

DISCLAIMER

This paper was submitted to the *Bulletin of the World Health Organization* and was posted to the Zika open site, according to the protocol for public health emergencies for international concern as described in Christopher Dye et al. (<http://dx.doi.org/10.2471/BLT.16.170860>).

The information herein is available for unrestricted use, distribution and reproduction in any medium, provided that the original work is properly cited as indicated by the Creative Commons Attribution 3.0 Intergovernmental Organizations licence (CC BY IGO 3.0).

RECOMMENDED CITATION

Lessler JT, Ott CT, Carcelen AC, Konikoff JM, Williamson J, Bi Q et al. Times to key events in the course of Zika infection and their implications: a systematic review and pooled analysis [Submitted]. *Bull World Health Organ E-pub*: 1 Apr 2016. doi: <http://dx.doi.org/10.2471/BLT.16.174540>

Times to key events in the course of Zika infection and their implications: a systematic review and pooled analysis

Justin T Lessler,^a Cassandra T Ott,^a Andrea C Carcelen,^a
Jacob M Konikoff,^a Joe Williamson,^a Qifang Bi,^a Lauren M Kucirka,^b
Derek AT Cummings,^c Nicholas G Reich^d & Lelia H Chaisson^a

^a Johns Hopkins Bloomberg School of Public Health, 615 N Wolfe Street, Baltimore, MD 21205, United States of America.

^b Johns Hopkins University School of Medicine, 720 Rutland Avenue, Baltimore, MD 21205, USA.

^c University of Florida, 2055 Mowry Road, Gainesville, FL 32610, USA.

^d School of Public Health and Health Sciences, University of Massachusetts Amherst, Amherst, MA 01003, USA.

Correspondence to Justin Lessler (email: justin@jhu.edu).

(Submitted: 31 March 2016 – Published online: 1 April 2016)

ABSTRACT

Objective

Evidence suggests that Zika virus has driven a 10-fold increase in babies born with microcephaly in Brazil, prompting the WHO to declare a Public Health Emergency of International Concern. However, little is known about the natural history of infection. These data are critical for implementing surveillance and control measures such as protecting the blood supply.

Methods

We conducted a systematic review and pooled analysis to estimate the distribution of times from Zika infection to symptom onset, seroconversion, and viral clearance, and analyzed their implications for surveillance and blood supply safety.

Findings

Based on 25 cases, we estimate the median incubation period of Zika virus infection is 5.9 days (95% CI: 4.4-7.6), and that 95% of those who do develop symptoms will do so by 11.1 days post-infection (95% CI: 7.6-18.0). On average seroconversion occurs 9.0 days (95% CI, 7.0-11.6) after infection, and virus is detectable in blood for 9.9 days (95% CI: 6.8-21.4). In 5% of cases detectable virus persists for over 18.9 days (95% CI: 12.6-79.5). The baseline (no screening) risk of a Zika infected blood donation increases by approximately 1 in 10,000 for every 1 per 100,000 person-days increase in Zika incidence. Symptom based screening reduces this by 7% (RR 0.93, 95% CI 0.86-0.99), and antibody screening by 29% (RR 0.71, 95% CI: 0.28-0.88).

Conclusions

Symptom or antibody-based surveillance can do little to reduce the risk of Zika contaminated blood donations. High incidence areas may consider PCR testing to identify lots safe for use in pregnant women.

RESEARCH IN CONTEXT

There is strong evidence that Zika virus outbreaks are driving significant increases in microcephaly rates, prompting international efforts to control spread and protect pregnant women. Little is known about the natural history of infection including the incubation period, time to seroconversion, and time to viral clearance. These parameters are key in designing effective surveillance and control measures. The WHO states the incubation period of Zika virus is unclear, but likely "a few days." Likewise, the US Centers for Disease Control and Prevention (CDC) states that the incubation period of Zika is unknown but probably "a few days to a week", and the European Centre for Disease Prevention and Control (ECDC) estimates 3-12 days. These estimates are based predominately on what is known about other arboviruses.

