


Research Article

Hepatitis E Virus (HEV) Seroprevalences in Pigs and among pork Butchers in two regions of Northern Togo

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Abstract

In the last decade in West Africa, the number of autochthonous cases of hepatitis E has significantly increased. Some hepatitis E virus (HEV) infections have been attributable to zoonotic transmission. This study was carried out to assess the seroprevalence of HEV among the exposed human and local pig populations in the cities of Kara and Sokodé (Togo), and the surrounding localities. A total of 89 breeders-butchers (5 women and 84 men), were recruited from November 2021 to February 2022 and their HEV serological status and socio-demographic status were assessed. In addition, 176 serum samples from slaughtered pig belonging to these breeders-butchers were collected. All human and swine sera were tested for the presence of HEV antibodies. We used the serological survey data from the general population cohort to compare with the current butcher/breeder cohort. The association between anti-HEV status and potential risk factors was evaluated. HEV IgM and IgG antibodies were detected in 20.22% (95% CI: 19.33 - 21.10%) and 5.6% (95% CI: 5.09 - 6.10) of the butcher serum samples, respectively. No specific behavior of the butchers was associated with seropositivity in butchers ($p \geq 0.05$). Total anti-HEV antibody seropositivity was 80.11% (95% CI: 79.66 - 80.55) in pigs. These results from asymptomatic population suggest 1) circulation of HEV in the pig butcher population and 2) pigs as the virus reservoir with probable zoonotic transmission in these areas. These data could provide evidence to understand the epidemiology and clues to control transmission in Kara, Sokodé and their surrounding localities in Togo.

Keywords: Hepatitis E Virus (HEV), seroprevalence, pork butchers, swine, Togo

Introduction

Hepatitis E Virus (HEV) is one of the leading causes of acute hepatitis in the world. HEV is a small icosahedral virus of the family *Hepeviridae*, which includes two genera *Orthohepevirus* and *Piscihepevirus*. The genus *Orthohepevirus* contains four species designated as *Orthohepevirus A* through *D*. Within *Orthohepevirus A*, 8 distinct mammalian genotypes (gt) have been identified to date (HEV 1-8) [1]. The gt 1 and 2 infect only humans and cause epidemics in developing countries in Africa and Asia, as well as in Mexico. They are primarily transmitted via the fecal-oral route due to fecal contamination of drinking water. Thus, it is related to the absence or failure of a safe water supply [2-3]. In contrast, gt 3 and gt 4 viruses have proven zoonotic potential and infect humans and several other animal species, such as pigs (*Sus scrofa domesticus*), wild boar (*Sus scrofa scrofa*), and deer (*Cervidae*)

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Table I: Sociodemographic characteristics of butchers

Population:	Breeder-Butcher
	N=89(%)
Age	
≤25	35 (39.3)
26-35	24 (27.0)
36≥	30 (33.7)
Gender	
Female:	5 (15.6)
Male:	84 (94.4)
Number of years in activity	
[0 - 5]	46 (51.7)
[5 - 10]	10 (11.2)
[10 - 15]	11 (12.4)
[15 - 20]	8 (9.0)
[20 - 25]	7 (7.8)
≥ 25	7 (7.8)
Level of study	
Primary	27(30.3)
Secondary	46 (51.7)
University	16 (18.0)
Area of residence	
Urban:	15 (16.1)
Rural:	74 (83.1)
Profession	
Breeder-Butcher:	89(100)
Butcher No Breeder	0(0)
Other:	0(0)
Religion	
Christian:	29 (32.6)
Muslim:	0 (0)
animist:	60 (67.4)
Source of water consumed	
Well:	32 (36.0)
Tap:	57 (64.0)
Mineral:	0(0)
Water treatment before consumption (chlorine or slurry)	
No:	89(100)
Yes:	0(0)
Cooking method of the meat before consumption	
Cooked to medium rare:	89(100)
No medium rare:	0(0)
Consume pork meat	
No:	0(0)
Yes:	89(100)
Hands washing with detergent	

