

Seroprevalence and risk factors associated with West Nile infection in human in Madagascar: a cross-sectional serological survey

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Primum vitare ! D'abord prévenir !

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Research Article

Keywords: West Nile virus, serological survey, humans, Madagascar

Posted Date: April 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-388847/v1>

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Abstract

Background: West Nile virus (WNV) is a vector-borne virus maintained through an arthropod-borne transmission cycle involving bird hosts and *Culex* mosquito species. The disease affects humans in North America, Europe, Africa and Indian Ocean islands including Madagascar. In Madagascar, despite the surveillance of WNV, unreported WNV encephalitis (WNE) are suspected. In order to improve WNE surveillance efficiency and optimize the available resources we aimed to estimate WNV human seroprevalence in different areas of the island, and to identify the environmental factors and areas favorable to WNV transmission in human.

Methods: Between 2011 and 2013, during a national cross-sectional serological survey 1,680 individuals were sampled. Sera were tested for IgG directed against WNV using ELISA and Hemagglutination Inhibition assays. Then, we analyzed human serological results using Generalized Linear Mixed Models, with the individual serological status as the response, and environmental variables and human related factors as explanatory variables.

Results : The overall human WNV seropositivity rate was 12.7% (95% CI [11.2-14.4]). Human seroprevalence was positively associated with humid environment ($p < 0.005$) whereas cold and highlands environment had a significant negative effect on seroprevalence ($p < 0.001$). Outdoor work had also a significant positive effect on seroprevalence ($p < 0.01$, OR = 1.99).

Conclusions: Our results indicate a heterogeneous circulation of WNV in human and highlights that humid environment of the northwestern part of the island is at-risk for WNV circulation whereas cold environment of the highlands is not favorable for WNV transmission in human. The identification of at-risk environments for WNV circulation in humans should help to improve the surveillance of the disease in Madagascar and is a first step to target future studies on acute encephalitis.

Trial registration: The protocol was approved by the Malagasy competent authorities, the Malagasy Ethic National Committee (authorization N°066/MSAMP/CE, 26th July 2011).

Background

Madagascar is a large island of the South West Indian Ocean, well known by its exceptional flora and fauna biodiversity and its high diversity of bioclimatic regions. This variety of bioclimatic regions is now known to harbor different epidemiological mechanisms of transmission of vector-borne diseases such as plague, malaria and Rift Valley fever (RVF) [1–4]. These variety of epidemiological mechanisms are likely linked to close relationship between the vectors of the diseases and their environment but also human behavior and hosts distribution [4]. Amongst vector borne diseases circulating in Madagascar, several arboviruses have been detected including RVF, Dengue, Chikungunya and West Nile [5–9].

West Nile virus (WNV) belonging to *Flaviviridae* family affects mainly human and horses in North America, Europe, Africa and Indian Ocean islands. The virus is maintained through an arthropod-borne

transmission cycle involving bird hosts (*e.g.* Charadriiformes and Passeriformes orders) and *Culex* mosquito species. Humans and horses are considered as incidental dead-end hosts [10]. In humans, symptoms of infection range from mild fever to severe or fatal neuroinvasive disease, including encephalitis [11]. In Madagascar, human infection with WNV has been documented [8, 12, 13], and the virus has been detected in horses, domestic birds and different mosquito species [14–16]. Since former studies suggested a heterogeneous circulation of WNV in Madagascar island [8, 13], WNV distribution at the national scale is poorly documented. Moreover, the high seroprevalences observed in some area suggest that WNV infection reports are probably underestimated [12, 15].

A surveillance sentinel network of fevers has been implanted in Madagascar in 2007 aiming at rapidly detect an epidemic and identify circulating arboviruses [17]. Despite the monitoring of WNV circulation amongst this surveillance system, unreported WNV encephalitis (WNE) cases are suspected [12]. In order to improve WNE surveillance efficiency and optimize the available resources by using a targeting strategy, the identification of at-risk environments is essential. Herein, the objectives of our study were to estimate WNV human seroprevalence in different areas of the island and to identify the environmental factors favorable to WNV transmission in human.