This study uses all available published reports of Zika virus infections containing sufficient information to inform the time to key events in Zika's natural history including symptom onset, seroconversion and viral clearance. We find that, while only one in five people infected with Zika will develop symptoms, those that do will do so on average six days after infection. Antibodies against Zika virus become detectable in the blood on average nine days after infection, and virus is recoverable from the blood for an average of ten days. Only one out of every one-hundred people will have virus detectable in their blood 24 days or longer after infection, though this value is uncertain due to limited evidence. Given these estimates, screening potential blood donors based on symptoms or serologic testing of donated blood would do little to protect the blood supply, reducing the risk of an infected donation by at most 30%. Direct testing for Zika virus in blood donations in areas with active circulation, and conservative waiting periods based upon the

estimated distribution of time to viral clearance for travelers, may be the best way to ensure blood supply safety.

INTRODUCTION

The explosion of Zika cases in Central and South America, combined with growing evidence that the virus is responsible for an epidemic of microcephaly in Brazil, has prompted the World Health Organization (WHO) to declare a Public Health Emergency of International Concern (1). As of February 29, 2016, there have been at least a half-million Zika virus infections in the Americas (2, 3). Although clinical disease is generally mild or asymptomatic (4), there is increasing evidence of a link between Zika virus infection and severe microcephaly in infants born to women infected during pregnancy, including a ten-fold increase in microcephaly cases in Brazil in the wake of the 2015 Zika epidemic (5). Zika virus infection has been linked to Guillain-Barre in adults (5, 6).

The severity of these complications highlights the need to protect pregnant women from infection and to ensure that blood supplies remain safe both in areas experiencing ongoing Zika virus transmission and in locations with travelers returning from affected areas. That large proportion of Zika infections that remain asymptomatic (4), inadequacy of current diagnostics, and uncertainties regarding the duration of viremia and viral shedding have raised concerns about the potential threat of transmission through blood transfusion. In a 2013-2014 outbreak in French Polynesia, researchers found that 3% of asymptomatic blood donors were infected with Zika virus (7), and several cases of possible Zika transmission through blood transfusion are currently being investigated in Brazil (8). As a result, some agencies now recommend halting blood donations in areas with active Zika transmission (9, 10). If implemented, these bans could result in severe blood supply shortages. Research to determine the duration of viremia and time to antibody seroconversion is therefore vital to quantify the risk to blood supplies, and develop efficient strategies for protection. Furthermore, more detailed estimates of key distributions in the natural history of Zika virus infection, including the incubation period and probable infectious period, are essential to designing evidence based surveillance systems and informing public health policy (11, 12).

In order to better characterize the natural history of Zika infection and inform disease prevention, surveillance, and blood supply safety, we performed a systematic literature review and pooled analysis of available data to estimate the incubation period, time to seroconversion, and length of shedding of Zika virus in infected populations.

METHODS

The systematic review was conducted according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (13) and the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines (14) where applicable (see Supplemental Material).

Search strategy and selection criteria

We searched PubMed, Scopus, and Web of Science on February 8, 2016 for all publications containing the word "Zika" in any field, with no restriction on date of publication or language. The search was updated on February 25, 2016 to identify additional relevant publications.

We included publications that provided information on time of exposure to Zika virus and 1) time of symptom onset, 2) time of sample collection for virologic Zika virus testing (e.g. PCR or culture) and test results (positive/negative), and/or 3) time of collection of samples for antibody testing and test results (positive/negative). We excluded publications if they did not provide sufficient information to determine a bounded time of exposure to Zika virus, contained no data from humans, were not in English or French, or reported only perinatal transmission of Zika virus.

Two reviewers (CTO, LHC, JW, AC) independently screened titles and abstracts for relevance. We excluded publications from full text review if they were not about Zika virus or if they definitively met one of the exclusion criteria. Two reviewers (CTO, LHC, JW, AC) independently performed full text reviews to identify publications with sufficient data for analysis; we contacted authors via email to obtain additional information for publications that were relevant but did not provide sufficient data for analysis. Discrepancies were resolved by discussion and consensus.