No:	0 (0)
Yes:	89 (100)
Have knowledge of hepatitis A, B and C	
No:	
Yes:	46 (51.7)
	43 (48.3)
Already screened for hepatitis B	
No:	60 (67.4)
Yes:	29 (32.6)
Already vaccinated against hepatitis B	
No:	73 (82.0)
Yes:	16 (18.0)

HEV Seropositivity

The table II shows the seroprevalence of the different study populations. A total of 18 samples (20.22%) (95% CI: 19.33-21.10) of the 89 pork butchers were positive for IgM antibodies, indicating a recent HEV infection, and 5 samples (5.6%) (95% CI: 5.09-6.10) were positive for IgG antibodies, indicating a recovering or old infection. There were 2 double positive IgG+IgM+. Because the risk of IgG false positive associated to Malaria fever was found in much serological analysis. We also evaluate the Malaria status of the human population thus five samples (27.7%) (95% CI: 5.09-6.10) of the anti-IgM HEV positive were found to be positive for the Rapid Diagnostic Test *Plasmodium falciparum* versus none in the anti-IgG HEV positive lot. Regarding detection in pigs 141/178 (80.1%) (95% CI: 79.66-80.55) were positive for total HEV antibodies.

Whatever was the geographical origin of the pigs, the HEV seroprevalence is the same (Table III), indicating that circulation of HEV is probably an old story but with some silence period as the youngest animals have already the very same prevalence as the oldest one. In Bohou there was a tendency to a prevalence increase with age that may signed a continuous virus circulation.

Risk factors associated with HEV infection

Observation of the risk factor analysis shows that no factor was significantly associated with anti-HEV IgM in butchers ($p \geq 0.05$). To avoid confounding factors with risk factors associated with infection, the 5 individuals positive for both anti-IgM HEV and plasmodium in butchers were excluded from the data. In the general population, 1% (3/289) of butchers who did not touch pigs or handle pig carcasses were observed and 37.0% (107/289) were involved in raising poultry, goats, cattle, dogs and cats. The ratio of anti-IgM VHE of butchers and the general population was (OR: 2) with $p = 0.202$. Tables IVa and IVb present successively the risk factors and behavioral risk factors associated with anti-IgM HEV infection in both the general population and butchers and those associated with anti-IgG HEV infection in butchers.

Table II: Results of HEV total and M immunoglobulin detection in butchers / pigs

Serological characteristics	% (n/N)	IC 95%
Total antibodies positives (IgM+ or IgG+)		
Human:	23.6 (21/89)	22.7-24.5
Anti-IgM positives (human)	20.2 (18/89)	19.3-21.1
Anti-IgG positives (human)	5.6 (5/89)	5.1-6.1
Anti-IgM positives and IgG negative		
Human:	17.9 (16/89)	17.1-18.8
Anti-IgM negative and IgG positive		
Human:	3.3 (3/89)	3.0-3.8
Anti-IgM positives and Ag Malaria Pf Positive Human:	27.7 (5/18)	46.4-8.9
Anti-IgG positives and Ag Malaria Pf Positive Human:	0 (0/5)	-
Total antibodies positives (IgM+IgG) Pigs:	80.1(141/178)	79.7-80.5

IC: Confidence interval, n: size, (-): Not calculated

Table III: Seroprevalence of pigs (P) tested per sample site

Sampling site	Seroprevalence	Seroprevalence		
	% (n/N)	(Age in months)		
		[3-6]	[6-12]	≥12
Sotouboua	81(13/16)	100(4/4)	71(5/7)	80(4/5)
Adjengré	77(14/18)	67(4/6)	100(5/5)	71(5/7)
Sokodé	75(12/16)	80(4/5)	86(6/7)	50(2/4)
Bohou	86(24/28)	75(6/8)	80(8/10)	100(10/10)
Tchitchao	72(13/18)	67(4/6)	60(3/5)	86(6/7)
Pyä	85(22/26)	100(7/7)	80(8/10)	78(7/9)
Tcharè	77(23/30)	75(6/8)	75(9/12)	80(8/10)
Kouméä	83(20/24)	86(6/7)	78(7/9)	88(7/8)
Total	80(141/176)	80(41/51)	78(51/65)	82(49/60)

