Title

Plasma transfusion-transmission of Zika virus in mice and macaques.

Journal

Transfusion, 63(3)

Authors

Van Rompay, Koen

Coffey, Lark

Yee, JoAnn

et al.

Publication Date

2023-03-01

DOI

10.1111/trf.17243

Peer reviewed

HHS Public Access

Author manuscript

Transfusion. Author manuscript; available in PMC 2024 March 01.

Published in final edited form as:

Transfusion. 2023 March ; 63(3): 574–585. doi:10.1111/trf.17243.

Plasma transfusion-transmission of Zika virus in mice and macaques

Koen K.A. Van Rompay[1,2], **Lark L. Coffey**[2], **JoAnn L. Yee**[1], **Anil Singapuri**[2], **Jackson Stuart**[2], **Marion C. Lanteri**[3], **Felicia Santa Maria**[3], **Kai Lu**[4], **Inderdeep Singh**[4], **Sonia Bakkour**[4], **Mars Stone**[4,5], **Phillip C. Williamson**[6], **Marcus O. Muench**[4,5], **Michael P. Busch**[#*,4,5], **Graham Simmons**[#*,4,5]

[1]California National Primate Research Center, University of California, Davis, CA, United States of America

[2]Department of Pathology, Microbiology and Immunology, University of California, Davis, CA, United States of America

[3]Cerus Corporation, Concord, CA, United States of America

[4]Vitalant Research Institute, San Francisco, California, United States of America

[5]Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, United States of America

[6]Creative Testing Solutions, Tempe, AZ, United States of America

[#] These authors contributed equally to this work.

Abstract

BACKGROUND: Zika virus (ZIKV) epidemics with infections of pregnant women are associated with severe neurological disease in newborns. Although an arbovirus, ZIKV is also blood transfusion transmitted (TT). Greater knowledge of the efficiency of ZIKV TT would aid decisions on testing and pathogen reduction technologies (PRT).

STUDY DESIGN AND METHODS: Plasma units from ZIKV RNA-reactive blood donors were used to study infectivity *in vitro*, in mice and in macaques. Furthermore, plasma units were subjected to PRT using amotosalen/UV light (A/UVA) prior to transfusion.

Graham Simmons, Vitalant Research Institute, 360 Spear St., Suite 200, San Francisco, CA. 94105. gsimmons@vitalant.org. Tel. 415 901 0748.

[*]These authors contributed equally

AUTHOR CONTRIBUTIONS:

Study was designed by GS, MPB, KKAR, LLC, JLY, MOM and MS. Sample acquisition, processing, experiments, and data analysis were performed by KKAR, LLC, JLY, AS, JS, MCL, FSM, KL, IS, SB, MS, PCW, MOM, MPB and GS. GS, KKAR and LLC prepared figures and GS, KKAR, LLC, ML and MPB edited the manuscript. All authors critically contributed to the finalization of the paper.

MCL and FSM were/are employees of Cerus Corporation. MCL is currently an employee of Creative Testing Solutions. SB is currently an employee of Grifols Diagnostic Solutions Inc

Conflict of interest statement

Felicia Santa Maria is and Marion C. Lanteri employees and shareholders of Cerus Corporation which markets the INTERCEPT Blood System

RESULTS: *In vitro* infectivity of ZIKV RNA-reactive plasma varied between 100 and 1,000 international units (IU) of ZIKV RNA. Immunodeficient mice were more sensitive with as low as 32 IU sufficient to infect 50% of mice. 50–5,500 IU of RNA led to TT in macaques using dose escalation of three different RNA-positive, seronegative plasma units. In contrast, RNA-reactive units collected post-seroconversion were not infectious in macaques, even at a dose of 9 million IU RNA. After A/UVA PRT, transfusion of plasma containing up to 18 million IU was no longer infectious *in vitro* and did not result in ZIKV TT in macaques.

CONCLUSION: Significant risks of ZIKV TT are likely confined to a relatively short viremic window prior to seroconversion, and that sensitive nucleic acid amplification testing (NAT) likely identifies the majority of infectious plasma. PRT was demonstrated to be effective at preventing ZIKV TT. Considering that there is no approved ZIKV vaccine, these data are relevant to mitigate the risk of TT during future ZIKV outbreaks.

INTRODUCTION

Zika virus (ZIKV) infection is usually characterized by mild febrile disease, although rare complications, such as Guillain-Barre syndrome, have been reported[1]. From 2014–2016, the virus rapidly spread throughout the Pacific and the Americas[2]. During this epidemic, large numbers of infants with severe congenital syndromes and neurological abnormalities, including microcephaly, were born to mothers infected with ZIKV during pregnancy[3].

ZIKV and related flaviviruses such as dengue (DENV) and West Nile (WNV) are predominantly mosquito-borne, however a small number of cases have been linked to transmission of these viruses via transfusion of infected blood or blood products [4,5,6]. High rates of ZIKV RNA have been documented in blood donors[7,8], often with high peak viremias[9]. Exquisitely sensitive NAT has been developed and employed for routine testing of blood donations for ZIKV RNA[10,11], while amotosalen (A) and ultraviolet A light (UVA) pathogen reduction technology (PRT) (INTERCEPT® Blood System for Platelets and Plasma, Cerus Corporation) was authorized by the Food and Drug Administration (FDA) to reduce the risk of transfusion transmission (TT) of ZIKV in lieu of NAT[12].

With implementation of the above measures, the risk of ZIKV TT associated with the majority of ZIKV positive blood donations capable of transmitting infection could be mitigated. However, many blood-borne pathogens, such as HIV and HBV, have been demonstrated to be highly transmissible, with minimal infectious doses (MIDs) close to the limits of detection (LOD) of all but the most sensitive NAT. Thus, we set out to determine the MID for ZIKV TT in order to demonstrate whether NAT cut-offs or the multi-log reduction of titers seen with PRT are sufficient each on their own, or together, to fully protect the blood supply.

STUDY DESIGN AND METHODS

Plasma samples, viruses, and standard assays

Zika virus RNA reactive blood donations in Puerto Rico were identified during routine screening. Upon notice of a reactive result, plasma units were frozen at −80°C, shipped to Creative Testing Solutions on dry ice, and stored at −80°C until required. Residual

volume from the routine NAT screening was used to characterize all reactive donations for viral load and IgM status[9]. Viral loads were established using a qRT-PCR assay and reported in international units (IU) per mL, with 1 IU/mL corresponding to 0.9 genome copies/mL[9]. Anti-ZIKV IgM serology was performed using the CDC MAC immunoassay, while Bio-Techne (Minneapolis, MN) and InBios (Seattle, WA) immunoassays were used for anti-ZIKV and anti-DENV IgG serology respectively. Neutralizing antibody titers were calculated using reporter virus particles (RVPs) from Integral Molecular (Philadelphia, PA)[13]. Viral infectivity was quantitated by plaquing on Vero cells[14].

Five reactive plasmas (PR1 - PR5) were selected to represent a range of titers and serostatus (Table 1). Similarly, an aliquot of frozen plasma was obtained from a previously described Zika virus RNA reactive blood donation in Brazil[5]. This aliquot was subsequently thawed and sub-aliquoted in DMEM (Gibco) supplemented with 5% fetal bovine serum and 1X glutamax (Gibco).

Inoculation of mice with ZIKV

Mouse experiments were performed with the approval and oversight of the Institutional Animal Care and Use Committee at Labcorp Early Development Laboratories Inc. (San Carlos, CA) under Animal Welfare Assurance A3367–01. Adult C57BL/6J mice were obtained from Jackson Laboratory (Sacramento, CA) and maintained and bred under ABSL-2 barrier conditions at Vitalant Research Institute as described[15]. The evening before infection, mice were transferred to an ABSL-3 laboratory and given pretreatment with 1 mg / mouse *InVivo*Plus anti-mouse interferon alpha/beta receptor subunit 1 (IFNAR-1) (clone MAR1–5A3; Bio-X-Cell; West Lebanon, NH) by intra-peritoneal (i.p.) injection. The following day, mice were infected with ZIKV given i.p. in 100 μL. Prior to use, serial dilutions of ZIKV were prepared for injection using phosphate buffered saline pH 7.8 containing 1% bovine serum albumin and 0.005% F-127 and snap frozen in aliquots. Infection was determined based on the presence of ZIKV RNA in small-volume blood samples collected by tail-tip clipping on day 2 post-infection and from terminal blood collections performed by orbital enucleation on day 5.