Data abstraction

Two reviewers (CTO, LHC, JW, AC) independently abstracted data using a standardized form and resolved discrepancies by discussion and consensus. We abstracted data necessary to estimate: 1) the incubation period of Zika virus, 2) the time and duration of viral shedding, and 3) the time to antibody seropositivity. We reviewed text, tables, and figures for information that allowed us to bound the time of: 1) exposure to Zika virus, 2) symptom onset, 3) collection of samples for Zika virus testing, and 4) collection of samples for antibody testing. For all virologic and serologic samples, the reported test result was recorded, and IgM specific serologies were noted when available. When possible, the exact timing of events was used, otherwise timing of the event was bounded based upon available information (e.g., travel dates to Zika endemic regions). We further recorded basic demographic characteristics, the type of sample collected (e.g., blood, urine), and, when available, the mode of transmission.

Extracted data was used to construct a data set bounding the time of key events. The time of Zika infection was bounded by the earliest and latest potential time of Zika virus exposure consistent with the case report. When no latest time of exposure could be determined (e.g., the case developed symptoms while in a Zika endemic area) the latest possible time of symptom onset was considered to be the latest possible time of exposure, the most conservative assumption we could make. Time of symptom onset was bounded based on the case report, and in most cases was specified to the nearest day. The earliest possible time of seroconversion was considered to be immediately after the last negative serological test, and the latest possible time of seroconversion was immediately before the first positive serological test. If there was only a positive serological test, the earliest possible time of seroconversion was considered to be the same as the earliest possible time of exposure; when only negative tests were performed, time to seroconversion was considered to be right censored. Similarly, the earliest possible time of viral clearance was the time of the last positive test (by PCR or viral culture), and the latest was the time of the first subsequent negative test. Missing virologic tests were treated the same as in the serological data.

Statistical Methods - Pooled Analysis

Bounding periods were used to construct doubly interval censored data sets for each distribution (15), and distributions were fit using an adaptation of techniques previously described (15, 16). Briefly, MCMC techniques were used to simultaneously fit the incubation period distribution (log-normal), distribution of time to IgM seroconversion (Weibull), and time to viral clearance (Weibull) to the doubly interval censored data. Given a time of infection the distributions were considered to be independent. The mean incubation period, and the times by which we expect 5%, 25%, 50%, 75%, and 95% of those who do develop Zika symptoms to become symptomatic were estimated. Full details are available in the supplemental appendix.

Statistical Methods - Blood Supply Safety

The impact of key distributions on blood supply safety was calculated assuming a constant incidence rate. The number of possibly infected donors per 100,000 if no screening occurs was calculated as: (daily incidence rate per 100,000) \times (mean time to viral clearance). This estimate was adjusted for symptom based screening based on mean time to symptom onset, assuming that 80% of the

population remains asymptomatic. The effect of serological based screening was calculated based on the mean time to the first of seroconversion or viral clearance (assuming independence), as the former cases would be successfully screened, and the latter would no longer be infectious. We assumed that any screening protocol would treat equivocal IgM ELISA results as being seropositive. Full equations are provided in the supplement.

Sensitivity to key model assumptions was assessed (see Supplement for results). Analyses were performed using JAGS and the R Statistical Language (17, 18). All data and code is available on GitHub (<https://github.com/HopkinsIDD/ZikaLitReview>).

RESULTS

Systematic Review Results

We identified 964 articles discussing Zika indexed by Pubmed, Scopus and/or Web of Science as of February 25, 2016 (Figure 1). After abstract and title screening, 846 articles were excluded based on predetermined criteria (480 duplicates, dead links, or non-English or French; 366 not about Zika in humans or lacking appropriate data). Among 118 articles selected for full text review and possible data abstraction, 86 did not have sufficient exposure information or dates of onset to estimate key distributions, and 11 reported suspected perinatal transmission. Authors were contacted for four articles lacking sufficient information on one or more cases; additional information was returned on one case, but we were still unable to bound the time of exposure. We extracted data from 21 articles that provided information on 25 unique Zika cases (Table 1). The analytic data set included: 25 individuals with a bounded time of symptom onset, 49 virologic tests on 22 individuals, and 62 serologic tests on 22 individuals.

