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Short Communication

Low seroprevalence of hepatitis E on Reunion island

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Running Head title: Seroprevalence of hepatitis E

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ABSTRACT

Objective. Hepatitis E virus (HEV) has been scarcely investigated in the Indian Ocean. Following a nationwide serosurvey among blood donors, we conducted a population-based serosurvey to assess the magnitude of HEV exposure on Reunion Island.

Methods. Four hundred and sixty-six archived frozen human sera from the 2009 CoPanFlu-RUN cohort were analysed using the Wantai HEV IgG enzyme immunoassay. HEV seropositivity was defined as an IgG titre ≥ 5 UI/ml. Raw and weighted seroprevalences were assessed to account for the discrepancy between the CoPanFlu-RUN subset and the general community. Prevalence proportion ratios (PPR) were measured using log-binomial models.

Results. The raw and the weighted seroprevalences of HEV were 9.01% (95% CI 6.41-11.61) and 6.73% (95% CI 4.47-8.98), respectively. The presence of HEV IgG antibodies was associated with increasing age ($P < 0.001$). In a survey-adjusted model minimizing the sampling bias and adjusting for age, males were more likely to be seropositive than females (adjusted PPR 2.59, 95% CI 1.07-6.25). Seropositivity was spatially heterogeneous across the island ($P < 0.01$). Living in the neighbourhood of a pig farm within a low to intermediate slope area was associated with seropositivity in several models adjusting for age, gender, altitude of residency and interaction between slope and pig farms.

Conclusion. Reunion Island is a low endemic area for HEV exposure. Despite limitations related to the retrospective study design, our findings confirm the roles of cumulative lifetime exposure and male gender in HEV exposure. The risk associated with neighbouring pig farms might also suggest environmental contamination in this setting.

Keywords: serology, immunoassay, seroepidemiological study, serosurvey, stored frozen serum, hepatitis E, pig farm, zoonosis, prevalence, general population.

1. Introduction

Hepatitis E virus (HEV) infection is a ubiquitous prevalent disease newly recognized as the most common cause of acute viral hepatitis (Kamar et al., 2017). Four major genotypes of HEV have been thus far identified. HEV-1 and HEV-2 are waterborne pathogens of mainly medical concern in developing countries, where they cause sporadic cases or large outbreaks linked with faecal contamination of drinking water, flooding, or iatrogenic transmission (Kamar et al., 2014). HEV-3 and HEV-4 are zoonotic foodborne pathogens with endemic transmission in developed countries, where they are usually acquired by consumption of undercooked pork or game meat, direct contact with infected animals (*e.g.*, domestic pigs and wild boars), or blood transfusions (Spahr et al., 2017). HEV-1 and HEV-2 are almost absent from the western world (except HEV2 also found in Mexico), and their burden is higher in monsoon Asia than in drought areas of Sub-Saharan Africa or the Middle East. By contrast, HEV-3 is prevailing in the western world (but also in Korea, Mongolia, and western China; both with HEV-4 in Japan and eastern China) (Kamar et al., 2017; Rein et al., 2012; Hakim et al., 2016).

The first clinical descriptions of hepatitis E in the southwestern Indian Ocean (SWIO) islands date back to the last decade and were almost exclusively reported among travellers returning from endemic areas (Milon et al., 2004; Parola et al., 2006). Importantly, in January 2009, an autochthonous case linked to the genotype 3f was identified in Mayotte, Comoros archipelago, from a 46-year-old healthy man originating from mainland France who developed an acute hepatitis within six months after his arrival on the island (Epelboin et al., 2011). Interestingly, in December 2010, a new subtype of the genotype 3 was recovered from pig herds in Madagascar, where HEV seroprevalence in swine was 71.2% and seroprevalence in slaughterhouse workers was 14.1%, suggesting a zoonotic cycle (Temmam et al., 2013). On Reunion Island, autochthonous HEV infections were first diagnosed about fifteen years

ago, both in healthy and immunocompromised subjects, accounting for 2% of all the infections notified nationally in France in 2008 (Nicand et al., 2009). In 2011-2012, a nationwide serosurvey among blood donors aged 18 to 70 years reported on Reunion Island a prevalence of 0.7% for HEV IgM antibodies and within the 0-10% range for HEV IgG antibodies (Mansuy et al., 2016).