Methods

Dataset

The dataset contained data from a national cross-sectional serological survey conducted from November 2011 to April 2012 and from October 2012 to May 2013 in 56 cities or villages (Fig 1). The protocol of sampling has been previously described [4,18]. In each of the 56 sites, 30 adults were randomly chosen and sampled on a voluntary basis (Fig 1). Socio-professional categories (butcher, farmer, health worker, teacher, student, administrative worker, retired), worker in contact with water or forest and contact with poultry were documented through a dedicated questionnaire.

The protocol was approved by the Malagasy competent authorities, the Malagasy Ethic National Committee (authorization N°066/MSAMP/CE, 26th July 2011). After reading of the informed consent letter, written and oral consent was obtained from volunteering individuals. Participants were sampled by qualified investigators and the data were analyzed anonymously.

Figure 1: Sampling sites and IgG serological results.

Covariates

Assuming that (1) WNV is likely to be endemic in Madagascar island [14,16,19] and (2) the introduction risk of WNV by migratory birds is low [16], we considered only covariates potentially involved in the amplification cycle of WNV and not those involved in the introduction events. The following covariates were selected according to their putative influences on mosquitoes' and wild birds' density, their population dynamics, and risk of contact with human:

- Surface covered by water bodies and landscape categories (e.g. rural/urban area, agricultural area, forest, shrubland). Density and population dynamics of vectors and presence of reservoir birds are influenced by environmental factors such as climate, presence of water bodies and other landscape features [16,20]. *Culex* mosquito genus is considered as the principal mosquito vector responsible for the transmission of WNV [21]. The presence of this genus is associated with a large variety of temporary and permanent water bodies, including rice fields, [16,19,22–24];
- Rainfall, Normalized Difference Vegetation Index (NDVI) and temperatures. The WNV infection risk has been shown to vary according to rainfall, NDVI and temperatures [25,26];
- Poultry contact and density. WNV is known to circulate amongst poultry [27,28];
- Human related factors: habitat, gender, profession (working environment, contacts with live poultry) and socio-economic status (SES) are potential risk factors for WNV infection in human [29–31].

Human related factors. Age of the individuals was categorized in 4 groups: [18 to 26], [27 to 37], [38 to 46] and more than 47 years old. Sampling sites were divided as urban, sub-urban and rural sites according the work of Andriamandimby et al. [18] (Fig 1). Working environments were characterized by household, indoor and outdoor environments. Daily or weekly work in rice field was considered as a frequent contact with rice field. As well, daily or weekly work in forest was considered as a frequent contact with forest environment. Contact with poultry was categorized according the number of poultry owned by household. We considered that a frequent contact occurs when household owned 11 or more poultry. The SES of each individuals were previously categorized by Andriamandimby et al. using principal component analysis and hierarchical cluster analysis [18]. Three cluster were used to describe SES : low socio economical level described by wooden combustion use, roof made in plants, light of the petroleum lamp, dirt floor in the bedroom and not equipped with toilet; intermediated socio-economical level described by wood charcoal combustion, sheet roof, electricity light, TV and cement floor and high socio-economical level described by computer owning, flush toilet owning, internet access, car and refrigerator owning.

Poultry density. For each of the 1,578 Malagasy communes, poultry density was estimated using the global distribution maps for poultry produced by the Food and Agriculture Organization of the United Nations [32]. Poultry density of the related communes was classified in 4 categories: below 15.6, [15.6 to 37.2], [37.3 to 75.9] and 76 and more.

Environmental variables. As environmental variables, we used data previously published and characterizing Malagasy environment through an integrated analysis [4]. In this study dedicated to RVF, the environment of the 1,578 Malagasy communes were characterized through a Multiple Factor Analysis (MFA) using climatic variables (the annual means of day and night LST, the annual mean and seasonality of precipitation) and landscape variables (the percentage of the surface of the commune covered by each landscape category and the annual mean and seasonality of NDVI). The value of each factor was computed for each of the Malagasy communes. Four MFA factors were described [4]:

- Factor 1 opposed dry environments in south-west to wet environments in the east of Madagascar;

- Factor 2 opposed cold environments in the highlands to warmer environments in the north-west and south of the island;
- Factor 3 opposed areas with high rainfall in the middle-west to areas with low rainfall in the south-west of Madagascar;
- Factor 4 opposed humid areas situated in the north-western part and eastern coast to dry environment in the center and south of the island.