Of the cases in the final data set, 23 were infected while traveling in endemic areas, one via sexual transmission, and one through experimental infection. The vast majority of cases occurred after 2008 and were among residents of the United States or Europe (Table 1). None of the reported infections were among children, and there were roughly equal numbers of males and females (14/25 male).

Key Distributions

We estimate the median incubation period of Zika virus to be 5.9 days (95% CI 4.4-7.6), with a dispersion of 1.46 (95% CI 1.23-1.94). Hence, 5% of cases will develop symptoms by 3.2 days after infection (95% CI 1.7-4.6), 25% by 4.6 days (95% CI 3.1-6.0), 75% by 7.6 days (95% CI 5.8-10.4), and 95% by 11.2 days (95% CI 7.6-18.0) (Figure 2A).

We estimate the mean time to viral clearance, defined as having no detectable virus in the blood, to be 9.9 days (95% CI 6.9-21.4). We estimate that 5% of cases will have no detectable virus by 2.4 days after infection (95% CI 0.09-5.9), 25% by 5.8 days (95% CI 1.4-9.2), 75% by 12.7 days (95% CI 9.2-25.9), and 95% by 18.9 days (95% CI 13.6-79.4) (Figure 2B).

We estimate the mean time to seroconversion is 9.1 days (95% CI 7.0-11.6). We estimate that 5% of cases will have detectable antibodies by 4.4 days after infection (95% CI 1.3-7.0), 25% by 7.1 days (95% CI 4.0-9.2), 75% by 10.1 days (95% CI 8.7-14.6), and 95% by 13.7 days (95% CI 10.6-21.7) (Figure 2C).

Implications for Surveillance and Blood Supply Safety

The mean time to viral clearance from the blood is 9.9 days, hence, in settings with ongoing transmission, if no screening of any type were performed, there would be a 9.9 per 100,000 donors increase (95% CI 6.9-21.4) in the risk of a blood donation being infected with Zika for every 1 in 100,000 increase in daily Zika incidence. Preventing those with recent symptoms of possible Zika

infection from donating would only decrease this risk by 7% (RR 0.93, 95% CI 0.89-0.99), as 80% of individuals with Zika infection are asymptomatic, and even those who do develop symptoms will be infectious but asymptomatic for an average of six days (assuming blood donations can transmit Zika virus from the moment of infection). Serological screening is more effective, reducing the risk by 29% (RR 0.71, 95% CI 0.28-0.88), but still only marginally improves blood supply safety.

Since it may not be practical to stop blood donations until the Zika epidemic has passed, countries may consider virologic (i.e., nucleic acid) testing of particular lots of donated blood for targeted use in pregnant women. Still, even nucleic acid testing is imperfect; we did find a single case of a negative virologic blood test followed by a positive one, though this was in the context of a perinatal transmission and not part of our main analysis (38).

In settings where the risk is solely from imported Zika cases, ensuring blood supply safety is far easier. By 23.4 (95% CI 14.3-154.3) days after infection, 99% of infections are expected to no longer have detectable virus in their blood. While this number cannot be estimated with confidence given the low number of observations it is based upon, it can serve as the basis for a risk averse donation rule (e.g. no donation for 300 days after travel to a Zika endemic regions, over twice the upper limit of the confidence interval for this estimate).

It is important to note that here we assume that not having detectable virus in blood implies safe blood donation; however, risk to the blood supply when virus is present in other fluids cannot be ruled out. We found four cases in which virus was no longer detectable in blood but a saliva, nasal, or urine sample tested positive (Table 1). While we have inadequate data to estimate the time to viral clearance in these fluids, we estimate the latest of these positive tests was 12.0 days after infection (95% CI 10.1-18.2) for the individuals in our dataset. Duration of viremia in other fluids may be relevant to other public health recommendations (e.g., how long to abstain from sex with a potentially pregnant partner).

DISCUSSION

As of time of writing, the WHO reports the incubation period of Zika virus as unclear, but likely “a few days” (39). Likewise, the US Centers for Disease Control and Prevention (CDC) states that the incubation period of Zika is unknown but probably “a few days to a week” (40), and the European Centre for Disease Prevention and Control (ECDC) estimates 3-12 days (41). Our analysis substantially clarifies the true incubation period for Zika virus infection and the amount of uncertainty that remains. We similarly illuminate the distribution of time to seroconversion and time to viral clearance.