Following this nationwide serosurvey, we conducted a population-based serosurvey to assess the magnitude of HEV exposure on Reunion Island. The aim was to verify whether human exposure on Reunion Island might be less than that observed in mainland France, and whether residential proximity to pig farms could be considered as an exposure at risk.

2. Materials and methods

2.1 Setting and population

La Réunion is a tropical island (2,512 km²), located in the SWIO region, 700 km east of Madagascar. Its hydrographic network is composed of 13 perennial rivers and a multitude of streams with torrential flow given the steep slopes. The pig livestock in 2009 was 10,000 sows distributed over more than 300 farms (75% grouped in cooperative), which ensured 100% of fresh swine meat consumption, and covered half of the total swine meat consumption on the island. Three quarters of the 816,000 inhabitants present in 2009 were supplied with surface drinking water. The structure by age, gender, and microregion (*i.e.*, 4 administrative regional subdivisions) of the general population is presented in [table S1](#).

The study population was a subset of the CoPanFlu-RUN cohort dedicated to the 2009 pandemic flu ([Dellagi et al., 2011](#)). A two-stage sampling option selected households according to the presence or absence of pig farms in the neighbourhoods then participants. A sample size of 500 sera was deemed to be adequate to detect a seroprevalence of 5% within a precision range of 1.5 to 11.5% with a 100% confidence ([Mansuy et al., 2016](#)). Age, gender, and residence data were retrieved for each participant and compared to the community to assess the representativeness of the study sample ([table S1](#)). Exposure to pig herds was defined by the presence of one or more swine farms within the residence neighbourhood, without distance limitation. Neighbourhood was defined at the IRIS (*Ilots Regroupés pour Information Statistique*) level, which in France generally makes it possible to obtain multiple layers of aggregated data.

2.2 Ethics and funding

The CoPanFlu-RUN cohort study was funded by the French National Institute of Health and Medical Research (INSERM). It was conducted in accordance with the Declaration of Helsinki and the French law for biomedical research (n°ID RCB AFSSAPS: 2009-A00689-

48). It was approved by the Ethic Committee of Bordeaux 2 University which allowed the use of serum samples for further investigation of other infectious diseases (Jaubert et al., 2019). Written consent was obtained from all participants or from the legal representative of all underage participants.

2.3 Serology

Four hundred and sixty-six sera were tested using the Wantai® HEV IgG enzyme immunoassay (Wantai pharmacy enterprise, Beijing, China) (Yan & Wang, 2008). HEV seropositivity was defined as an IgG titre ≥ 5 UI/ml.

2.4 Statistical analysis

Statistical analyses were performed using Stata 14.2® (StataCorp, College Station; Texas, USA). The seroprevalence rates were weighted on age, gender and the microregion to account for a potential difference between the CoPanFlu-RUN subset and the general population. Associations between HEV IgG seropositive individuals and age, gender, geographic subdivision and proximity to pig herds were determined using weighted chi-square tests. Survey-adjusted log-binomial regression models were used to estimate prevalence proportion ratios (PPR) and 95% confidence intervals (95% CI) while minimizing the sampling bias. Cluster effects were tested within households and neighbourhoods using GEE (generalized estimating equations) models, using a repeated statement with an exchangeable correlation matrix. All interaction terms between the variables included in these models were tested using the Mantel-Haënszel method. Further analyses using multiple logistic regression models tested the hypothesis that neighbouring a pig farm was a risk factor potentially affected by the altitude or the slope. For all these analyses, a *P* value <0.05 was considered significant.

3. Results

The study population covered 146 neighbourhoods distributed over 26 age-, gender- and area-level-corrected strata. This represented a third of the participating households and nearly a quarter of the participants of the CoPanFlu-Run cohort (Figure 1). Among these neighbourhoods, 63% of the pig farms (n=92) that could be geocoded were included. Altogether, this represented 53 neighbourhoods, 122 households and 180 individuals exposed to pig farms. Of note, the study population was skewed towards middle-aged and older adults, females, and residents from the southern and western microregions.