Laboratory analysis

Due to potential cross-reactivity with other viruses from the *Flaviviridae* family, the samples were tested for the presence of both WNV and dengue virus antibodies.

Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis. The serum samples were first tested for IgG antibody using ELISA as previously described [33]. Serum samples diluted at 1/100 were incubated on plates coated the day before with crude antigens (cellular antigens, donation from the Institut Pasteur of Laos). Conjugate anti-human IgG peroxidase-marked (Jackson Immunoresearch Europe LTD) was used to detect IgG. On each plate, a positive control was tested, as well as three negative controls.

Hemagglutination Inhibition (HI) analysis. HI tests were performed according to Clarke and Casals protocol [34]. Antigens for WNV and the four serotypes of dengue virus were produced at IPM, according to the sucrose-acetone extraction method [35]. Titers of antigen were checked at each experiment and titers obtained were corrected according to it.

Discrimination of positive samples. Positive sample on ELISA against WNV (Optical Density (OD) >0.02) were tested using HI assays. In addition, due to potential cross-reactivity with other viruses from the *Flaviviridae* family, positive samples on ELISA against Dengue virus (OD >0.08) were also tested for WNV using HI. A sample was considered positive for WNV if its titer in HI was at least of 1:80. To overcome cross-reactivity, if a sample had titers against the four Dengue virus serotypes higher than for WNV, then this sample was considered as positive for Dengue virus and consequently, negative for WNV. That corresponded to an implicit hypothesis that the seropositivity for the 5 viruses was deemed improbable, given the estimated low probability of these 5 co-infections.

Statistical analysis

As a first step, univariate analyses of association between suspected risk factors and human WNV serological status were undertaken using Chi square tests for categorical factors and generalized linear models for quantitative factors. Risk factors with significance level ≤ 0.20 were then included as explanatory variables in generalized linear mixed models (GLMMs), with human individual serological status as response. To account for interdependency of serological status of individuals sampled in the same locality, the commune administrative unit were included in the models as a random effect. In the models, it was assumed that the relationships between serological prevalence and quantitative factors were linear on the logit scale. Multicollinearity among variables was assessed using Variance Inflation

Factors (VIF), we assumed that a VIF under 10 did not reveal any multicollinearity [36]. Collinear variables were not included in a same model. The selection of the best models was based on the Akaike Information Criterion (AIC). A multi-model inference approach was used to estimate Model-Averaged Fixed Effects (MAFE; full average) and the weight of each explanatory variable [37]. Within the set of models tested, only those with an AIC within 2 units difference from the best model were considered [37]. Internal validity of sets of models was evaluated using the Receiver Operating Characteristic curve method [38].

Data analyses were performed using R software package version 3.0.1 [39–44].

Results

Description of data and univariate statistical analysis

Overall, 1,680 individuals were sampled. Descriptive analyses of the data are presented in the Table 1. Age of volunteers ranged from 18 to 99 years (mean age 37.6 years) with a male/female ratio of 1.03. A total of 27%, living in urban areas, 30% in sub-urban areas and 43% in rural areas (Fig 1). About 50.4% of the individuals were classified as low SES, 44.4% as intermediate and 5.2% as high. About 55% of the individuals work outdoor, 30% indoor and 15% at the household. Frequent contact with poultry has been recorded for 30% of the individuals. We noted that 38% and 31% declared working frequently in rice field and forest respectively.

MFA factor values of the 48 communes ranged from -1.86 to 3.29 (Factor 1), -1.87 to 2.77 (Factor 2), -5.08 to 1.75 (Factor 3) and -0.75 to 4.51 (Factor 4).