IC: Confidence interval, n: size, (-): Not calculated

Table IVa: Anti-HEV immunoglobulins associated with risk factors

Population	Pigs butchers						General population		
	Anti-IgM HEV			Anti-IgG HEV			Anti-IgM HEV		
	Neg: n (%)	Pos: n (%)	Pvalue	Neg: n (%)	Pos: n (%)	Pvalue	Neg: n (%)	Pos: n (%)	Pvalue
Age									
≤25	31 (91.1)	3 (8.9)		32 (94.1)	2 (5.9)		146(85.3)	25(14.7)	
26-35	18 (78.2)	5 (21.8)	0.406a	20 (86.9)	3 (13.1)	0.149b	88(92.6)	7(7.4)	0.190c
36≥	22 (81.4)	5 (18.6)		27 (100)	0 (0)		21(91.3)	2(8.7)	
Gender									
Female:	4 (80.0)	1 (20.0)	0.578a	5 (100)	0(0)	0.730b	111(94)	7(5.9)	0.011*c
Male:	67 (84.8)	12 (5.2)		74 (93.6)	5(6.4)		144(84.2)	27(15.8)	
Number of years of activity									
]0-5[40 (88.8)	5 (12.2)		42 (93.3)	3 (6.7)				
[5-10[8 (88.8)	1 (12.2)	0.122a	9 (100)	0 (0)	0.590b	-	-	-
[10-15[7 (63.6)	4 (36.4)		9 (81.8)	2 (18.2)				
[15-20[6 (85.7)	1 (15.3)		7 (100)	0 (0)				
[20-25[5 (100)	0 (0)		5 (100)	0 (0)				
≥25	5 (71.4)	2 (28.6)		7(100)	0 (0)				
Level of study									
Primary	22 (81.5)	4 (18.5)		23 (88.4)	3 (11.6)				

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Secondary	36 (78.3)	7 (21.7)	0.560aa	42 (97.6)	1 (2.4)	0.7111b	-	-	-
University	13(81.3)	2 (18.8)		14 (93.3)	1 (6.7)				
Area of residence									
Urban:	14 (93.3)	1 (6.7)	0.273a	14 (93.3)	1 (6.7)		238(87.5)	34(12.5)	0.121c
Rural:	57 (82.6)	12 (17.4)		65(94.2)	4 (5.8)	0.636b	17(100)	0(0)	
Religion									
Christian:	28 (96.6)	1 (3.4)	0.223a	8 (96.6)	1 (3.4)	0.433b	106(86.8)	16(13.2)	0.623c
Muslim:	0(0)	0(0)		0(0)	0(0)		144(88.8)	18(11.2)	
Other:	43 (78.1)	12 (21.9)		51 (92.7)	4 (7.3)		5(100)	0(0)	
Presence of animals in the house									
Yes:	58 (86.5)	9 (14.6)	0.248a	63 (94.0)	4 (6.0)	0.735b	123(85.4)	21(14.5)	0.138c
No:	13 (76.5)	4 (23.5)		16 (94.1)	1 (5.9)		132(91)	13(9)	
Breeding practice									
No:	13 (76.5)	4 (23.5)		16 (94.1)	1 (5.9)		162(89)	20(11)	0.593c
Yes (with pork):	58 (86.5)	9 (14.6)	0.248a	63 (94.0)	4 (6.0)	0.735b	0(0)	0(0)	
Yes (no pork):	0(0)	0(0)		0(0)	0(0)		93(86.9)	14(13.1)	

