

Persistence of Zika virus and higher viral load in cutaneous capillaries than venous blood

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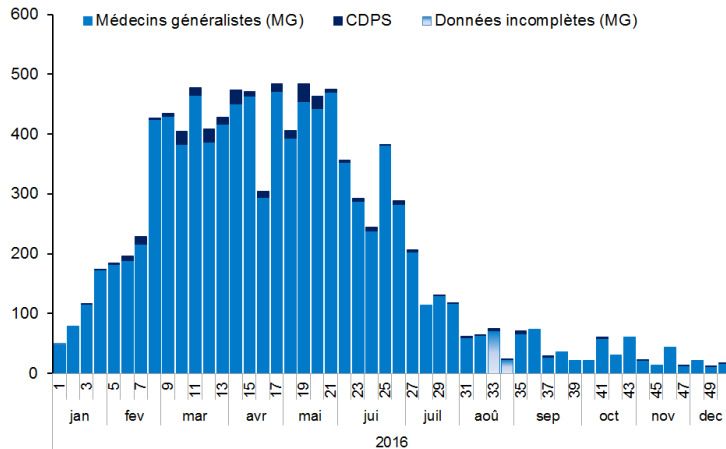
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Zika outbreak: Context in French Guiana

- French territory of South America (≈ 250,000 inhabitants)
- High frequency of Dengue outbreaks
- First confirmed Zika case: **December 2015**
- Declaration of outbreak phase: **January 2016**
- End of outbreak: **September 2016**



Weekly estimated number of people with Zika symptoms in French Guiana from January to December 2016

- Estimated number of people with Zika symptoms: **9500**
- Number of Zika-infected pregnant women: **1519**

⇒ **16** cases associated with brain malformations, including **4** with microcephaly

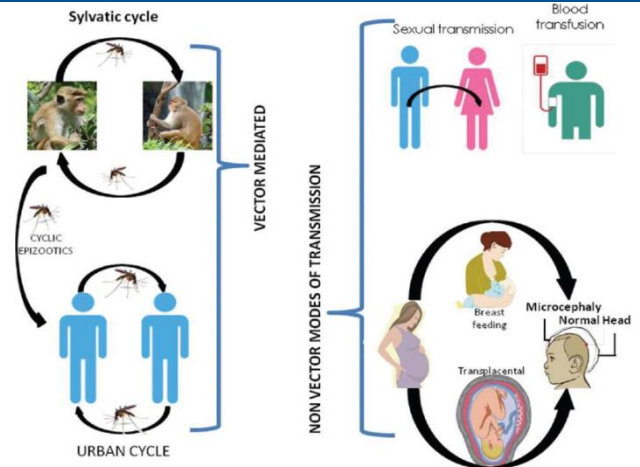
Context and design of study

- **Set up a descriptive and prospective study of Zika virus disease from a cohort of military personnel**
- **Inclusion of consenting patients with cutaneous rash, with or without fever, for ZIKV diagnosis (serum and/or urine samples)**
- **Enrollment of patients with a confirmed ZIKV diagnosis in a longitudinal cohort survey to:**
 - ⇒ **Characterize the clinical and biological parameters of the acute infection and its evolution**
 - ⇒ **Analyze the immune response to ZIKV (IgM and IgG antibodies) over the course of one year**
 - ⇒ **Study the kinetics of ZIKV in different biological compartments: semen, venous blood, and capillary blood**

Knowledge of ZIKV transmission

- Zika transmission to humans:

- Mosquito bites
- Sexual transmission
- Mother to fetus

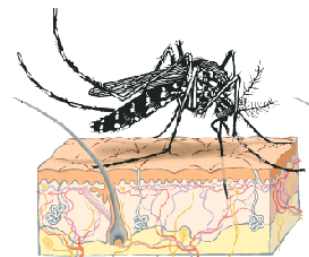


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- Approach to limit vectorial transmission: isolation of positive patients during the entire **viremic phase**
- Viremic phase **only** measured in venous blood, whereas mosquitoes transmit the virus into cutaneous capillary blood

⇒ Interest to compare viremia between the two blood compartments:

Venous blood versus capillary blood



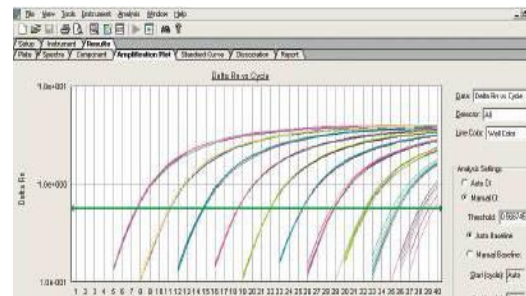
Patients

- Inclusion of **21 symptomatic and consenting patients** with confirmed ZIKV diagnosis in venous and/or urine samples by real time PCR
- Median age: **40 years** (range 28-63 years)
- Sex ratio: **1.6**
- All patients were negative for DENV and CHIKV
- No comorbidities
- Collection of **sequential serum samples** from:
 - ⇒ **Venous compartment**
 - ⇒ **Cutaneous capillary blood compartment**
- Duration of collection: **Day 1 to day 18** after the onset of disease