Inoculation of macaques with ZIKV

All experiments with macaques were performed at the California National Primate Research Center (CNPRC), University of California Davis, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Animal care was performed in compliance with the 2011 *Guide for the Care and Use of Laboratory Animals* provided by the Institute for Laboratory Animal Research. The animals in this study were healthy adult Indian-origin rhesus macaques (Macaca mulatta), 5 to 15 years of age, from the type D retrovirus-free, Herpes B virus free, SIV-free and simian lymphocyte tropic virus type 1-free colony, and confirmed to be antibody negative for WNV using a simian WNV immunoassay (Xpress Bio). Macaques were housed indoor in stainless steel cages (Lab Product, Inc.) whose sizing was scaled to the size of each animal, as per national standards, and were exposed to a 12-hour light/dark cycle, 64–84°F, and 30–70% room humidity. Animals had free access to water and received commercial chow (high protein diet, Ralston Purina Co.) and fresh produce supplements. The study was approved

by the Institutional Animal Care and Use Committee of the University of California, Davis. When necessary, including for plasma infusions, macaques were immobilized with ketamine HCl (Parke-Davis) at approximately 10 mg/kg and injected intramuscularly after overnight fasting. Blood samples were collected using venipuncture. At the end of the study, ZIKV-inoculated animals were euthanized with an overdose of pentobarbital, followed by necropsy with extensive tissue collection.

Macaque serology

Anti-ZIKV IgM and IgG antibody was determined using commercially available reagents (Xpress Bio, Frederick, MD). For IgM detection the anti-monkey IgG secondary antibody was replaced with horseradish peroxidase conjugated goat anti-monkey IgM (Kirkegaard and Perry, Gaithersburg, MD).

Pathogen reduction

18 mL of two ZIKV viremic plasma donations were aliquoted and dosed with amotosalen to a final concentration of approximately 150 μM. An aliquot from each plasma was collected following amotosalen addition (pre-illumination) and the remaining plasma volume was treated with a target 3.0 J/cm^2 UVA light dose. Amotosalen adsorption was not performed after treatment due to lack of volume. However, this is not expected to affect inactivation. No acute symptoms following transfusion into animals was observed. After UVA treatment, an aliquot from each unit was collected (post-illumination) and the pre- and post-illumination samples were kept on ice and tested the same day (i.e. without any extra freeze-thaw cycle) for *in vitro* infectivity by plaque assay. In addition, one and ten milliliter aliquots of each of the treated plasmas were immediately shipped on ice by same-morning courier service to the California National Primate Research Center (CNPRC) and transfused into two adult macaques (total of 4 animals), who were then sampled at regular intervals. Plasma samples harvested from transfused animals were tested using ZIKV RT-PCR (LOD=200 ZIKV RNA copies/mL) as previously described[14].

RESULTS

Characterization of donor plasma

To study the infectivity of plasma collected during the acute phase of ZIKV infection, we characterized a panel of ZIKV RNA reactive plasmas for viral load, flavivirus serology and *in vitro* infectivity (Table 1). Four plasmas collected from Puerto Rican blood donors (termed PR1 to 4) were identified during routine NAT screening, while a fifth plasma from Brazil (termed BZ) that was identified following a case of ZIKV TT[5]. Viral loads varied from 3.0×10^4 to 1.9×10^6 IU/mL.

Specific anti-ZIKV and cross-reactive flavivirus antibodies may impact the infectivity of RNA-reactive plasma. BZ and PR1 and PR2 were seronegative for both anti-ZIKV IgM and IgG, while PR3 was positive and PR4 was equivocal (see Table 1 footnote) for anti-ZIKV IgM only. Because of the extensive cross-reactivity between ZIKV and DENV, and the fact the plasmas were collected in DENV endemic regions, we also examined anti-DENV IgG. BZ and PR1 and PR2 were seronegative for DENV IgG, while PR3 and PR4 were positive.

PR4 was able to potently neutralize all four DENV serotypes, and weakly neutralized ZIKV. This may be due to either the equivocal anti-ZIKV IgM or cross-reactive anti-DENV responses. Surprisingly, given the ZIKV and DENV IgM and IgG seronegativity, BZ also neutralized ZIKV and all four DENV serotypes, although only weakly. It is possible that this donor had previous exposure to DENV in the distant past, or demonstrated some level of non-specific cross-reactivity.

In order to confirm that our *in vivo* infectivity results with human plasma were not impacted by handling required for routine plasma collection, we also collected plasma from an experimentally infected macaque and froze aliquots immediately. The animal was infected with 5,400 IU of RNA of BZ plasma, and blood was then collected on days 1, 4 and 10 post-infection (termed Macd1, Macd4 and Macd10 respectively). VL was detectable at all time-points, and peaked in the day 4 plasma at 1.0×10^6 IU/mL. Days 1 and 4 were seronegative, while day 10 plasma had detectable anti-ZIKV IgM but not IgG and demonstrated detectable neutralizing antibodies to ZIKV.

Plasma infectivity

BZ, PR1 and PR2, as well as Macd4 were titrated for *in vitro* infectivity using Vero cell plaque assays, with titers up to 6,400 PFU/mL. In comparison a minimally passaged stock of ZIKV isolated from the BZ plasma (termed TC) gave a titer 500-fold higher than the parental plasma (Table 1). However, when the relative infectivity was evaluated by calculating the ratio of RNA copies to PFUs, the parental plasma appeared much more infectious compared to the tissue-culture expanded virus (Figure 1A). Generally, the plasma virus was more infectious on a per genome basis, apart from the macaque plasma.

Serial dilutions of the human plasma, together with tissue-culture expanded virus, were performed in PBS containing BSA and a surfactant. These dilution panels were then delivered i.p. to mice, with at least 6 mice per dilution group. The mice were monitored at days 2 and 5 for ZIKV RNA in plasma, with the number of positive and negative mice in each group used to calculate 50% infectious doses based on international units of viral RNA. Generally the mice appeared much more susceptible than the cell line, with as low as 32 – 140 IU sufficient to infect 50% of mice for the various plasmas (Figure 1b). As with *in vitro* titration the BZ plasma was more infectious than the tissue-culture adapted stock.

Dilution panels of plasma were generated using uninfected macaque citrate plasma as a diluent, with two-fold, three-fold or half-log dilutions used for different experiments. Panels were snap frozen in aliquots (Figure 2). Starting with the BZ plasma dilution panel, dose escalation studies were performed in a macaque (animal number Rh-BZ1; Table 2). A dilution estimated to be below 1 PFU of *in vitro* infectivity, was infused and the animal monitored on days 3, 7 and 11 for viremia. If the days 3 and 7 samples were negative for ZIKV RNA, the next more concentrated dilution was thawed and infused on day 11. An aliquot of each thawed dilution was also titrated *in vitro* to enumerate input infectious virus in PFUs. The same general time course was followed until an animal became viremic. The first BZ plasma dilution that led to infection was the equivalent of 8,010 IU of RNA and 64 PFUs (Table 2 and Figure 3). A repeat experiment using fresh aliquots of the dilution panel resulted in infection from the preceding dilution (the equivalent of 2,700 IU of RNA and 21

PFUs) in animal Rh-BZ2 (Table 2). A third animal (Rh-BZ3) became infected when given a dose of 5,400 IU or 42 PFU diluted from a separate aliquot of the original BZ plasma in order to confirm infectivity (Figure 3). This animal was used for collection of plasma for subsequent macaque-to-macaque transfusions. Surprisingly, despite the *in vitro* and mouse data showing lower (Figure 1A), or similar (Figure 1B), relative infectivity compared to BZ, PR1 was significantly more infectious in macaques (Figure 1C) and the initial dilution selected for dose escalation, 248 IU RNA, was infectious in macaque Rh-PR1a (Table 2). Thus PR1 was repeated (animal Rh-PR1b), with infection resulting from 51 IU RNA. This dose (and the 248 IU dose) were below the LOD of the plaque assay (<1 Vero cell plaque), Back-calculation of the titer of 248 IU based on successful plaquing of a more concentrated dilution confirmed it was less than 1 PFU. The third seronegative plasma, PR2, resulted in infection after a dose similar to BZ - 2178 IU (animal Rh-PR2; Table 2; Figure 3). Back calculation led to an estimation of 5 PFU for this minimal infectious dose.