Table IVb: Anti-HEV immunoglobulins associated with comported risk factors

Population	Pigs butchers						General population		
	Anti-IgM HEV			Anti-IgG HEV			Anti-IgM HEV		
	Neg: n (%)	Pos: n (%)	Pvalue	Neg: n (%)	Pos: n (%)	Pvalue	Neg: n (%)	Pos: n (%)	Pvalue
Wearing gloves									
No:	63 (86.3)	10 (13.7)	0.226a	70 (95.8)	3 (4.2)				
Yes:	8 (72.7)	3 (27.3)		9 (81.8)	2 (18.2)	0.126b	-	-	-
Source of water consumed									
Well:	25 (83.3)	5 (16.7)	0.527a	28 (93.3)	2 (6.7)	0.590b	101(85.5)	17(14.5)	0.380c
Tap:	46 (85.1)	8 (14.9)		51 (94.4)	3 (5.6)		148(89.6)	17(10.4)	
Mineral:	-	-		-	-		6(100)	0(0)	
Hand washing after the sale									
No:	0 (0)	0 (0)	-	0 (0)	0 (0)				
Yes:	71 (84.5)	13(15.4)		79 (94.0)	5 (6.0)	-	-	-	-
Hands washing with detergent									
No:	0 (0)	0 (0)	-	0 (0)	0 (0)	-	14(93.3)	1(6.7)	0.529c
Yes:	71 (84.5)	13 (15.4)		79 (94.0)	5 (6.0)		241(87.9)	33(12.1)	
Have knowledge of hepatitis A,B and C									
No:	36 (83.7)	7 (16.3)	0.538a	41 (95.3)	2 (4.7)	0.477b	116(87.8)	16(12.2)	0.863c
Yes:	35 (85.3)	6 (14.7)		38 (92.6)	3 (7.4)		139(88.5)	18(11.6)	
Already screened for hepatitis B									
No:	49 (85.9)	8 (14.1)	0.408a	54(94.7)	3 (5.3)	0.519b	77(90.6)	8(9.4)	0.423c
Yes:	22 (81.4)	5 (18.6)		25 (92.5)	2 (7.5)		178(87.2)	26(12.8)	
Already vaccinated against hepatitis B									
No:	57 (82.6)	12 (17.4)	0.273a	65 (94.2)	4 (5.8)	0.636b	230(88.1)	31(11.9)	0.856c
Yes:	14 (93.3)	1 (6.7)		14 (93.3)	1 (6.7)		25(89.2)	3(10.8)	

*Significant (p<0.005), n: size, (-): Not calculated

a: Comparison of butchers' exposure to Anti-IgM VHE+ and the risk factor

b: Comparison of butchers' exposure to Anti-IgG VHE+ and the risk factor

c: Comparison of general population exposure to Anti-IgM VHE+ and the risk factor

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Discussion

In previous studies in Togo, it was established evidence of the diffusion of HEV in the general human [14]. Although contamination of drinking water is the main source of virus spread, numerous studies have shown that zoonotic transmission is also one of the sources of HEV spread. Pork meat, which is highly appreciated by consumers because of its taste, is increasingly becoming part of the eating habits of most Togolese, especially those living in the northern part of the country. Pork butchers find it a great opportunity to increase their income. Unfortunately, the activity is unstructured and poorly organized, so that pigs are slaughtered in makeshift slaughterhouses without any health regulations. We observed that the pork butchery attracts mostly males, 84 (94.4%) who reside in the outskirts of towns 74 (83.1%) with a secondary education level 46 (51.7%). This result was similar to that reported in Benin where 92.8% were male [15] and in Chad where 77% were educated [16]. These data suggest that, like any activity requiring a great deal of physical effort, men were the most solicited and the essential link in this economic activity.

In the previous study, the anti-IgM and IgG seroprevalence of HEV in the general population in 2023 was 11.7% and 5.6% respectively. In the present study, the anti-IgM HEV seroprevalence was 20.22% with ratio (OR: 2; $p= 0.202$), and that of IgG was 5.6%. These data suggested that HEV circulates more actively in this population group of swine butchers and they had twice the chance of contracting the virus. These results could be explained by the lack of hygiene around this activity, handling meat, organs and feces of pigs without protection. The high IgM to IgG ratio in these studies may suggest that there are several foci of transmission with a higher frequency of new infections or that those tested had associated autoimmune diseases or had malaria at the time of testing with a possible interaction. The difference could be due to the high sensitivity of the anti IgM kits, the versions of the tests to detect low concentrations of IgG, but also to differences in the type of IgG antibodies present in the sera [17]. Five (5; 27.7%) were positive for antibodies to *Plasmodium falciparum* out of 18 positives for HEV anti-IgM. As some studies have shown, antibodies to *Plasmodium* Ag may interact nonspecifically with antigens of other agents such as HEV that could test positive, resulting in false positives to anti HEV. Thus, this false positive rate could abnormally increase the seroprevalence of HEV immunoglobulin. Certainly, a study with a higher number of samples could help to understand this difference. In Burkina Traoré et al. (2015) reported a similar seroprevalence which was 1% for anti-IgM and 76% for total anti-HEV antibodies in the population of pig butchers. This reflects that HEV is endemic and circulating in this population. The seropositivity for gender was 20.2% (95% CI: 19.4 - 21.0) in men and 20.0% (95% CI: 6.0 - 34.0) in women, this result translates that they