Understanding what is known about key distributions in the natural history of Zika virus infection is an important component of designing and evaluating screening and surveillance protocols, as we illustrate with an analysis of screening for Zika infection in blood donors. While the risk is quite low, it scales with Zika incidence, which in turn is hard to measure due to the high number of asymptomatic cases. Screening is important, but only a direct antigen test can have any hope of substantially reducing risk, though serologic tests may be able to offer a marginal (~30%) improvement.

This analysis is based on published data that was collected for reasons other than estimation of these key distributions; as such we were required to make several assumptions. We assumed that the virologic testing of blood or sera is 100% sensitive for detecting Zika virus; however there is evidence that viral shedding can continue far longer in urine and other bodily fluids, raising concerns that virus may exist in the blood below the limit of detection. We assumed that the distribution of time to seropositivity is independent of previous infection with other flaviviruses (those with prior

flavivirus infections will likely seroconvert more quickly). Since the majority of the cases included in our analysis were travelers returning to countries with little endemic flavivirus circulation, it is likely our estimates of time to seroconversion are conservative (i.e., long). Further, the majority of our data comes from presumed mosquito infections, and these distributions may differ for other routes of infection (e.g., perinatal, sexual). Likewise, all of the cases we report were symptomatic, and the distribution of time to seroconversion and viral clearance may differ in asymptomatic individuals. However, the biggest limitation of our analysis is the small number of cases, which both increases uncertainty and the potential for bias.

Despite the limitations of this analysis, our estimates are the most detailed, quantitative estimates to date for the natural history of Zika virus. These estimates can be used to target surveillance in both endemic settings and for returning travelers as well as guide empirical efforts to study basic features of this pathogen.

CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

ACKNOWLEDGEMENTS

The Department of Epidemiology and the Office of Public Health Practice and Training at the Johns Hopkins Bloomberg School of Public Health funded this study. The funder had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

We would like to thank Prof. Alfonso Javier Rodriguez-Morales for providing additional data on reported cases. We also thank Joshua Sharfstein for helping to bring together the team to perform this rapid review.

REFERENCES

1. WHO Director-General summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome [Internet]. World Health Organization; 2016. WHO statement on the first meeting of the International Health Regulations (2005) Emergency Committee on Zika virus and observed increase in neurological disorders and neonatal malformations; 2016 Feb 1. Available from: <http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/>
2. PAHO, WHO. Cumulative Zika suspected and confirmed cases reported by countries and territories in the Americas, 2015-2016 Washington, D.C.: PAHO/WHO; 2016 [updated 2016 Feb 25; cited 2016 Feb 28]. Available from: http://ais.paho.org/hip/viz/ed_zika_cases.asp.
3. Protocol for surveillance and response to the occurrence of microcephaly Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis., 2016 Jan 22. Report No.: 1.3.
4. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med*. 2009;360(24):2536-43. Epub 2009 Jun 12. doi: 10.1056/NEJMoa0805715. PubMed PMID: 19516034.
5. WHO. Zika virus microcephaly and guillain-barré syndrome. Situation Report: World Health Organization; [updated 2016 Feb 26; cited 2016 Feb 28]. Available from: <http://www.who.int/emergencies/zika-virus/situation-report-26-02-2016.pdf?ua=1>.
6. Cao-Lormeau V-M, Blake A, Mons S, Lastère S, Roche C, Vanhomwegen J, et al. Guillain-Barre Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *The Lancet*. Epub 2016 Feb 29. doi: 10.1016/S0140-6736(16)00562-6.
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(14). Epub 2014 Apr 18. PubMed PMID: 24739982.

8. Boadle A. Brazil reports Zika infection from blood transfusions: Reuters; [updated 2016 Feb 10; cited 2016 Feb 28]. Available from: <http://www.reuters.com/article/us-health-zika-brazil-blood-idUSKCN0VD22N>.