2 ml of Macd1 (day 1 plasma from animal Rh-BZ3) equalling ~125 IU of RNA followed sequencially by the remaining volume (8 mL) of was transfused into an uninfected animal (Rh-MacA). The total RNA content of the second infusion was approximately 500 IU (Figure 3). After the animal failed to become infected, an aliquot of Macd4 was thawed and diluted in uninfected macaque plasma to give the equivalent to 1,000 IU/mL. One mL was immediately infused and led to infection. On back calculation this was equivalent to just less than 1 PFU. In a second animal (Rh-MacB), after unsuccessful transfusion with seropositive plasma (see below), escalating doses of Macd4 resulted in no infection after 1,000 IU, but the animal became subsequently infected with a dose of 2,000 IU (Table 2; Figure 3).

Seropositive plasma

We also tested transfusion of large volumes of two seropositive plasma (PR3 and PR4). Despite high viral loads and only early humoral immune responses, these two plasmas were both non-infectious in tissue culture (Table 1) and to macaques after transfusion. In the case of PR3, doses of 9.5×10^5 IU (0.5 mL) and 9.5×10^6 IU (5 mL) were infused into macaques (Rh-PR3a and Rh-PR3b) without producing infection based on subsequent RNA testing, while 1×10^6 IU (2.3 mL) was not infectious for PR4 (animal Rh-PR4). All three animals also failed to seroconvert for IgM or IgG after inoculation (Figure 4A) and were demonstrated to be fully susceptible to ZIKV as a subsequent successful inoculation with PR1 (1.8×10^4 IU per animal) induced infection with viremias indistinguishable from those of naive animals (e.g. Rh-BZ3 and Rh-PR1a). Similarly, seropositive macaque plasma (Macd10) was also not infectious, although due to the low VL even transfusing all 10 mL of this plasma resulted in only 2,000 IU in the inoculum. However, the animal (Rh-MacB) was subsequently successfully infected with 2,000 IU of seronegative Macd4 (Figure 3). In order to evaluate the role of prior flavivirus infection of transmission, we performed dose escalation on PR5, a ZIKV RNA reactive, ZIKV IgM and IgG seronegative plasma with specific neutralizing antibodies to DENV-2 (Table 1). Infection was achieved with a dose of 3×10^4 IU RNA (Figure 4B).

Pathogen reduction

Finally we tested whether A/UVA PRT treatment of plasma was sufficient to fully prevent TT in macaques using high (PR1) and low (PR2) VL plasma. Plasmas were treated as described in the methods and infused into four macaques(Figure 5), with two macaques receiving an estimated 1.8×10^6 or 1.8×10^7 IU RNA of PR1 and two receiving 3×10^4 or 3×10^5 IU RNA of PR2. None of the four animals became viremic, nor developed serological responses to ZIKV. Five weeks after this initial transfusion, these animals were reinoculated with PR1 (1.8×10^4 IU per animal) and became infected, with viremias within the range of those observed for naive animals (Figure 4C).

DISCUSSION

We used plasma from ZIKV NAT reactive donors, as well as from an experimentally infected macaque, in tissue culture, immunodeficient mice, and rhesus macaques in order to define relative infectivity and to calculate the MID required for ZIKV TT. *In vitro* and immunocompromised mice demonstrated that ZIKV RNA reactive but seronegative plasmas were more infectious on a per international unit of viral RNA level compared to tissue-culture expanded virus derived from the Brazilian plasma. Anti-IFNAR treated mice in particular proved to be highly sensitive to TT, with ~40 IU of RNA (well below 1 PFU for both) proving to be infectious for BZ and PR1. This supports previous findings of the extreme susceptibility of immunocompromised mice[16].

In order to efficiently move to a larger animal model of TT, we performed dose escalation studies using serial dilutions of human plasma in rhesus macaques. Only the MID of the most infectious plasma (PR1, 51 IU RNA) came close to matching the MIDs from blood-borne pathogens such as HIV and SIV, where as few as 20 RNA copies from a pre-seroconversion acute ramp-up infection (similar to our Macd1 sample) was found to be infectious[17]. The MIDs for the three different plasma units were highly variable from a low of ≤51 IU to a high of ≤8,010 for PR1 and BZ respectively. PR2 fell between these two extremes with an MID of ≤2,178, as did a repeat of BZ (≤2,700) and a single dose challange of PR1 (≤248). It is not clear what the reasons for this variability were, however BZ did demonstrate a very weak degree of neutralization against ZIKV. Similarly, this donor had evidence from neutralization, but not ELISA, of previous DENV infection. Thus, it is possible that previous flavivirus infection in the donor impacts transmissibility. Indeed, a DENV seropositive plasma, lacking any specific ZIKV reactivity, had a MID four-fold higher than even BZ. However, we were only able to perform dose escalation of one DENV seropositive sample in one animal, limiting conclusions. This data also doesn't exclude the possibility that at certain titers, pre-existing anti-flavivirus antibodies to could enhance ZIKV TT. It is also possible that handling and shipping of these routine plasma collections also contributed to the variability. However, the fact that the MIDs in mice were similar for BZ and PR1 but were the most divergent in macaques, suggests something other than absolute infectivity underlies the differences in MIDs in macaques. The acute macaque plasma, for which handling was controlled, was subjected to freeze/thaw cycles in a similar manner to routine handling of fresh frozen plasma, resulting in an MID of either ≤1,000 or ≤2,000 IU of RNA. Other variables such as protein or lipid content or low levels of

specific or cross-reactive antibody and other immune factors may affect residual infectivity or transmissibility. Flaviviruses are rapidly cleared from the circulation, likely through multiple host mechanisms[18], limiting the window for establishing transmission. Thus, it may be that there is a great deal of innate variability in establishment of infection following TT, and the wide range of MIDs seen in macaques, should be taken as an indication of this.

As expected from previous studies[19], the dynamics of acute plasma viremia following infection were somewhat delayed in animals given doses at, or close to, the MID compared to animals given very large doses (Figure 6). None of the infected animals had any apparent clinical signs during ZIKV infection. In addition, when animals were euthanized, no significant gross pathological lesions were found. These findings are consistent with previously described findings in ZIKV-infected rhesus macaques [14].

Despite the variability of MIDs, the impact of NAT detection and PRT on the risk of TT can be evaluated. For example, an MID of ~8,000 IU RNA in a 200 ml unit of plasma would be approximately 40 IU/ml. The 95% LOD of the most sensitive NAT assays are~14 IU/mL[20], which would equate to ~84 or ~224 IU/mL in minipools of 6 or 16 respectively. Thus, even the highest MID is potentially on the edge of detectability for minipools. This conclusion relies on the assumption that when the MID is dispersed throughout an entire transfused unit it remains as infectious as the limited volume allowed for in our macaque experiments.

Simulated minipool testing (1 in 6 dilution) on ZIKV reactive donations in Puerto Rico found that 30% (96 out of 319) of donations would not have been identified as reactive if tested only by minipool[9]. The majority (81/96) of the ID-NAT only reactive samples were also seropositive for anti-ZIKV antibodies, indicating they were from the tail-end of acute infection. Thus, we also tested TT of plasma collected from donors that retained relatively high viral loads, but were ZIKV-IgM positive. Despite administration of many multiples of the calculated MID in macaques, these seropositive plasmas were not infectious. This is particularly important given antibody-dependent enhancement of ZIKV infection has been reported both *in vitro* and *in vivo*[21]. A model of ZIKV TT using pregnant IFNAR-deficient mice as recipients and infected mice as donors found plasma was infectious only if it was collected at an early stage of infection, despite late stage plasma retaining significant VL and *in vitro* infectivity[22]. Although seroconversion in these samples was not analyzed, it again highlights the greatest threat for TT comes from early acute phase plasma.