were exposed to the same source of contamination and were unaware of the hygiene rules. Many studies suggest that zoonotic transmission of HEV may occur through frequent contact with biological samples (feces, blood) and organs of HEV-infected animals [18]. In agreement with many similar studies in the world, we have observed high seroprevalences in pork butchers who have direct contact with pigs or pork meat [19-21]. Comparing this study to other studies conducted in other countries of the sub-region, such as Burkina Faso [9], the seroprevalence observed among butchers was higher than that observed in the general population. The data from these studies suggested that zoonotic contamination could be one of the main sources of HEV infection; however, our data suggest that in Togo, in addition to the zoonotic source of contamination, there are other reservoirs of contamination that are totally unknown and that fuel the spread of infection. Although in this study no risk factors such as gender, age, education, area of residence, number of years in business, husbandry practices and hygiene were associated with anti-IgM and IgG HEV seroprevalences, studies have reported that they may constitute significant risks in the contamination and spread of HEV [22]. In addition, the seroprevalence of total anti-HEV antibodies observed in pigs at threshing age was 80.11%. This result suggests that HEV is endemic in pig populations in Togo. This result was similar to those observed in Ghana 62.4% [23] and Burkina 80% [9], which could be a source of contamination and spread of HEV for professionals in the sector [10,22]. In addition, the study revealed similar seroprevalences according to the age of the pigs which were 80%, 78% and 82% respectively in 3-6 months, 6-12 months and over 12 months of age. This suggests that, pigs at slaughter age could be at the same risk of HEV contamination.

Limitations of this study

This study has limitations. The study did not allow the detection of HEV RNA, and the characterization of HEV genotypes circulating in the pig and pork butcher population was not performed. The genotypic characterization of HEV to be determined in pigs and humans will provide a more accurate picture of the zoonotic transmission of this virus.

Conclusion

This study showed the presence of HEV antibodies in the serum samples from butchers and pigs slaughtered for consumption, and could reflect that they were in contact with HEV. The relatively low IgG seroprevalence in the butchers reflects a very low endemic status of the infection that is not different from the general population which is in contrast to the observed situation in Burkina. We did not know if this was might be due to a lack of sensitivity or the current test compared to the old test used in Burkina by our group. Whatever the case, however, the high seroprevalence of IgM might reflects a major risk of HEV transmission in this

population and a largely changing status. In addition, the high seroprevalence of total antibodies observed in pigs confirms their HEV reservoir characteristics and their potential role as a source of contamination and virus propagation. Further studies are needed to characterize the circulating genotypes in pork butchers and pigs in order to establish the link between them and to identify other factors fueling the HEV status in the human population.

Consent to Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

KVMS, KAT, JBO, ET, PR, SDK, and NB participated in the study design. KVMV, BKN, JA, GK and KAT performed serological analyses, KVM, KAT, BLO, and PR performed the statistical analysis, and all authors participated in drafting and revising the manuscript. All authors read and approved the final version.

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Ethical Approval

The study was approved by the Bioethics Committee for Health Research of the Ministry of Health and Public Hygiene of Togo under No.: 040/2019/CBRS, and furthermore the study protocol complies with the ethical guidelines of the 1975 Declaration of Helsinki, as evidenced by the approval of the Ministry of Health and Public Hygiene (Togo) under No.:027/2020/MSHP/CAB/SG/DGAS/DPML/CBRS. The administrative authorization was also obtained from the Polyclinic and the Regional Blood Transfusion Center of Sokodé. Informed consent was obtained from all study participants after they were informed of the project.

Availability of Data and Materials

Not applicable.

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