Overall, the human WNV seropositivity rate was 12.7% (95% CI [11.2-14.4]; Table 1). Frequent work in rice-field or forest, habitat, poultry density category, working environment, Factor 1, Factor 2, Factor 3 and Factor 4 were statistically associated with human seroprevalence by univariate analysis ($p \leq 0.20$; Table 1) and thus included in a multivariate model.

Table 1: Descriptive and univariate analyses.

Multivariate analysis

The multicollinearity test did not detect any correlation ($VIF < 3$) between variables included in the multivariate analysis. According to AIC, 10 models were considered as suitable for describing seroprevalence in humans and thus were analyzed using a multi-model inference approach. These models included working environment, frequent working in rice-field, frequent contact with poultry, poultry density, Factor 1, Factor 2, Factor 3 and Factor 4 as explanatory variables (Table 2). Factor 4 had a significant positive effect on seroprevalence ($p < 0.005$, weight=1) whereas Factor 2 had a significant negative effect on seroprevalence ($p < 0.001$, weight=1). Outdoor work had a significant positive effect on seroprevalence ($p < 0.01$, weight=1) with an Odd-Ratios of 1.99 (95% CI [1. 23-3.23]). The other variables were not significantly associated with human seroprevalence.

Internal validity of human set of models were satisfactory with an Area Under the Curve of 0.81 (95% CI [0.78-0.84]).

Table 2: Results from the multi-model inference approach for dataset analysis.

Discussion

In Madagascar, WNV circulation has already been described several times [8,12,13]. Our study brings recent and national-wide distribution of its circulation in human. The overall human seroprevalence was estimated at 12.7% (95% CI [11.2-14.4]). This relatively high result is not surprising since WNV seroprevalence in Madagascar was estimated at 46.2% in horses in 2010 [14], and at 29.9% and 33.8% in human [8,13].

The overall seroprevalence observed is higher compared to other African countries such as Union of the Comoros (7.3%), Kenya (9.5%), and Cameroon (6.6%) but lower than adult seroprevalence in Ghana (27.9%) [45–48].

Our results showed that WNV has circulated in all the island, though with lower seroprevalences in the highlands, as suggested previously in 1990 and 2003 [8,49]. This heterogeneous circulation seems to be linked with environmental factors. Indeed, our results showed that higher circulation is associated with high value of Factor 4 (large surface of permanent wetlands, marshlands and irrigated lands) which suggests that WNV circulation in human is positively associated to humid environment. These environments are mainly situated in the north-western part of Madagascar and are favorable to *Culex* mosquitoes as well as water birds hosts of WNV [16,23]. These results are consistent with the known at-risk environment for WNV circulation in Europe, Tunisia and the United States of America [25,50–52]. Conversely, cold environments mainly situated in the highlands (Factor 2) seem to be protective against WNV infection for human. A previous study showed that risk for WNV infection decrease with increasing elevation [52].

Regarding human individual related factors, the absence of significant relationship between age and seroprevalences, first supports the hypothesis of a non-endemic circulation of WNV in human population. Indeed, if the virus was circulating endemically in humans, a positive association of seroprevalence with age would be observed. Since the introduction of WNV through migratory birds in Madagascar seems to be unlikely [16], we can suggest that WNV circulates endemically between birds and mosquitoes and that human are occasionally infected by the virus. Secondly, *the seroprevalence is higher in individuals working outdoors. This is not surprising since the persons working outside are more exposed to exophagic mosquitoes and several Culex WNV vector species are known to have this behavior* [28,54].

The observed heterogeneous circulation of WNV in human also suggests that WNV transmission is different according to ecoclimatic regions. These environmental-associated mechanisms of transmission could be modulated by (1) the presence of bird hosts, (2) the presence of vectors and (3) the vector competency. To date, 16 mosquito species have been found naturally infected by WNV in Madagascar,

including four *Anopheles* (*Anopheles coustani*, *An. pauliani*, *An. brunnipes*, *An. maculipalpis*), one *Mansonia* (*Mansonia uniformis*), one *Aedeomyia* (*Aedeomyia madagascariensis*), four *Aedes* sp. (*Aedes albocephalus*, *Ae. madagascariensis*, *Ae. circumluteolus*, *Ae. aegypti*) and four *Culex* species (*Cx. quiquefasciatus*, *Cx. scottii*, *Cx. tritaeniorhynchus*, *Cx. Univittatus*; [16]. Some of these species are specifically distributed in some regions of Madagascar and others are found in all the island [23]. However, the competency of a same mosquito species to transmit WNV could be modulated in different ecosystems by climatic factors such as temperature [55,56].

