Since October 2013, French Polynesia has experienced the largest documented outbreak of Zika virus (ZIKAV) infection. To prevent transmission of ZIKAV by blood transfusion, specific nucleic acid testing of blood donors was implemented. From November 2013 to February 2014: 42 (3%) of 1,505 blood donors, although asymptomatic at the time of blood donation, were found positive for ZIKAV by PCR. Our results serve to alert blood safety authorities about the risk of post-transfusion Zika fever.

ZIKAV has been isolated from several *Aedes* mosquito species, notably including *Ae. aegypti* [9] and *Ae. albopictus* [10]. *Ae. aegypti* is widespread in the tropical and subtropical regions of the world and *Ae. albopictus* is now established in many parts of Europe, especially Mediterranean countries [11]. Recent reports of imported cases of ZIKAV infection from south-east Asia or the Pacific to Europe [12] or Japan [13] highlight the risk of ZIKAV emergence in parts of the world where the vector is present.

**Sample collection**

According to the procedures of the blood bank centre of French Polynesia, all blood donors have to fill in a pre-donation questionnaire and have a medical examination before blood donation. Blood is taken only from voluntary donors who are asymptomatic at the time of donation. A signed informed consent statement was obtained from all blood donors and publication of data related to ZIKAV testing was approved by the Ethics Committee of French Polynesia (reference 66/CEPF).

ZIKAV nucleic acid testing (NAT) of samples of all donations was implemented routinely from 13 January 2014. In February, samples of donations collected from 21 November 2013 to 12 January 2014 were retrospectively tested. We report here the results of ZIKAV testing for all donors who donated blood from 21 November 2013 to 17 February 2014.

**Laboratory and clinical findings**

On the basis of protocols implemented for WNV NAT [14], blood donor samples were tested in minipools. In order to increase the sensitivity of detection and to reduce the occurrence of false-negative results, sera from no more than three blood donors were included in each minipool.
Detection of Zika virus RNA in blood samples from asymptomatic donors

RNA was extracted from 200 µL minipooled or individual sera using the Easymag extraction system (bio-Mérieux, France) as previously reported [15]. ZIKAV real-time reverse-transcription PCR (RT-PCR) was performed on a CFX Biorad real-time PCR analyser using two real-time primers/probe amplification sets specific for ZIKAV [16]. The sensitivity of the assay was controlled by amplifying serial dilutions of an RNA synthetic transcript that covers the region targeted by the two primers/probe sets. A sample was considered positive when amplification showed a cycle threshold (Ct) value <38.5. However, in order to avoid false-negative results due to the pooling, each minipool showing a Ct value >40 with at least one primer/probe set was controlled by individual RT-PCR. Even if the two primers/probe sets did not react with the four DENV serotypes [16], the specificity of the amplified product from two donors whose blood was ZIKAV positive by RT-PCR was controlled by sequencing [1]. The sensitivity of the assay was the same as that previously reported (25 to 100 copies per assay) [16].

From 533 minipools tested from blood donated during 21 November 2013 to 17 February 2014, 61 were found positive, with at least one of the Ct values >40. The constitutive blood plasmas of these 61 ZIKAV-positive minipools were tested individually and revealed 34 minipools in which one of the donors was ZIKAV positive; in four minipools, two of the three donors were positive.

In total, 1,505 blood donors were tested: 42 (2.8 %) were confirmed positive by individual testing (28 with the two primer/probe sets and 14 with one primer/probe set).

The two sequenced samples were confirmed as ZIKAV (GenBank accession numbers KJ680134 and KJ680135)*, sharing 99.6% similarity with the sequence initially reported at the beginning of the outbreak (GenBank accession number KJ579442) [1].

Detection of Zika virus in culture

Sera from 34 ZIKAV RT-PCR-positive donors were inoculated on Vero cells in order to detect replicative viral particles; there was insufficient serum available for the remaining eight RT-PCR-positive donors. Of the 34 inoculated, three were positive in culture. However, the culture was conducted retrospectively and sample storage conditions were not optimal for viral culture (several freeze/thaw cycles), leading potentially to some false-negative results.

