cameroon showing the two tested regions and the seroprevalence of HEV in pigs.



In black are the proportions of positive and in grey are the proportions of negative.

Table 1. Results of detection of specific anti-hepatitis E virus antibodies.

Antibody results	North N (%)	West N (%)	Total N (%)
IgG positive and IgM positive	0	4 (5.1)	4 (2.5)
IgG positive, IgM negative	3 (3.6)	3 (3.8)	6 (3.7)
IgG negative, IgM positive	39 (46.4)	21 (26.9)	60 (37.0)
IgG negative and IgM negative	42 (50)	50 (64.1)	92 (56.8)
Total	**84**	**78**	**162**

were 37% (60/162), 2.5% (4/162) and 3.7% (6/162), respectively. Both regions had similar IgM and IgG positivity profile with higher frequency of recent infection. Ninety-two out of 162 samples (56.8%) had a negative result for both anti-HEV antibodies.

Discussion

In this study, we report results of serological analysis for HEV in pigs in two regions of Cameroon at 43.2%. Several reports of higher hepatitis E seroprevalence in pigs have been observed in other studies. In Madagascar [14] and Scotland [15] HEV seroprevalence of 71% and 61.4% were respectively noted in slaughter-age pigs. This can be explained by the fact that, in those studies was used an ELISA that can detect 3 serological markers (IgM, IgG and IgA) comparing to only two serological markers used in this study. The proportion of past HEV infection as reflected by the presence of IgG antibodies was lower than that reported by other studies [16]. Also, a review by Salines *et al*. (2017) reported that anti-HEV IgM were more detected in younger pigs while anti-HEV IgG were more detected in older ones [17]. Other studies have observed similar IgM and IgG profiles [18]. In our study, the high proportion of 37.0% of anti-HEV IgM positive samples can thus be attributed to a recent infection since all pigs had attained slaughter age. Studies showing a higher prevalence of anti-HEV IgM antibodies compared to IgG are rare in the literature. However, a study published by Seminati *et al*. (2008) shows that pigs older than 12 weeks of age have a higher IgM seroprevalence (50%), compared to IgG seroprevalence (33.9%) [19].

Though the Northern and the western regions are not the main markets for pig trading in Cameroon, they however constitute the major towns from which live pigs are obtained [20]. A significant difference was noted in the seroprevalence of HEV within the two regions of Cameroon similarly to reports by García-Hernández *et al*. [16] where higher seroprevalence was noted in the Northern part of the country. In depth studies are required in order to determine the serporevalence of this infection in other regions of the country where pig farming is of less economic importance or where farming practices differ since high population density, and shorter production cycles have been shown to significantly increase the risk for HEV infection [20].

In Cameroon, although hepatitis of viral origin is widely recognized, HEV remains largely unknown because of the lack of investigation on the disease as well as limited data on HEV infection in animals and humans. Today, only three HEV seroprevalence studies have been conducted in humans in Cameroon [2,21,22]. In animals, one study was conducted on the genome characterization of HEV in Cameroonian pigs [23] while no study had yet been performed on the seroprevalence of HEV in this population. Our study reported a high seroprevalence of recent HEV infection in pigs and thus pigs could be considered the reservoir of the disease with the possibility to transfer the infection to humans. Moreover, there has been a previous detection of HEV genotype 3 in pigs from Cameroon [23] who have been incriminated in zoonotic transmission and could thus support this hypothesis.

Conclusion

In Cameroon, HEV seroprevalence in pigs is high. Importantly, our study showed the highest seroprevalence of the recent HEV infection (presence of IgM alone). Thus, future studies are required to confirm the high seroprevalence of anti-HEV IgM in pigs and the probability of pig-to-human zoonotic transmission.

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