Complete inactivation of infectivity in macaques was demonstrated using amotosalen and ultraviolet A illumination in the INTERCEPT system, with a dose of 1.8×10^7 IU RNA of PR1 failing to cause infection after inactivation. Thus, the level of inactivation observed is many orders of magnitude above the MID calculated for PR1 (≤51 or ≤248 IUs depending on the animal). Even for the less infectious PR2, a titer over 3 logs above the MID for the plasma was successfully inactivated.

The highest VLs in seronegative blood donors during the epidemic approached 10^8 IU RNA/mL[23], with 2% (4 out of 209) of Puerto Rican ZIKV RNA reactive/seronegative donors identified in 2016/17 having viral loads above 10^7 IU [9]. Using an average of the *in vitro* infectivity to VL ratio for our three human plasmas (183 IU RNA/PFU), ~10^8 IU of ZIKV

RNA would equate to ~5 × 10^5 PFU/mL, although in the current and previous[14] studies it is important to note that RNA/PFU ratios are not consistent over the course of acute infection. Given the lowest MID we observed (51 IU RNA) equates to less than 1 PFU, pathogen reduction treatment would need to reduce *in vitro* infectivity in a full plasma unit by up to 6 orders of magnitude. The constraints of transfusing large volumes of human plasma into macaques prevent the testing of large volumes and titers of treated plasma; however the results of all of the various arms of this study suggest that only the absolute highest titer, seronegative plasma has any remaining possibility of fully evading PRT.

The risk of TT is further complicated by the fact that detection of ZIKV RNA persists in non-plasma components, such as packed red blood cells[24], raising the possibility that such components may remain infectious after the plasma detection window. We did not have access to stored human components other than plasma. Thus, whether persistent viremia associated with blood components is infectious remains an unanswered question, although we suspect that as persistence occurs after seroconversion and viral RNA levels are low, the efficiency of ZIKV TT from other components would be very low.

Overall, these studies support the fact that measures put in place to secure the safety of the blood supply during and after the 2016 ZIKV epidemic were largely sufficient to greatly reduce, if not eliminate, the risk of ZIKV transmission to at-risk individuals. However, these studies suggest that minipool-NAT screening may elevate the risk of undetected ZIKV RNA reactive units above the threshold where they may cause infection. Our studies also support the use of pathogen reduction technologies to limit the TT risk of plasma units with high RNA reactivity. Considering that there is no approved ZIKV vaccine yet, the importance of these measures will be emphasized during future outbreaks of ZIKV.

Acknowledgements:

This study was funded in whole or in part by Federal funds from the National Heart, Lung, and Blood Institute, National Institutes of Health, Department of Health and Human Services (contract number HHSN268201100001I. Additional funding was provided by a grant from Grifols Diagnostic Solutions, Inc. Research reported in this publication was supported by the Office of Research Infrastructure Program, Office of The Director, National Institutes of Health under Award Number P51OD011107.

We thank the expert assistance of the following staff at CNPRC: Vanessa Bakula, Miles Christensen, Amanda Carpenter, Bryson Halley, Peter Nham, Amal Sayedi, Jodie Usachenko, Wilhelm von Morgenland, Jennifer Watanabe, and the staff of Animal Care and Research Services, Clinical Laboratories, Primate Medicine and Pathology. We also thank Christine Crasto for help developing figures.

REFERENCES

1. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastère S, Valour F, et al. Zika virus infection complicated by Guillain-Barré syndrome case report French Polynesia, December 2013. Eurosurveillance [Internet]. March 2014;19(9). Available at: https://doi.org/10.2807%2F1560-7917.es2014.19.9.20720
2. Fauci AS, Morens DM. Zika Virus in the Americas–Yet Another Arbovirus Threat.. N Engl J Med. 2016;374:601–4. [PubMed: 26761185]
3. Heymann DL, Hodgson A, Sall AA, Freedman DO, Staples JE, Althabe F, et al. Zika virus and microcephaly: why is this situation a PHEIC?. Lancet. 2016;387:719–21. [PubMed: 26876373]
4. Petersen LR, Busch MP. Transfusion-transmitted arboviruses.. Vox Sang. 2010;98:495–503. [PubMed: 19951309]

5. Barjas-Castro ML, Angerami RN, Cunha MS, Suzuki A, Nogueira JS, Rocco IM, et al. Probable transfusion-transmitted Zika virus in Brazil.. Transfusion. 2016;56:1684–8. [PubMed: 27329551]

6. Motta IJ, Spencer BR, Cordeiro da SSG, Arruda MB, Dobbin JA, Gonzaga YB, et al. Evidence for Transmission of Zika Virus by Platelet Transfusion.. N Engl J Med. 2016;375:1101–3. [PubMed: 27532622]

7. Musso D, Nhan T, Robin E, Roche C, Bierlaire D, Zisou K, et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014.. Euro Surveill. 2014;19.

8. Kuehnert MJ, Basavaraju SV, Moseley RR, Pate LL, Galel SA, Williamson PC, et al. Screening of Blood Donations for Zika Virus Infection - Puerto Rico, April 3-June 11, 2016.. MMWR Morb Mortal Wkly Rep. 2016;65:627–8. [PubMed: 27337368]

9. Williamson PC, Biggerstaff BJ, Simmons G, Stone M, Winkelman V, Latoni G, et al. Evolving viral and serological stages of Zika virus RNA-positive blood donors and estimation of incidence of infection during the 2016 Puerto Rican Zika epidemic: an observational cohort study.. Lancet Infect Dis. 2020;20:1437–45. [PubMed: 32673594]

10. Williamson PC, Linnen JM, Kessler DA, Shaz BH, Kamel H, Vassallo RR, et al. First cases of Zika virus-infected US blood donors outside states with areas of active transmission.. Transfusion. 2017;57:770–8. [PubMed: 28229475]

11. Galel SA, Williamson PC, Busch MP, Stanek D, Bakkour S, Stone M, et al. First Zika-positive donations in the continental United States.. Transfusion. 2017;57:762–9. [PubMed: 28164310]

12. Donor Screening Recommendations to Reduce the Risk of Transmission of Zika Virus by Human Cells, Tissues, and Cellular and Tissue-Based Products - Guidance for Industry [Internet]. https://www.fda.gov/files/vaccines,%20blood%20&%20biologics/published/Donor-Screening-Recommendations-to-Reduce-the-Risk-of-Transmission-of-Zika-Virus-by-Human-Cells--Tissues--and-Cellular-and-Tissue-Based-Products--Guidance-for-Industry.pdf; Available at: https://www.fda.gov/files/vaccines,%20blood%20&%20biologics/published/Donor-Screening-Recommendations-to-Reduce-the-Risk-of-Transmission-of-Zika-Virus-by-Human-Cells--Tissues--and-Cellular-and-Tissue-Based-Products--Guidance-for-Industry.pdf

13. Whitbeck JC, Thomas A, Kadash-Edmondson K, Grinyo-Escuer A, Stafford LJ, Cheng C, et al. Antigenicity, stability, and reproducibility of Zika reporter virus particles for long-term applications.. PLoS Negl Trop Dis. 2020;14:e0008730.

14. Coffey LL, Pesavento PA, Keesler RI, Singapuri A, Watanabe J, Watanabe R, et al. Zika Virus Tissue and Blood Compartmentalization in Acute Infection of Rhesus Macaques.. PLoS One. 2017;12:e0171148.

15. Blessinger SA, Tran JQ, Jackman RP, Gilfanova R, Rittenhouse J, Gutierrez AG, et al. Immunodeficient mice are better for modeling the transfusion of human blood components than wild-type mice.. PLoS One. 2020;15:e0237106.

16. Aliota MT, Caine EA, Walker EC, Larkin KE, Camacho E, Osorio JE. Characterization of Lethal Zika Virus Infection in AG129 Mice.. PLoS Negl Trop Dis. 2016;10:e0004682.