The raw seroprevalence of HEV was 9.01% (95% CI 6.41-11.61%) and the weighted seroprevalence was 6.73% (95% CI 4.47-8.98%), which fits with the seroprevalence range of 0-10% for HEV IgG antibodies reported in blood donors on Reunion Island (Mansuy et al., 2016).

HEV IgG antibodies were absent in sera from people younger than 20 years old and seroprevalence increased with age (adjusted PPR 1.03, 95%CI 1.01-1.06, $P<0.001$) in all multivariate models. Seropositivity was also associated with male gender (adjusted PPR 2.59, 95%CI 1.07-6.25) in a survey-adjusted model minimizing the sampling bias (Table 1).

Spatial distribution of HEV exposure was inconsistent between the four microregions depending on whether we adjusted for differences between the study population and the community distribution or not. Raw seroprevalence was the highest in the South (Table S2) and the weighted seroprevalence was the highest in the North (Table 1), while the lowest seroprevalence was consistently measured in the East. However, there was a strong spatial heterogeneity in HEV exposure across the island ($P<0.01$, Figure S1), observed at the neighbourhood level (weighted seroprevalence range: 0-62.25%) as well as the strata level (weighted seroprevalence range: 0-28.57%). Living in the neighbourhood of a pig farm (Figure S2) was associated with the presence of HEV IgG antibodies (Table S2), both in

bivariate analysis (crude PPR 1.92, 95% CI 1.08-3.43) and in a model adjusting for age and gender but not the sampling bias (adjusted PPR 1.82, 95%CI 1.01-3.25). Similar findings were obtained from the GEE models adjusting for the cluster effects within households or within neighbourhoods (data not shown). Interestingly, the likelihood of being HEV seropositive when neighbouring a pig farm was modified by the mean slope of the area (Mantel-Haënszel OR 2.23; $P=0.014$), but not by altitude (Mantel-Haënszel OR 1.90; $P=0.077$). The risk associated with living near a pig farm was restricted to the range of low to intermediate slopes, *i.e.* 61-200 meters of height difference, in both stratified (stratum OR 6.99; $P<0.001$) and several multivariate logistic regression models (Table 2).

4. Discussion

Our population-based serosurvey confirms the low exposure (<10%) of Reunion Island community to HEV, as previously suggested from healthy blood donors ([Mansuy et al., 2016](#)).

Hepatitis E has been rarely observed for the last two decades on Reunion Island, despite notified HEV infections representing 2% of all autochthonous infections reported nationally in 2008 ([Nicand et al., 2009](#)), and the pork industry being prominent in the island. Together with the scarcity of clinical cases, hallmark of a low incidence, and the low proportion of HEV IgG antibodies previously reported in blood donors ([Mansuy et al., 2016](#)), the low seroprevalence (raw and weighted) observed herein suggest that Reunion Island is a low endemic area for HEV transmission. Few HEV seroepidemiological studies have been conducted within general populations, as compared to studies among blood donors ([Capai et al., 2019](#); [Tengan et al., 2019](#); [Yue et al., 2019](#)). The figures observed in our study are in strong contrast with the seroprevalence averaging 25% in hyperendemic industrialized countries from the northern hemisphere, including France, Germany, the Netherlands and China ([Capai et al., 2019](#); [Yue et al., 2019](#)). They are more in agreement with seroprevalence below 10% reported, for instances, from Brazilian or Finnish communities ([Kantala et al., 2016](#); [Tengan et al., 2019](#)).

Our findings confirm the association between HEV exposure and increasing age ([Zheng et al., 2006](#); [Rein et al., 2012](#); [Mansuy et al., 2016](#); [Kamar et al., 2014](#); [Teshale et al., 2015](#); [Kamar et al., 2017](#); [van Gageldonk-Lafeber et al., 2017](#); [Cangin et al., 2019](#)), which might indicate a lifetime cumulative exposure ([van Gageldonk-Lafeber et al., 2017](#)) or a past higher exposure risk ([Teshale et al., 2015](#)). However, the absence of HEV exposure in young people (≤ 20 years old) may be related to a sampling bias, given the underrepresentation of this age group in our study population. This could also suggest the presence of a non-epidemic