9. Stramer DS. Red Cross Statement on the Zika Virus: American Red Cross; [updated 2016 Feb 17; cited 2016 Feb 28]. Available from: <http://www.redcross.org/news/press-release/Red-Cross-to-Implement-Blood-Donor-Self-Deferral-Over-Zika-Concerns>.

10. New blood donation rules protect Canadian blood supply from Zika virus: Canadian Blood Services; [updated 2016 Feb 3; cited 2016 Feb 28]. Available from: <https://www.blood.ca/en/media/new-blood-donation-rules-protect-canadian-blood-supply-from-zika-virus>.

11. Fraser C, Riley S, Anderson RM, Ferguson NM. Factors that make an infectious disease outbreak controllable. Proc Natl Acad Sci U S A. 2004;101(16):6146-51. Epub 2004 Apr 9. doi: 10.1073/pnas.0307506101. PubMed PMID: 15071187; PubMed Central PMCID: PMC15395937.

12. Lessler J, Brookmeyer R, Reich NG, Nelson KE, Cummings DA, Perl TM. Identifying the probable timing and setting of respiratory virus infections. Infect Control Hosp Epidemiol. 2010;31(8):809-15. Epub 2010 Jun 24. doi: 10.1086/655023. PubMed PMID: 20569117.

13. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. Jama. 2000;283(15):2008-12. Epub 2000 May 2. PubMed PMID: 10789670.

14. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097. Epub 2009 Jul 22. doi: 10.1371/journal.pmed.1000097. PubMed PMID: 19621072; PubMed Central PMCID: PMC15395937.

15. Reich NG, Lessler J, Cummings DA, Brookmeyer R. Estimating incubation period distributions with coarse data. *Stat Med*. 2009;28(22):2769-84. Epub 2009 Jul 15. doi: 10.1002/sim.3659. PubMed PMID: 19598148.
16. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DA. Incubation periods of acute respiratory viral infections: a systematic review. *Lancet Infect Dis*. 2009;9(5):291-300. Epub 2009 Apr 28. doi: 10.1016/s1473-3099(09)70069-6. PubMed PMID: 19393959; PubMed Central PMCID: PMCPCmc4327893.
17. Plummer M. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. 2003.
18. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; 2016.
19. Bearcroft WG. Zika virus infection experimentally induced in a human volunteer. *Trans R Soc Trop Med Hyg*. 1956;50(5):442-8. PubMed PMID: 13380987.
20. Chen LH. Zika Virus Infection in a Massachusetts Resident After Travel to Costa Rica: A Case Report. *Ann Intern Med* [Internet]. 2016 Feb 25 [cited 2016 Feb 25]. Available from: <http://dx.doi.org/10.7326/L16-0075>
21. Fonseca K, Meatherall B, Zarra D, Drebot M, MacDonald J, Pabbaraju K, et al. First case of Zika virus infection in a returning Canadian traveler. *Am J Trop Med Hyg*. 2014;91(5):1035-8. Epub 2014 Oct 9. doi: 10.4269/ajtmh.14-0151. PubMed PMID: 25294619; PubMed Central PMCID: PMCPCmc4228871.
22. Foy BD, Kobylinski KC, Chilson Foy JL, Blitvich BJ, Travassos da Rosa A, Haddock AD, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis*. 2011;17(5):880-2. Epub 2011 May 3. doi: 10.3201/eid1705.101939. PubMed PMID: 21529401; PubMed Central PMCID: PMCPCmc3321795.
23. Ginier M, Neumayr A, Gunther S, Schmidt-Chanasit J, Blum J. Zika without symptoms in returning travellers: What are the implications? *Travel Med Infect Dis*. 2016;14(1):16-20. Epub 2016 Feb 16. doi: 10.1016/j.tmaid.2016.01.012. PubMed PMID: 26876061.