17. Ma ZM, Stone M, Piatak MJ, Schweighardt B, Haigwood NL, Montefiori D, et al. High specific infectivity of plasma virus from the pre-ramp-up and ramp-up stages of acute simian immunodeficiency virus infection.. J Virol. 2009;83:3288–97. [PubMed: 19129448]

18. Fuchs A, Lin TY, Beasley DW, Stover CM, Schwaeble WJ, Pierson TC, et al. Direct complement restriction of flavivirus infection requires glycan recognition by mannose-binding lectin.. Cell Host Microbe. 2010;8:186–95. [PubMed: 20709295]

19. Best K, Barouch DH, Guedj J, Ribeiro RM, Perelson AS. Zika virus dynamics: Effects of inoculum dose, the innate immune response and viral interference.. PLoS Comput Biol. 2021;17:e1008564.

20. Stone M, Lanteri MC, Bakkour S, Deng X, Galel SA, Linnen JM, et al. Relative analytical sensitivity of donor nucleic acid amplification technology screening and diagnostic real-time polymerase chain reaction assays for detection of Zika virus RNA.. Transfusion. 2017;57:734–47. [PubMed: 28194799]

21. Pinto AK, Hassert M, Han X, Barker D, Carnelley T, Branche E, et al. The Ability of Zika virus Intravenous Immunoglobulin to Protect From or Enhance Zika Virus

Disease. Frontiers in Immunology [Internet]. September 2021;12. Available at: https://doi.org/10.3389%2Ffimmu.2021.717425

22. Tai W, Voronin D, Chen J, Bao W, Kessler DA, Shaz B, et al. Transfusion-Transmitted Zika Virus Infection in Pregnant Mice Leads to Broad Tissue Tropism With Severe Placental Damage and Fetal Demise.. Front Microbiol. 2019;10:29. [PubMed: 30728813]
23. Stramer SL, Lanteri MC, Brodsky JP, Foster GA, Krysztof DE, Groves JA, et al. Mitigating the risk of transfusion-transmitted infections with vector-borne agents solely by means of pathogen reduction.. Transfusion. 2022;62:1388–98. [PubMed: 35726756]
24. Stone M, Bakkour S, Lanteri MC, Brambilla D, Simmons G, Bruhn R, et al. Zika virus RNA and IgM persistence in blood compartments and body fluids: a prospective observational study.. Lancet Infect Dis. 2020;20:1446–56. [PubMed: 32673593]

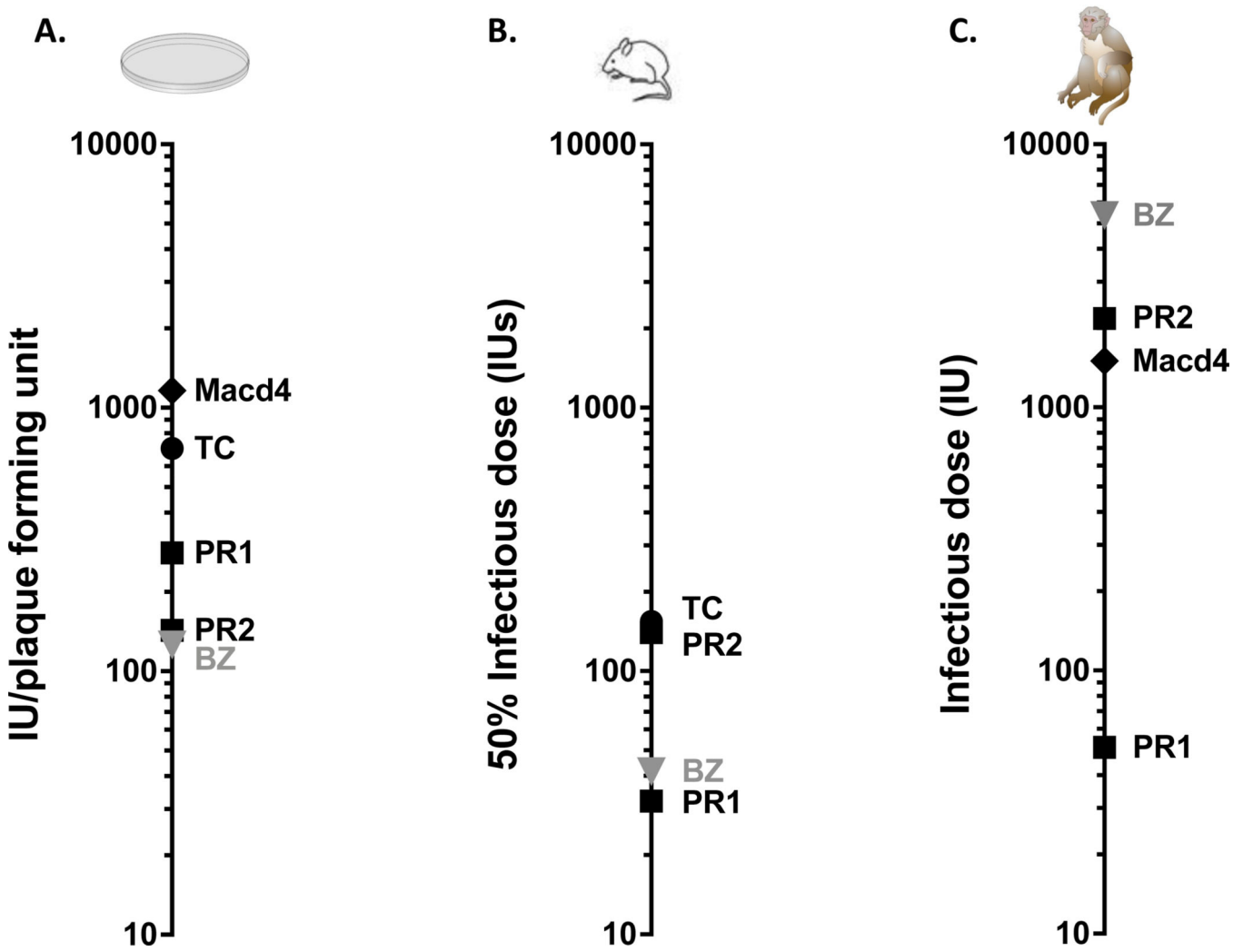

Figure 1. Relative infectivity in tissue culture, mice and macaques.

Serial dilutions of tissue-culture expanded BZ isolate (TC), Brazilian plasma (BZ), Puerto Rican plasmas 1 and 2 (PR1, PR2) and macaque plasma (Macd4) were used to infect Vero cells, anti-IFNAR treated mice or macaques. **A.** Plaque forming units (PFU) per ml for each plasma or TC isolate were calculated on Vero cells and a ratio of international units (IU) of RNA over plaque forming units calculated. **B.** Serial dilutions of plasmas or TC isolate were made and infused into mice i.p., with at least six mice per dilution. Mice were scored positive based on plasma viral load (VL) at day 2 and 5 and 50% infectious doses calculated based on the input IU. **C.** Escalating doses of serial dilutions of plasmas were infused into macaques and they were monitored for subsequent infection. The infecting dose is displayed as IU of RNA.

