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Short communication

Detection of Zika virus in saliva

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ABSTRACT

Background: During the largest Zika virus (ZIKV) outbreak ever reported that occurred from October 2013 to March 2014 in French Polynesia, we observed that several patients presenting the symptoms of acute phase Zika fever were tested negative in blood by ZIKV real-time PCR (RT-PCR).
Objectives: As we have previously detected ZIKV RNA in the saliva of a young child, we investigated the use of saliva as an alternative sample for routine ZIKV RNA detection.
Study design: Over a 6 month period, 1,067 samples collected from 855 patients presenting symptoms of Zika fever (saliva only, blood only or both samples) were tested using a specific ZIKV RT-PCR. A medical questionnaire was available for most of the patients.
Results: ZIKV was more frequently detected in saliva compared to blood. For the 182 patients with both samples collected, tests were positive for 35 (19.2%) in saliva while negative in blood and tests were positive for 16 (8.8%) in blood while negative in saliva; the difference in mean days after symptoms onset and the percentage of the main symptoms of Zika fever for patients only positive in saliva or in blood was not significant.
Conclusion: The use of saliva sample increased the rate of molecular detection of ZIKV at the acute phase of the disease but did not enlarge the window of detection of ZIKV RNA. Saliva was of particular interest when blood was difficult to collect (children and neonates especially).

1. Background

Zika virus (ZIKV) is an arthropod-borne virus (arbovirus) of the family *Flaviviridae* and genus *Flavivirus* [1]. ZIKV was first isolated in 1947 in Uganda [2]; sporadic human cases were reported from the 1960s in Asia and Africa [3]. The first ZIKV outbreak outside Africa and Asia occurred in 2007 in Yap Island (Federated States of Micronesia, Pacific) [4] and the largest one from October 2013 to March 2014 in French Polynesia (FP), Pacific [5,6]. Clinical presentation of Zika fever is non-specific; the most common symptoms are rash, fever, arthralgia, myalgia, asthenia and conjunctivitis. Patients usually report mild symptoms, asymptomatic infections have also been described [7]. As there is no abrupt clinical onset, dating the beginning of the illness is subjective [8]. Laboratory Zika fever diagnosis is challenging because there is no "gold standard" diagnosis tool. The cross reactivity of antibodies between Flaviviruses [9] limits the use of serology, viral culture is not routinely performed and there is no antigenic detection test available. Acute phase diagnosis relies on molecular technologies [5,9]. During the FP outbreak, we used the real time reverse transcription-PCR (ZIKV RT-PCR) protocol described by Lanciotti et al. during investigations of the ZIKV outbreak on Yap Island [9]. The specificity of this protocol for ZIKV amplification has been confirmed against other arboviruses, especially DENV which was co-circulating in FP during the ZIKV outbreak.

2. Objectives

During the French Polynesian outbreak, we observed that several patients presenting the major symptoms of Zika fever were tested negative by ZIKV RT-PCR in blood collected during the first week after symptoms onset. On November 2014, unrealizable blood collection from a 1 year-old child suspected of being infected by ZIKV led us to collect a saliva sample which tested positive by ZIKV RT-PCR (unpublished data). Therefore, we decided to investigate

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Table 1
ZIKV RT-PCR results for patients with both samples collected.

		Saliva	
		Positive	Negative
Blood	Positive	52 (28.6%)	16 (8.8%)
	Negative	35 (19.2%)	79 (43.4%)

the use of saliva as an alternative sample for acute phase ZIKV detection.

3. Study design

From October 2013 to March 2014, we collected 1,067 samples from 885 patients presenting with Zika fever symptoms for ZIKV RNA detection: blood samples for 748, saliva samples for 319 and both samples collected at the same time for 182 patients. A standardized medical questionnaire form was available for most of the patients, numbers of days from the first reported symptom (days from symptoms onset) and main symptoms were recorded. Numbers of days post illness onset was the number of days after symptoms onset. Blood samples were collected from venous blood puncture and saliva samples with dry cotton swabs without transport media (N° 150C, Copan, Brescia, Italy).

Molecular detection of ZIKV in saliva was performed after RNA extraction using the NucliSENS® easyMAG® System (BioMérieux) according to manufacturer's recommendations. Briefly, 200 μl of serum was directly added to 2 ml of lysis buffer; oral swabs were first vortexed in 2 ml of lysis buffer, both were eluted by 50 μl of elution buffer and 5 μl of extracted RNA was used for amplification on a CFX96 Touch™ real-time PCR detection system (Biorad) using two real-time primers/probe amplification sets specific for ZIKV [5,9]. Results were reported as positive when the 2 amplifications occurred. The 182 patients with both samples collected tested negative for DENV using a multiplex DENV RT-PCR protocol as previously described [10]. Statistical analyses were performed by using GraphPad Prism 6.

4. Results

The overall number of RT-PCR positive ZIKV samples was 210 of 748 (28.1%) in blood and 182 of 319 (57.1%) in saliva. ZIKV RNA was more frequently detected in saliva than in blood samples (Fisher's exact test, $p < 0.0001$).

ZIKV RT-PCR results for the 182 patients with both samples are reported in Table 1. Thirty five patients (19.2%) tested positive in saliva while negative in blood and 16 patients (8.8%) tested positive in blood while negative in saliva, this difference was significant (McNemar test, $p = 0.0117$). Mean age of patients tested positive only in saliva and blood was, respectively, 31.1 and 28.4 years old, the difference was not significant (Man–Witney test, $p = 0.7363$). From the 137 patients with only saliva samples, 95 samples tested were positive for ZIKV, of which 38 were children less than 10 years old (including 7 neonates).