Finally, as observed for malaria, plague and RVF, we suggest that WNV epidemiology has to be considered differently according to Malagasy ecosystems. This implies that WNV infections surveillance, including acute encephalitis (AE) surveillance, should be adapted according to epidemiological conditions.

Conclusion

The importance of WNV-associated AE in Madagascar is probably underestimated and data regarding burden of WNV diseases are sparse in the country. Migliani et al. detected, between January 2001 and June 2002, 79 cases of AE in two hospitals of Antananarivo, the capital city of Madagascar, including 3% due to WNV infection [57]. A recent study described an imported case of AE due to WNV infection in La Réunion island and coming from the north-west of Madagascar [12]. A review performed by Jmor et al. [58] revealed a minimum incidence of AE syndrome for all age groups of 6.34 per 100,000 population in tropical countries. The increasing incidence of encephalitis due to emerging agents, such as Japanese encephalitis virus, WNV and tick-borne encephalitis virus is a new challenge for public health [58]. More work is needed to better estimate the real burden of AE associated to WNV infection. Thus, the identification of at-risk environments for WNV circulation in human in Madagascar island is a first step to target future studies on AE surveillance.

Abbreviations

AE: acute encephalitis

AIC: Akaike Information Criterion

CI: confidence interval

ELISA: Enzyme Linked ImmunoSorbent Assay

GLMM: Generalized linear mixed model

IPM: Institut Pasteur de Madagascar

MAFE: Model-Averaged Fixed Effects

NDVI: Normalized Difference Vegetation Index

OD: Optical Density

OR: Odd-Ratios

RVF: Rift Valley Fever

SES: socio-economic status

VIF: Variance Inflation Factors

WNE: West Nile Virus encephalitis

WNV: West Nile Virus

Declarations

Ethics approval and consent to participate

The protocol was approved by the Malagasy competent authorities, the Malagasy Ethic National Committee (authorization N°066/MSAMP/CE, 26th July 2011). After reading of the informed consent letter, written and oral informed consent was obtained from all participants. Participants were sampled by qualified investigators and the data were analyzed anonymously. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not Applicable

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author (soafy@pasteur.mg) on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the Institut Pasteur de Madagascar (Internal Project through the ZORA (ZOonoses, Rodent and Arboviruses) project. Moreover, this material is based upon work supported by the Centers for Disease Control and Prevention (CDC; Cooperative Agreement #U51/IP000327) and the U. S. Department of Homeland Security under the Center of Excellence of Emerging and Zoonotic Animal Diseases Grant Award Number 2010-ST061-AG0001. The views and conclusions contained in this paper

are those of the authors and should not be interpreted as necessarily representing the official policies, either explicit or implicit, of the Centers for Disease Control and Prevention and the U. S. Department of Homeland Security. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

MMO: Conceptualization, Field coordination and achievement, Methodology, Data management, Statistical analysis, Project administration, Project supervision, Original draft Writing, Draft manuscript & Editing

AB: Methodology, Laboratory analysis, Data management, Statistical analysis, Draft manuscript

SFA: Conceptualization, Field coordination and achievement, Data management, Methodology, Project administration, Project supervision, Draft manuscript

ACD: Methodology, Laboratory analysis

JPR: Field collection, Laboratory analysis

SA: Field collection

FR: Methodology, revised manuscript

CR: Conceptualization, Methodology, Validation, revised manuscript

JMH: Conceptualization, Methodology, Project administration, Project supervision, Validation, Visualization, Draft manuscript.

All authors have given final approval of the version to be published.