genotype (HEV-3 or HEV-4), given the consistent peak incidence in the second decade of life with HEV-1 and HEV-2 (Rein et al., 2012). Our findings confirm that males are at higher risk of HEV exposure, which has been observed both in other general populations (Zheng et al., 2006; Kamar et al., 2014; van Gageldonk-Lafeber et al., 2017) as in blood donors (Mansuy et al., 2016). This might be related to occupational differences, as it has been shown that the gender effect is abrogated when the population is stratified by the occupation (Yue et al., 2019) or when the study is conducted within occupations at risk (Kantala et al., 2016). It may also be driven by ethnic/cultural differences on Reunion Island, as previously observed in the United States with the rising incidence among non-Hispanic Asian females, which has abrogated the male predominance in recent years (Cangin et al., 2019). In this study, the gender effect was found only after controlling the sampling bias, which suggests a weak robustness of this observation in our local context.

This study also suggests a possible role of pig farms in HEV transmission to humans. Although no prevalence is available for swine herds on Reunion Island, it is reasonable to hypothesize that pork industry on Reunion Island is involved in HEV transmission. Indeed, HEV seroprevalence in French domestic pigs is high, both at farm- (65%) and individual-levels (31%), and there are similarities between HEV subtypes from pigs and humans, which corroborate the zoonotic origin of some HEV autochthonous infections (Rose et al., 2011). Whilst we did not manage to spatialize our data in relation to the location of swine herds, nor obtain data on pig densities, we evidenced an interaction between HEV prevalence and the presence of swine farms within the residence neighbourhood and low to intermediate sloping areas, which may suggest environmental contamination. Interestingly, people living in pig-dense areas in the Netherlands (where the relief is flat) do not display a higher risk of HEV infection (van Gageldonk-Lafeber et al., 2017), whereas, in eastern China, persons living in communities downstream of pig farms have a 29% higher risk of infection than persons living

in upstream communities (Zheng et al., 2006). Consistent with this hypothesis, the island holds rainfall records, and runoff or flooding may spread the virus present in effluents from swine farms to communities downstream, especially in low to intermediate sloping areas less prone to a wash away phenomenon.

In view of these elements, we believe this wash away phenomenon is likely to protect the population and that, in its absence, contamination of surface water could be more important. Otherwise, the low seroprevalence in humans reported herein might also indicate either different eating habits in the multicultural Reunionese society (*e.g.*, Muslim and Hinduist communities are traditionally low pork meat consumers), or low seroprevalence and low viral shedding in pigs that both need to be further investigated.

Other sources of HEV contamination seem far less possible. For instances, there is no wild boar on the island, and only one slaughterhouse, which makes these contributors unlikely.

This study has potential limitations. Firstly, despite weighting our sample to limit resampling bias, a bias might still occur as the sera were originally sampled for another purpose. Secondly, because this is a retrospective study using archived sera and given the impossibility to complete individual data, we cannot rule out information bias and residual confounding. Thereby, we cannot distinguish the foodborne from the environmental sources of HEV exposure. Nevertheless, a lesser consumption of pig meat in Reunion Island compared to mainland France (24 kgs *versus* 34 kgs per habitant in 2009, DAAF source), and late slaughter habits in the local pork industry (201 days on average), do not argue in favour of a massive contamination of foodborne origin. By contrast, effluent management is highly variable. Few livestock have slurry tanks, effluents are often spread on the crops (with or without composting) while uncontrolled releases are suspected (*e.g.*, in case of heavy rains) (Aubry et al., 2006).

Finally, our findings warrant a larger scale seroepidemiological study aimed at (i) assessing with more accuracy the HEV prevalence in both swine and human communities and (ii) better understanding the local transmission pathways. Meanwhile, special attention should already be paid to occupations at risk (swine farmers, butchers, slaughterhouse workers, veterinarians, *etc...*) and people living downstream pig farms, especially during periods of heavy rainfalls that may spread the virus.

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Data sharing

Data will be made available on request.

Disclaimer

The funder of the CoPanFlu-RUN program did not participate in the design, preparation, data analysis, or decision to publish the manuscript.