24. Gyurech D, Schilling J, Schmidt-Chanasit J, Cassinotti P, Kaeppli F, Dobec M. False positive dengue NS1 antigen test in a traveller with an acute Zika virus infection imported into Switzerland. *Swiss Med Wkly*. 2016;146:w14296. Epub 2016 Feb 10. doi: 10.4414/smw.2016.14296. PubMed PMID: 26859285.
25. Korhonen EM, Huhtamo E, Smura T, Kallio-Kokko H, Raassina M, Vapalahti O. Zika virus infection in a traveller returning from the Maldives, June 2015. *Euro Surveill*. 2016;21(2). Epub 2016 Jan 23. doi: 10.2807/1560-7917.es.2016.21.2.30107. PubMed PMID: 26794427.
26. Kutsuna S, Kato Y, Takasaki T, Moi M, Kotaki A, Uemura H, et al. Two cases of Zika fever imported from French Polynesia to Japan, December 2013 to January 2014 [corrected]. *Euro Surveill*. 2014;19(4). Epub 2014 Feb 11. PubMed PMID: 24507466.
27. Kwong JC, Druce JD, Leder K. Zika virus infection acquired during brief travel to Indonesia. *Am J Trop Med Hyg*. 2013;89(3):516-7. Epub 2013 Jul 24. doi: 10.4269/ajtmh.13-0029. PubMed PMID: 23878182; PubMed Central PMCID: PMC3771291.
28. Leung GH, Baird RW, Druce J, Anstey NM. ZIKA VIRUS INFECTION IN AUSTRALIA FOLLOWING A MONKEY BITE IN INDONESIA. *Southeast Asian J Trop Med Public Health*. 2015;46(3):460-4. Epub 2015 Nov 3. PubMed PMID: 26521519.
29. Maria AT, Maquart M, Makinson A, Flusin O, Segondy M, Leparac-Goffart I, et al. Zika virus infections in three travellers returning from South America and the Caribbean respectively, to Montpellier, France, December 2015 to January 2016. *Euro Surveill*. 2016;21(6). Epub 2016 Feb 24. doi: 10.2807/1560-7917.es.2016.21.6.30131. PubMed PMID: 26898198.
30. Shinohara K, Kutsuna S, Takasaki T, Moi ML, Ikeda M, Kotaki A, et al. Zika fever imported from Thailand to Japan, and diagnosed by PCR in the urines. *J Travel Med*. 2016;23(1). Epub 2016 Jan 20. doi: 10.1093/jtm/tav011. PubMed PMID: 26782128.
31. Simpson DI. ZIKA VIRUS INFECTION IN MAN. *Trans R Soc Trop Med Hyg*. 1964;58:335-8. PubMed PMID: 14175744.

32. Summers DJ, Acosta RW, Acosta AM. Zika Virus in an American Recreational Traveler. *J Travel Med.* 2015;22(5):338-40. Epub 2015 May 23. doi: 10.1111/jtm.12208. PubMed PMID: 25996909.
33. Tappe D, Nachtigall S, Kapaun A, Schnitzler P, Gunther S, Schmidt-Chanasit J. Acute Zika virus infection after travel to Malaysian Borneo, September 2014. *Emerg Infect Dis.* 2015;21(5):911-3. Epub 2015 Apr 22. doi: 10.3201/eid2105.141960. PubMed PMID: 25898277; PubMed Central PMCID: PMC4412240.
34. Tappe D, Rissland J, Gabriel M, Emmerich P, Gunther S, Held G, et al. First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. *Euro Surveill.* 2014;19(4). Epub 2014 Feb 11. PubMed PMID: 24507467.
35. Waehre T, Maagard A, Tappe D, Cadar D, Schmidt-Chanasit J. Zika virus infection after travel to Tahiti, December 2013. *Emerg Infect Dis.* 2014;20(8):1412-4. Epub 2014 Jul 26. doi: 10.3201/eid2008.140302. PubMed PMID: 25062427; PubMed Central PMCID: PMC4111184.
36. Zammarchi L, Tappe D, Fortuna C, Remoli ME, Gunther S, Venturi G, et al. Zika virus infection in a traveller returning to Europe from Brazil, March 2015. *Euro Surveill.* 2015;20(23). Epub 2015 Jun 19. PubMed PMID: 26084316.
37. Zammarchi L, Stella G, Mantella A, Bartolozzi D, Tappe D, Gunther S, et al. Zika virus infections imported to Italy: clinical, immunological and virological findings, and public health implications. *J Clin Virol.* 2015;63:32-5. Epub 2015 Jan 21. doi: 10.1016/j.jcv.2014.12.005. PubMed PMID: 25600600.
38. Besnard M, Lastere S, Teissier A, Cao-Lormeau V, Musso D. Evidence of perinatal transmission of Zika virus, French Polynesia, December 2013 and February 2014. *Euro Surveill.* 2014;19(13). PubMed PMID: 24721538.
39. Zika virus: Fact Sheet: World Health Organization; 2016 [updated 2016 Feb; cited 2016 Feb 28]. Available from: <http://www.who.int/mediacentre/factsheets/zika/en/>.