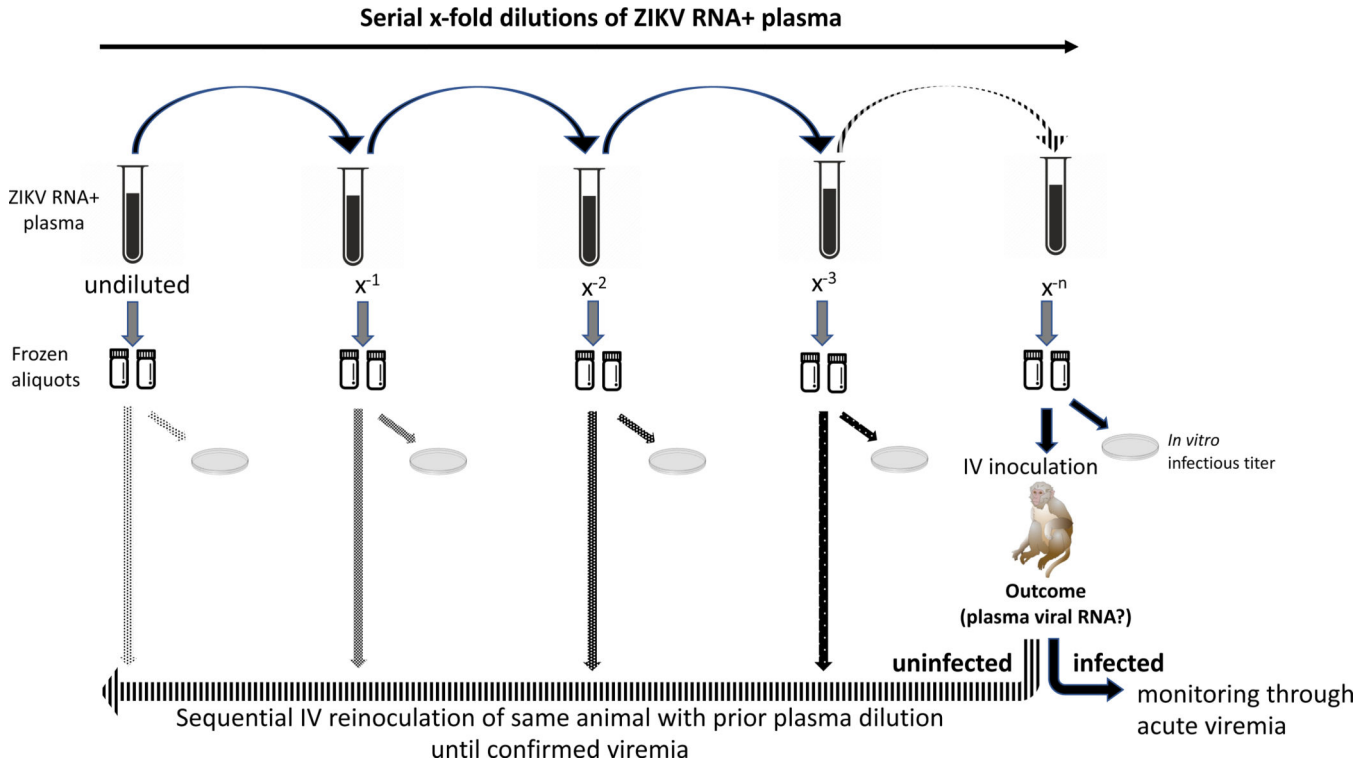

Figure 2.

Schematic of dilutions and inoculations for determining *in vitro* and *in vivo* titers in macaques.

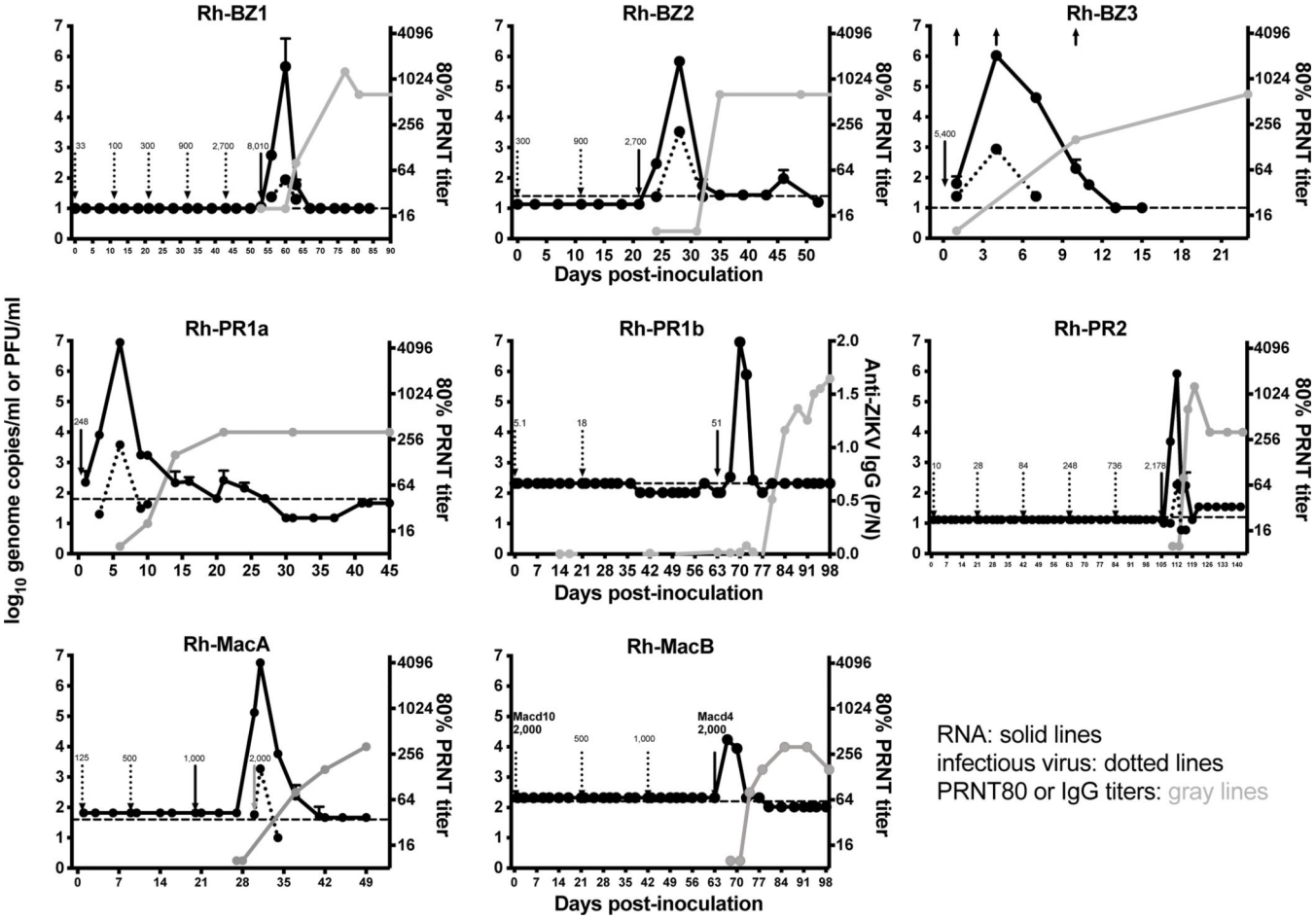

Figure 3. Kinetics of transfusion-transmission of ZIKV plasma in macaques.

Inoculations are denoted in downward arrows with a solid line for the infecting inoculation and dotted for non-infecting. Inoculum doses are in IU of RNA. Last day tested indicates last sampling time before the animal was euthanized. Dotted line shows limit of detection for genome copies. Viral loads are plotted on the left Y axis while PRNT80 titers are shown on the right Y axis for all except Rh-PR1b where PRNT was not performed, and IgG serology is displayed. Upward arrows denote collection of Macd1, Macd4 and Macd10 plasma. Rh-MacA and Rh-MacB were inoculated first with the entire volume of Macd1 or Macd10 respectively. Subsequent inoculations were with a dilution panel made with Macd4. Gray arrow represents an additional inoculation that was given before viral load results identified the animal as already infected.