Occurrence of Zika fever-like syndrome following blood donation

Blood donors positive for ZIKAV were contacted retrospectively by telephone to investigate the occurrence of ‘Zika fever-like syndrome’ (rash and/or conjunctivitis and/or arthralgia) after their blood donation. Of the 42 donors tested positive by RT-PCR, 11 declared that they had a Zika fever-like syndrome from 3 to 10 days after they gave blood.

Discussion

The main challenge in the prevention of arbovirus transfusion-derived transmission is the high rate of asymptomatic infections: this has been estimated at over 75% for DENV [17] and West Nile virus (WNV) [18]. For ZIKAV, there is no estimate available of the percentage of asymptomatic infections. Arbovirus transfusion-derived transmission has been reported principally for WNV [19], DENV [20] and chikungunya virus (CHIKV) [21,22]. For CHIKV, the risk was evaluated as high [21,22].

During the outbreaks of CHIKV infection in Italy (2007) [21] and in Réunion Island in the Indian Ocean (2005–07) [22], blood donation was discontinued and blood products were imported from blood bank centres elsewhere. In French Polynesia, due to its geographically isolated location, it was impossible to be supplied with fresh blood products from blood bank centres outside French Polynesia.

Due to the potential risk of ZIKAV transfusion-derived transmission, the need to continue blood donations and the lack of a licensed test for ZIKAV diagnosis, we decided to implement ZIKAV NAT as soon as possible, using a modified RT-PCR [16]. The protocol was implemented in November 2013, when agreement from the French Polynesian health authorities was obtained. The specificity of this RT-PCR assay has been previously evaluated and was confirmed by sequencing analysis conducted during the outbreak in French Polynesia [1] and its sensitivity was similar to that previously evaluated [16].

We detected an unexpectedly high number of positive asymptomatic blood donors (42/1,505; 3%). To date, no post-transfusion ZIKAV infection has been reported in recipients of ZIKAV-positive blood in French Polynesia; however, haemovigilance studies are still ongoing.

Due to concomitant circulation of DENV serotypes 1 and 3 since early 2013 [3], multiplex NAT testing for DENV has been implemented from April 2013: no DENV-positive donor has yet been detected. While this might be related to a low level of viraemia in asymptomatic donors, we consider it was probably due to the low level of DENV-1 and DENV-3 circulation. Pathogen inactivation of platelet concentrates using a photochemical treatment (amotosalen) of blood products and ultraviolet A light inactivation was also implemented [23].

The management of a dual outbreak of ZIKAV and DENV infection was challenging because we had to test all blood donors for both pathogens, which was time-consuming and expensive. In addition, in our blood bank
centre, the mean delay between blood donation and production of fresh blood product available for transfusion is generally 24 hours. During the outbreaks, the mean delay was three days.

This report serves as a reminder of the importance of quickly adapting blood donation safety procedures to the local epidemiological context. Moreover, it should help in anticipating the needs in other parts of the Pacific region, such as in New Caledonia (South Pacific), where an outbreak of ZIKAV infection started in February 2014 [24].

Our findings suggest that ZIKAV NAT should be used to prevent blood transfusion-transmitted ZIKAV. As recommended by the European Centre for Disease Prevention and Control, blood safety authorities need to be vigilant and should consider deferral of blood donors returning from areas with an outbreak of ZIKAV infection [2]. In areas endemic for Aedes species, a preparedness plan to respond to future outbreaks of ZIKAV infection should include emergency plans to sustain blood supply.

Conflict of interest
None declared.

Authors' contributions

* Addendum:

The GenBank accession numbers of the two ZIKAV sequenc-
es derived from the amplified PCR products from two blood donors whose blood was ZIKAV positive by RT-PCR, were added on 11 April 2014.

References