Acknowledgements

We especially thank the population of Madagascar who participated to the studies. We thank those who facilitated the survey, i.e., heads of fokontany, local administration authorities and health authorities from Ministry of Health. We also thank the Plague Unit at the Institut Pasteur de Madagascar for data collection and supporting (S. Telfer, C. Rahaingosoamamitiana, F.M. Andriamiarimanana, S. Rahelinirina, M. Rajerison), S. Andrimasinoro for the management of data, B.S. Rahoilijaona H.A. Rakotoarison, H. Raharimampianina and A.M. Rakotohaingomahefa for their field supports. We thank G. Gray from the Division of Infectious Diseases of Duke University for supporting field collection.

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Tables

Table 1: Descriptive and univariate analyses.

Tested variable		Positive	Number	% seroprevalence [95% CI]	Chi2 / generalized linear model P- value
Gender	Female	108	851	12.7 [10.5 - 15.1]	p >0.20
	Male	106	829	12.8 [10.6 - 15.3]	
Age	18 to 26	56	456	12.3 [9.4-15.6]	p >0.20
	27 to 37	48	423	11.3 [8.5-14.8]	
	38 to 46	49	361	13.6 [10.2- 17.5]	
	> 47	61	440	13.9 [10.8- 17.4]	
Habitat	Urban	35	450	7.8 [5.5 - 10.7]	p < 0.05
	Sub-urban	64	510	12.5 [9.8 - 15.7]	
	Rural	115	720	16.0 [13.4 - 18.9]	
socio-economic status	Low	148	847	17.5 [15.0 - 20.2]	p < 0.001
	Intermediate	61	746	8.2 [6.3 - 10.4]	
	High	5	87	5.7 [1.9-12.9]	
Working environment	Indoor	35	499	7.0 [4.9 - 9.6]	p < 0.001
	Outdoor	145	924	15.7 [13.4 - 18.2]	
	Household	34	257	13.2 [9.3-18.0]	
Frequent contact	No	150	1,182	12.7 [10.9 -	p >0.20

with poultry				14.7]	
	Yes	64	498	12.9 [10.0 – 16.1]	
Frequent work in rice field	No	117	1,038	11.3 [9.4-13.4]	p < 0.05
	Yes	97	642	15.1 [12.4-18.1]	
Frequent work in forest	No	122	1,162	10.5 [8.8-12.4]	p < 0.001
	Yes	92	518	17.8 [14.6-21.3]	
Poultry density	< 15.6 per km ²	70	420	16.7 [13.2-20.6]	p < 0.001
	15.6 to 37.2 per km ²	60	420	14.3 [11.1-18.0]	
	37.2 to 75.9 per km ²	60	450	13.3 [10.3-16.8]	
	> 76.0 per km ²	24	390	6.2 [4.0–9.9]	
Factor 1		/	/	/	p < 0.20
Factor 2		/	/	/	p < 0.001
Factor 3		/	/	/	p < 0.001
Factor 4		/	/	/	p < 0.001
Total		214	1,680	12.7 [11.2-14.4]	/

Table 2: Results from the multi-model inference approach for dataset analysis.

Variables		MAFE ^a	95% CI	p-value	weight	Number of models
Working environment	Indoor	Ref	/	/	1	10
	Outdoor	0.69	[0.21 - 1.17]	< 0.01		
	Household	0.48	[-0.07 - 1.04]	NS ^b		
Frequent contact with rice field	No	Ref	/	/	0.48	5
	Yes	0.14	[-0.13 - 0.70]	NS	/	/
Poultry density	< 17 per km ²	Ref	/	/	0.84	10
	18 to 37 per km ²	-0.41	[-1.27 - 0.29]	NS	/	/
	38 to 77 per km ²	0.02	[-0.72 - 0.77]	NS	/	/
	> 77 per km ²	-0.95	[-2.08 - -0.19]	NS	/	/
Factor 1		-0.09	[-0.42 - 0.04]	NS	0.46	4
Factor 2		-0.91	[-1.32 - -0.51]	< 0.001	1	10
Factor 3		0.10	[-0.15 - 0.57]	NS	0.47	6
Factor 4		0.53	[0.19 - 0.87]	< 0.005	1	10