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Potential Conflicts of interest

The authors declare that they have no competing interests.

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Figure 1. Study population

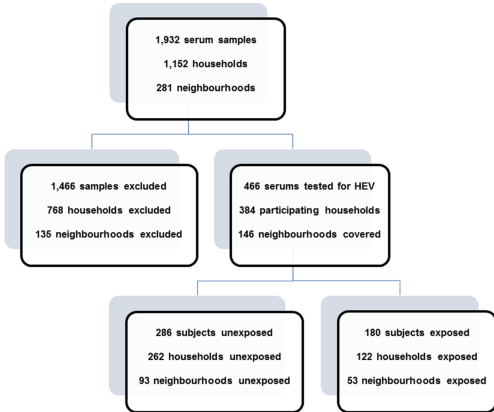


Table 1. Factors associated with HEV exposure in bivariate and multivariate weighted analyses, Reunion Island, 2009 (n=466)

<i>Hepatitis E virus IgG ≥ 5 UI/ml</i>							
Variables	n	Weighted %	P value	Survey crude PPR	95% CI	Survey adjusted PPR	95% CI
Age			0.156	1.03 **	1.00 - 1.06	1.03 **	1.01 - 1.06
≤ 20 years	0/24	0.00		-	-	-	-
21-40 years	4/137	9.15		-	-	-	-
41-60 years	23/200	10.22		-	-	-	-
> 60 years	15/105	14.95		-	-	-	-
Gender			0.167				
Female	24/313	4.26		1		1	
Male	18/153	8.80		2.07	0.73 - 5.80	2.59 *	1.07 - 6.25
Microregion **			0.337				
North	1/15	13.32		1	-	-	-
South	27/186	7.19		0.54	0.07 - 4.02	-	-
West	11/199	5.08		0.38	0.04 - 3.07	-	-
East	3/66	1.74		0.13	0.01 - 1.31	-	-
Exposed to pig herds†			0.628				
Yes	23/180	7.67		0.74	0.22 - 2.49	-	-
No	19/286	5.68		1	-	-	-

- 1 Data are numbers (n), weighted seropositive rates (%), crude and adjusted prevalence proportion ratios (PPR), and 95% confidence intervals (95% CI).
2 P values linked to variable names are given for overall design-based Pearson chi2 tests. P values linked to PPR are given with asterix for within-
3 each-category Wald tests. † Presence of one or more swine farms within the residence neighbourhood. * P<0.05. ** P<0.01.

Table 2. Effect of exposure to pig herds within low to intermediate slope areas in crude and multivariate logistic regression analyses, Reunion Island, 2009 (n=466)

Model	Odd ratio	95% CI	P value
Model 1			
Unweighted analysis	5.92	2.29 - 15.29	< 0.001
Survey-adjusted analysis	3.59	0.77 - 16.78	0.103
Model 2			
Unweighted analysis	5.72	2.10 - 15.53	0.001
Survey-adjusted analysis	3.16	0.75 - 13.27	0.116
Model 3			
Unweighted analysis	5.77	2.16 - 15.35	< 0.001
Survey-adjusted analysis	3.45	0.87 - 13.60	0.077
Model 4			
Unweighted analysis	5.37	2.01 - 14.33	0.001
Survey-adjusted analysis	3.62	1.06 - 12.39	0.040
Model 5			
Unweighted analysis	4.00	1.44 - 11.13	0.008
Survey-adjusted analysis	4.22	1.16 - 15.35	0.029

Data are odd ratios and 95% confidence intervals. Model 1 displays the crude (unadjusted) effect of the interaction between the presence of one or more swine farms within the residence neighbourhood and an a low to intermediate slope (second quartile, 62 to 200 meters) within the neighbourhood. In this model, the referent category is the absence of exposure to pig herds and the range of slopes is divided into four quartiles. Model 2 is model 1 + age adjusted. Model 3 is model 1 + age and gender adjusted. Model 4 is Model 1 + age, gender, and altitude of residency adjusted. Model 5 is Model 1 + age, gender, altitude of residency and microregion adjusted. Bold values: $p \leq 0.05$.