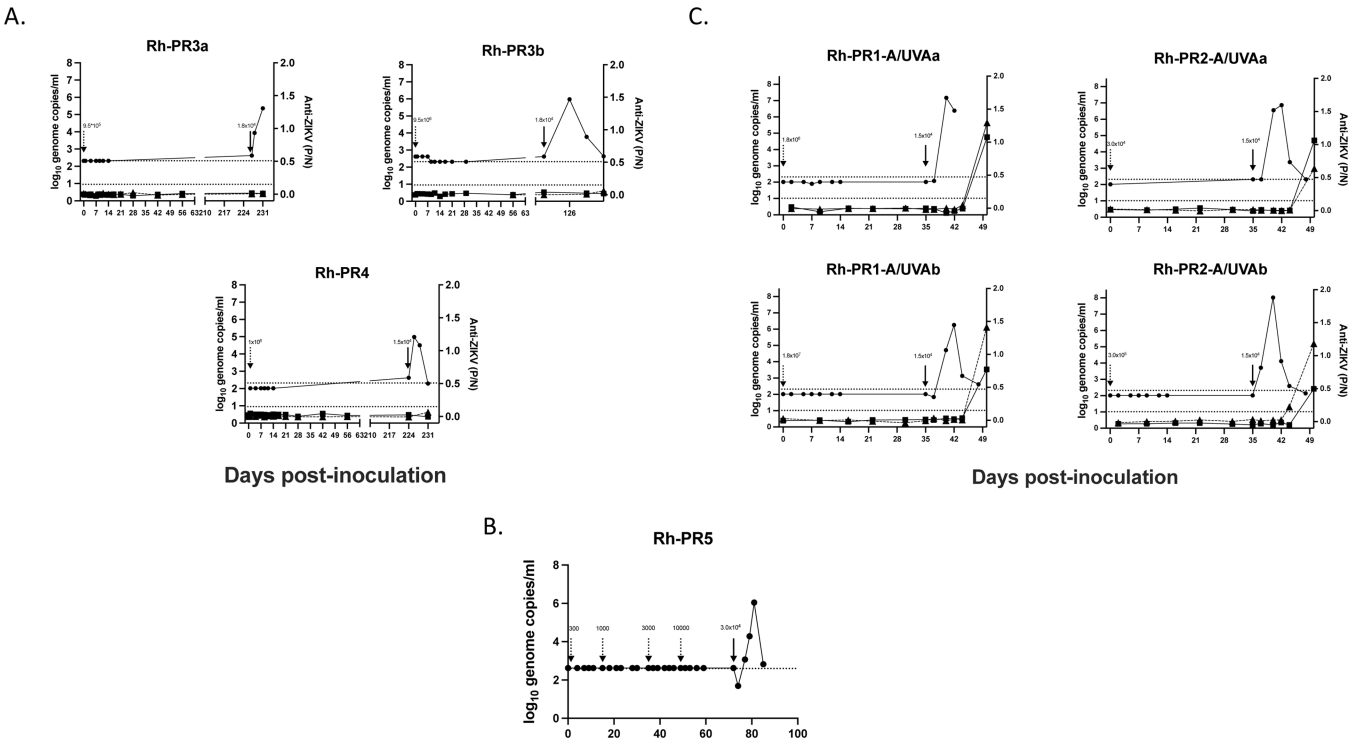

Figure 4. Timecourse of transfusion-transmission of ZIKV experiments in macaques.

(A) shows animals Rh-PR3a, Rh-PR3b and Rh-PR4 initially transfused with ZIKV RNA reactive, but seropositive human plasma. Arrows denote inoculations, with dotted for the seropositive plasma inoculation (PR3; top two graphs or PR4; bottom graph) and a solid line for subsequent infecting PR1 plasma at 1.8×10^4 IU. **(B)** shows does escalation of plasma PR5 in animal Rh-PR5. Inoculations are denoted in downward arrows with a solid line for the infecting inoculation and dotted for non-infecting. **(C)** shows the pathogen reduction experiments with ZIKV RNA reactive, seronegative plasma. The top two graphs represent 1 mL transfusions of inactivated plasma, while the bottom two graphs represent 10 ml transfusions. For all graphs inoculum doses are in IU of RNA. Upper dotted line shows limit of detection for genome copies, while the lower dotted line denotes cut-off for serology. Viral loads are plotted on the left Y axis (circles, solid line) while anti-ZIKV serology is on the right, with IgM (triangles, dotted line) and IgG (squares, solid line). Last day tested indicates last sampling time before the animal was euthanized.

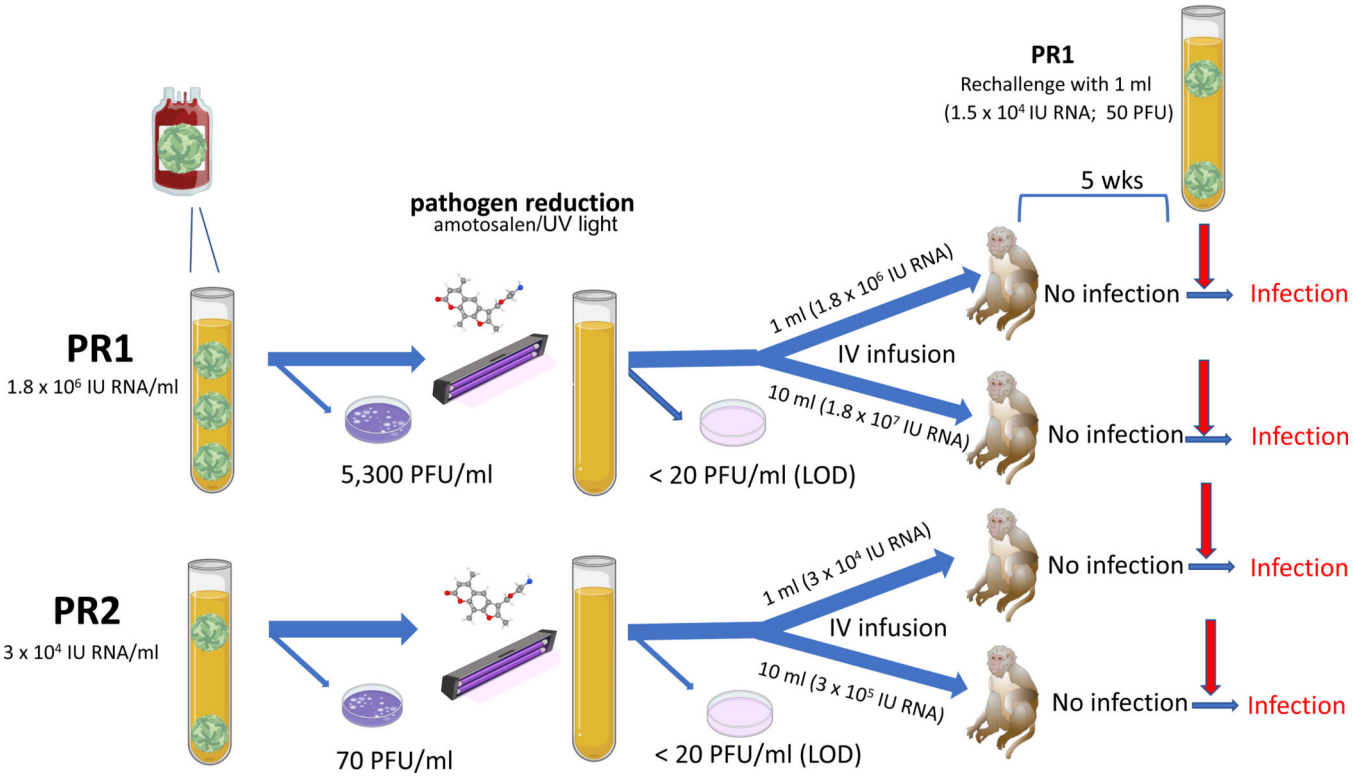

Figure 5. Schematic of pathogen reduction experiments and outcomes.

PR1 and PR2 plasma were thawed and an aliquot was placed on ice for same day *in vitro* titering, while the remainder was subjected to A/UVA PRT treatment. Following treatment a further aliquot was placed on ice for same day *in vitro* titering. The remaining plasma was separated into 1 and 10 mL aliquots for both PR1 and PR2 and inoculated into macaques. The animals were regularly monitored for viremia over 14 days and for serological responses over the course of five weeks. After 5 week no animal demonstrated evidence of infection and were therefore reinoculated with PR1 plasma (1.8×10^4 IU per animal).