^aMAFE = Model-Averaged Fixed Effects

^bNS = not significant

Figures

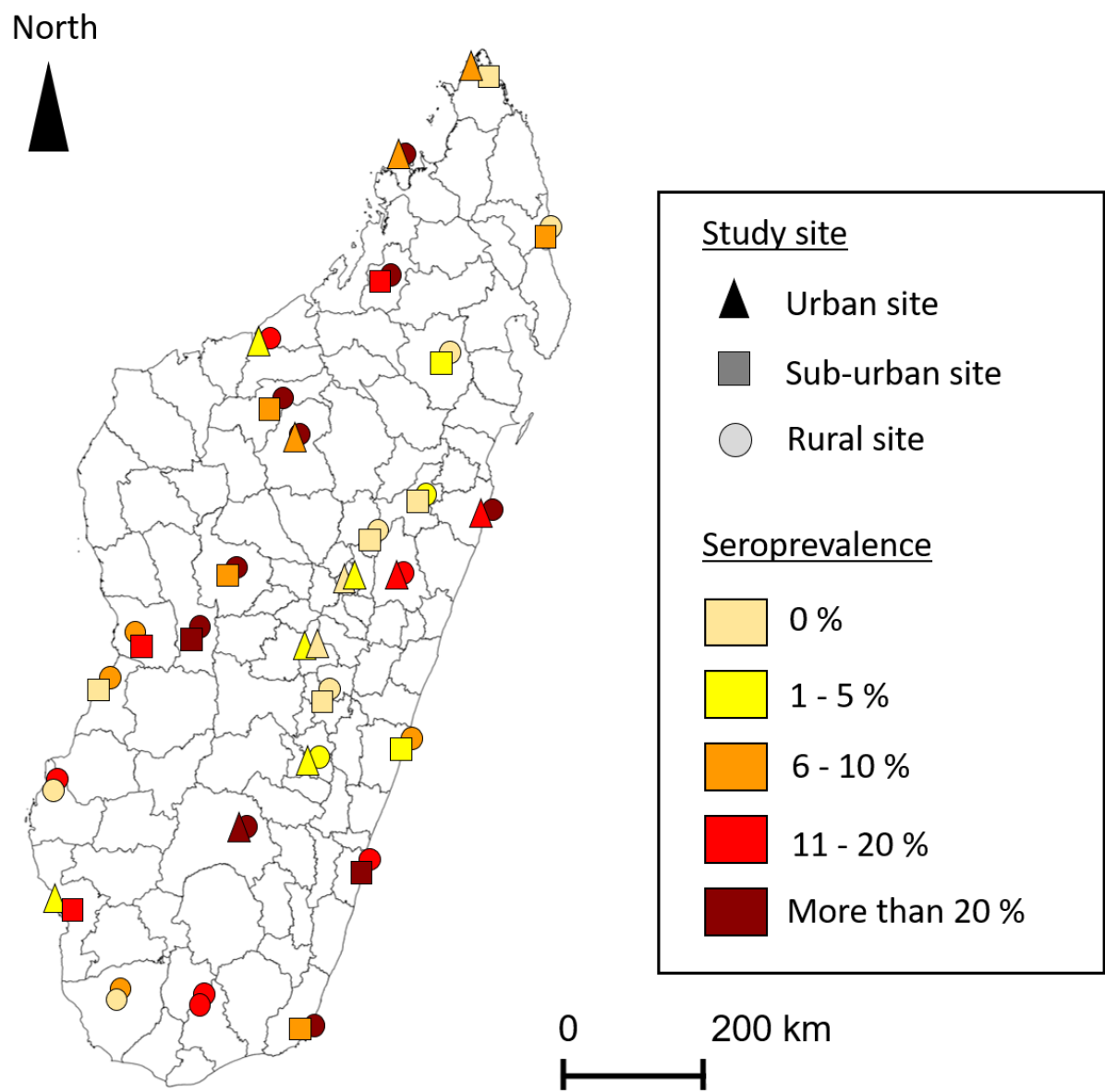


Figure 1

Sampling sites and IgG serological results.