Method

- All sequential serum samples collected from the two biological compartments analyzed by real-time PCR
 - Extraction using the Qiagen viral RNA kit
 - 150 μ L of serum from venous blood
 - Variable volume of serum from capillary blood
 - Inclusion of an internal control (6 μ L) in extraction step
 - ZIKV real-time PCR (Altona kit)
 - Use of quantitative ZIKV strain (Ref EVAg) to quantify RNA ZIKV loads
 - Viral load estimated as log copy number per mL



Results (1)

- Three patient profiles:
 - **57% (12/21)** of patients for whom the ZIKV RNA persisted longer in capillary than venous blood samples (95 CI= 34-78)

Patient	Age	Gender	Days after onset of symptoms	ZIKV load (Log copy number/mL)	
				Venous blood	Capillary blood
5	37	M	1	4.3	4.2
			3	< 1.8	2.9
			7	< 1.8	2.9
11	35	M	2	2.6	2.9
			3	< 1.8	2.6
			5	< 1.8	2.5
			8	< 1.8	2.4
16	63	F	3	3.9	3.4
			8	< 1.8	2.7
			10	< 1.8	2.5
19	35	M	4	< 1.8	3.8
			6	< 1.8	3.2
			8	< 1.8	3.7
			18	NA	1.9

Results (2)

- **Three patient profiles:**

- 57% (12/21) of patients with longer duration of ZIKV RNA in capillary blood samples than in venous blood samples (95 CI= 34-78)
- **33% (7/21) of patients with equal duration of detectable Zika RNA in the two compartments (95 CI=15-57)**
- 10% (2/21) of patients with longer viremia duration in venous blood samples than in capillary blood samples (95 CI=1-30)

Patient	Age	Gender	Days after onset of symptoms	ZIKV load (Log copy number/mL)	
				Venous blood	Capillary blood
10	41	M	3	3.3	3.4
			5	< 1.8	< 1.8
			10	< 1.8	< 1.8
18	32	F	1	4.3	NA
			3	3.0	3.1
			7	< 1.8	< 1.8
			15	< 1.8	< 1.8

Results (3)

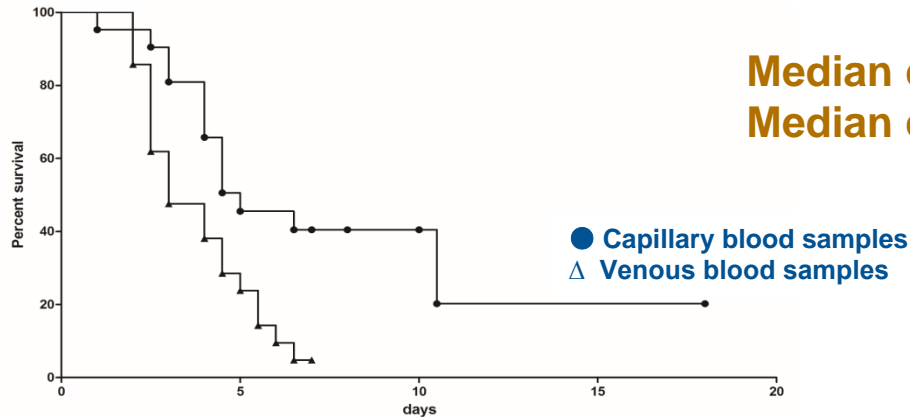
- **Three patient profiles:**

- 57% (12/21) of patients with longer duration of ZIKV RNA in capillary blood samples than in venous blood samples (95 CI= 34-78)
- 33% (7/21) of patients with equal duration of viremia (95 CI=15-57)
- **10% (2/21) of patients for whom longer duration of viremia in venous than capillary blood**

Patient	Age	Gender	Days after onset of symptoms	ZIKV load (Log copy number/mL)	
				Venous blood	Capillary blood
21	35	F	1	3.4	< 1.8
			3	< 1.8	< 1.8
			6	< 1.8	< 1.8
			13	< 1.8	< 1.8

Results (4)

- Median duration of detected ZIKV RNA significantly longer in capillary than in venous blood (Log rank test, $P = 0.005$; hazard ratio = 2.99, 95 CI 1.39–6.43)



- Duration of viremia in capillary blood underestimated because ZIKV was still present in the last capillary blood sample from 8 patients
- Correlation between ZIKV RNA loads in capillary and venous blood compartments (Spearman's correlation test, $r = 0.51$, $P < 0.0001$)
- But significantly higher in capillaries (except in two patients) (Wilcoxon signed rank test, $P = 0.0002$)

Conclusion and perspectives

- **Does the persistence of ZIKV RNA in cutaneous capillary blood reflect:**
 - The replication of viral particles?
 - Local Zika virus replication in permissive cells of the skin (human dermal fibroblasts, epidermal keratinocytes) or/and capillaries?
- **What is the implication of RNA viral load in the capillary compartment on vectorial transmission?**
 - Is the late viral load detected in the capillary compartment relevant for mosquito infection?
- **It may be useful to more thoroughly explore this neglected biological compartment and extend such studies to other arboviruses**
- **Capillary blood spot test: Alternative approach for the molecular diagnosis of Zika, especially in the field**

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An aerial photograph of a vast, dense tropical forest in French Guiana. The forest is covered in a thick layer of mist or low clouds, creating a hazy, ethereal atmosphere. The trees are a deep green color, and the mist is a pale, greyish-white. The landscape is flat, with the forest extending to the horizon. The sky is overcast and grey.

Thanks for your attention

and

Welcome to French Guiana !!!