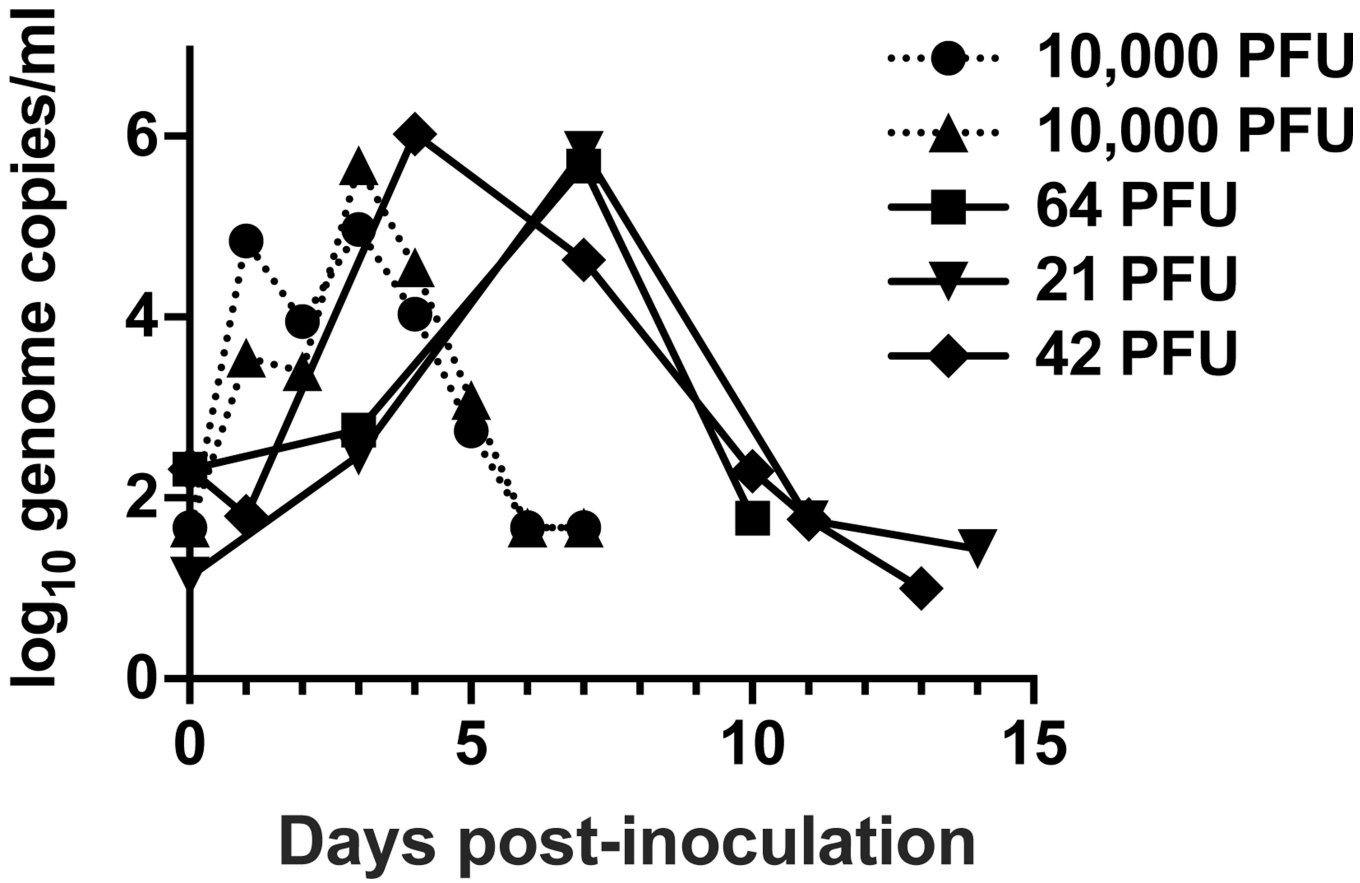

Figure 6. Dynamics of acute macaque infection.

Viremias of animals after the infecting inoculation using either BZ plasma at, or close to, the MID (Solid lines) or high titer tissue-culture expanded BZ (Dashed lines). Data for the 64, 21 and 42 plaque-forming unit (PFU) doses are from animals Rh-BZ1, Rh-BZ2 and Rh-BZ3 respectively. Data for high titer inoculations are from Coffey et al, 2017. PLoS One 12(1):e0171148.

Table 1.

Characteristics of Human and Macaque plasma samples

Code	Sample type	Origin	ZIKV RNA VL[a] (IU/mL)	ZIKV Titer (PFU/mL)	ZIKV IgM[b] (P/N)	ZIKV IgG[c] (OD)	DENV IgG (P/N)	ZIKV NT80[d]	DENV1 NT80	DENV2 NT80	DENV3 NT80	DENV4 NT80
TC	Tissue culture	Brazil[e]	2.1×10^9	3.0×10^6	N/A[f]	N/A	N/A	N/A	N/A	N/A	N/A	N/A
BZ	Human plasma	Brazil	7.3×10^5	5.8×10^3	1.07 [Neg]	−0.01 [Neg]	1.10 [Neg]	14	18	50	37	23
PR1	Human plasma	Puerto Rico	1.8×10^6	6.4×10^3	1.21 [Neg]	−0.02 [Neg]	1.10 [Neg]	<10	<10	<10	<10	<10
PR2	Human plasma	Puerto Rico	3.0×10^4	210	1.01 [Neg]	−0.04 [Neg]	1.18 [Neg]	<10	<10	<10	<10	<10
PR3	Human plasma	Puerto Rico	1.9×10^6	<10	3.27 [Pos]	−0.02 [Neg]	2.56 [Pos]	<10	42	86	33	92
PR4	Human plasma	Puerto Rico	4.5×10^5	<20	2.20 [Equiv][h]	0.04 [Neg]	3.55 [Pos]	34	803	2272	709	612
PR5	Human plasma	Puerto Rico	6.4×10^4	ND	1.26 [Neg]	−0.09 [Neg]	2.78 [Pos]	<10	<10	486	<10	<10
Macd1	Macaque plasma	Brazil[e]	6.3×10^1	<20	0.05 [Neg]	0.05 [Neg]	ND[f]	<20	ND	ND	ND	ND
Macd4	Macaque plasma	Brazil	1.0×10^6	860	−0.02 [Neg]	0.06 [Neg]	ND	ND	ND	ND	ND	ND
Macd10	Macaque plasma	Brazil	2.0×10^2	ND[g]	0.80 [Pos]	0.09 [Neg]	ND	160	ND	ND	ND	ND

[a] VL = viral load in international units (IU) per mL

[b] Human samples run with CDC MAC ELISA. Macaque samples run with Simian ZIKV NS1 IgM assay (Xpress Bio).

[c] Human samples run with ZIKV NS1 IgG assay (Biotechne). Macaque samples run with Simian ZIKV NS1 IgG assay (Xpress Bio).

[d] NT80 = 80% Neutralization titer (dilution of plasma required to give a 80% reduction in titer) calculated using reporter virus particles for human samples and plaquing for macaque samples

[e] Tissue-culture expanded virus was isolated from plasma BZ, while plasmas Mac1, Mac2 and Mac3 were collected on day 1, 4 and 10 respectively, from an animal inoculated with 5,400 RNA IUs of plasma BZ.

[f] N/A = not applicable; ND = Not done

[g] Titer was not done on this day 10 plasma, however a sample from the donor animal was below the limit of detection (23 PFU/ml) for day 7

[h] Equiv = equivocal (P/N of 2.00 to <3.00) on CDC MAC ELISA as per EUA instructions for use

Table 2.

Minimal Infectious Doses of Human and Macaque Seronegative Plasmas transfused into Rhesus Macaques

Animal	Plasma	Previous dose[a]		Minimal infectious dose[b]	
		RNA IU/dose	PFU/dose[c]	RNA IU/dose	PFU/dose
Rh-BZ1	BZ	2,700	21	8,010	64
Rh-BZ2	BZ	900	7	2,700	21
Rh-PR1a	PR1	N/A	N/A	248	<1
Rh-PR1b	PR1	18	<1	51	<1
Rh-PR2	PR2	736	1.7	2178	5
Rh-MacA	Macd1/Macd4	500	<1	1,000	<1
Rh-MacB	Macd4	1,000	<1	2,000	1.8

[a]= previous dose in the dilution panel, not leading to infection

[b]= dose leading to infection

[c]=pfu above the LOD of 20 are from titrations of the actual input, below the LOD are estimations based on a lower dilution in the panel

N/A – not applicable