The number of days after symptoms onset for the 103 patients with either saliva, blood or both samples tested positive for ZIKV is reported in Table 2. The difference in mean days after symptoms onset for patients only positive for ZIKV in blood and those only positive in saliva was not significant (Kruskal–Wallis test with Dunn's multiple comparisons post test, $p > 0.9$). The proportion of samples tested positive by ZIKV RT-PCR according to the number of days from symptoms onset for the 182 patients with both saliva and blood collected is reported in Fig. 1. The results suggest that testing saliva may be of particular interest on day 3.

Table 2
Number of days from symptoms onset for patients with both samples collected.

	Saliva positive	Blood positive	Saliva and blood positive
Mean	3.5	3.3	2.6
Median	3.0	3.0	2.0
SD[a]	1.5	1.8	1.3

[a] Standard deviation.

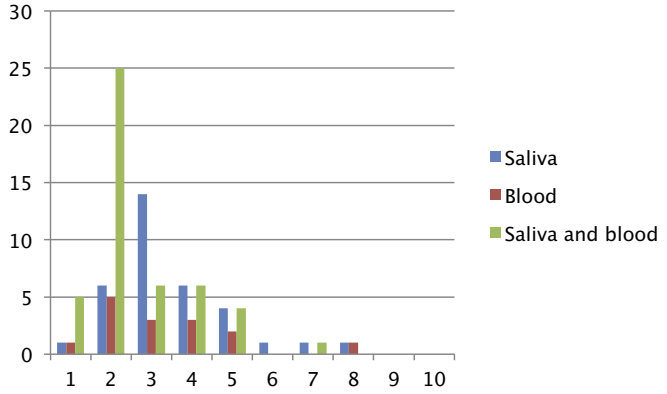

Fig. 1. Proportion of positive samples (Y axis in %) according to the number of days after symptoms onset (X axis) for the 182 patients with saliva, blood or both samples tested by ZIKV RT-PCR.

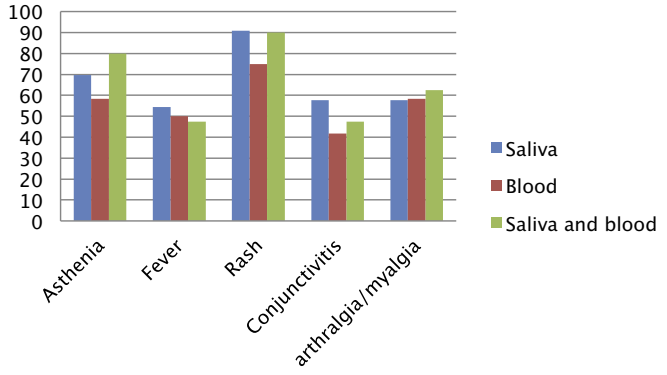

Fig. 2. Proportion (Y axis in %) of symptoms (X axis) reported for the 182 patients with saliva, blood or both samples tested positive by ZIKV RT-PCR.

The percentage of the main symptoms of Zika fever (Fig. 2) was not significantly different whether saliva or blood were tested positive for ZIKV (Chi-square, p was, respectively, 0.2889 for asthenia, 0.8348 for fever, 0.3054 for rash, 0.5545 for conjunctivitis and 0.9054 for arthralgia and myalgia), suggesting that the detection of ZIKV RNA was not related to a particular clinical presentation.

5. Conclusion

The use of alternative samples to blood for arbovirus diagnosis has already been described. Detection of DENV RNA in saliva has been reported with concomitant detection in either blood and urine [11] or in urine while negative in blood [12]. Detection of ZIKV in saliva was reported for a neonate and his mother, respectively, on days 3 and 2 post partum [13].

Detection of arbovirus RNA in urine has been reported for a longer time than in blood for ZIKV [8,13], DENV [14] and West Nile virus [15]. In New Caledonia, ZIKV RNA was detected in urine of 6 patients up to 20 days after viremia had reached an undetectable level [8].

We investigated the use of saliva collected on oral swabs for ZIKV RNA detection. Oral swab is a non invasive sample-collection device of particular interest when blood samples are difficult to collect,

in remote places where medical facilities are lacking, in community settings for surveillance and epidemiology, self collection is possible.

We found that the ability to detect ZIKV RNA in saliva was higher compared to blood; was neither found to be related to a particular clinical presentation nor to the age of the patient. The use of saliva improved the ability to detect ZIKV RNA within the first week from symptoms onset but it did not increase the window of detection in contrast to what was reported for urine.

However, as ZIKV RNA detection can be negative in saliva while positive in blood and blood sample is required for other laboratory tests, saliva cannot replace blood samples.

For acute phase, Zika fever diagnosis we recommend to collect both blood and saliva samples to increase the sensitivity of molecular detection of ZIKV, urine sample can be associated at the late stages of the disease. When blood collection cannot be performed, saliva collection alone should be considered. During the FP outbreak we adjusted our recommendations for acute phase Zika fever diagnosis for young children and neonates, for whom it seemed more appropriate to collect saliva as a first line for ZIKV RNA detection.

As dating of symptoms onset is difficult during Zika fever, when detection of ZIKV is of particular importance, using a combination of samples (blood/saliva/urine) is recommended.

The use of saliva as an alternative specimen for acute phase molecular diagnosis needs to be investigated for other arbovirus infections.

Competing interest

None declared.

Funding

None.

Ethics approval

The study has been approved by the Ethics Committee of French Polynesia (reference 66/CEPF). When additional samples were collected for the purpose of the study, written consents were obtained from the patients.

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