40. Symptoms, Diagnosis, & Treatment: Centers for Disease Control and Prevention; [updated 2016 Feb 23; cited 28 Feb 2016]. Available from:

<http://www.cdc.gov/zika/symptoms/>.

41. Factsheet for health professionals: European Centre for Disease Prevention and Control; [updated 2016 Feb 12; cited 2016 Feb 28]. Available from:

http://ecdc.europa.eu/en/healthtopics/zika_virus_infection/factsheet-health-professionals/Pages/factsheet_health_professionals.aspx.

Table 1. Characteristics of Zika cases included in pooled analysis (N=25)

First author (year)	Age	Sex	Place of origin	Probable location infected	Year exposed	Exposure window (days)	Days to symptom onset (min-max)	Days to seroconversion (min-max)	Days to viral clearance ^a (min-max)
Bearcroft (1956)(19)	34	Male	Europe	Nigeria ^b	–	<1	3-4	4-9	>6 ^c
Chen (2016)(20)	55	Male	USA	Costa Rica	2015	8	3-12	<39	–
Duffy (2009)(4)	–	Female	USA	Yap Island	2007	13	7-21	<34	–
Fonseca (2014)(21)	–	Female	Canada	Thailand	2013	16	1-18	<24 ^d	26-28
Foy (2011)(22)	36	Male	USA	Senegal	2008	24	5-30	<33	<33 ^e
	27	Male	USA	Senegal	2008	24	4-29	<33	<33 ^e
	–	Female	USA	USA ^f	2008	7	3-11	15-34	<16 ^e
Ginier (2016)(23)	51	Female	Switzerland	Guatemala, El Salvador	2015	14	3-18	<24	>23
Gyurech (2016)(24)	44	Female	Switzerland	Brazil	2015	1	4-17	19-23	<23
Korhonen (2016)(25)	37	Male	Finland	Maldives	2015	183	1-185	–	<191 ^g
Kutsuna (2014)(26)	Early 30s	Female	Japan	Bora Bora	2013-2014	10	5-16	<21	<21 ^h
Kwong (2013)(27)	52	Female	Australia	Indonesia	–	9	0-10	–	13-24
Leung (2015)(28)	27	Male	Australia	Indonesia ⁱ	–	6	2-9	–	<14 ^j
Maria (2016)(29)	60s	Female	France	Martinique Island	2015	22	1-24	<28	–
	20s	Male	France	Brazil	2015-2016	8	0-9	<17	–
	50s	Male	France	Colombia	2015-2016	29	0-30	31-37	– ^k
Shinohara (2016)(30)	Early 40s	Male	Japan	Thailand	2014	7	1-9	10-14	>10 ^d
Simpson (1964)(31)	28	Male	Europe	Uganda	–	76	0-77	<78	>2 ^c
Summers (2015)(32)	48	Male	USA	Ecuador, Peru, Bolivia, Chile, Easter Island, French Polynesia, Hawaii	2013	34	0-35	<45	–
Tappe (2015)(33)	45	Female	Germany	Malaysia	2014	22	5-28	29-33	<30
Tappe (2014)(34)	Early 50s	Male	Germany	Thailand	2013	12	0-12	<22	<22
Wæhre (2014)(35)	31	Female	Norway	Tahiti	2013	15	0-16	20-52	20-52 ^e
Zammarchi (2015)(36)	Early 60s	Male	Italy	Brazil	2015	12	0-13	<16	<16
Zammarchi (2015)(37)	Early 30s	Female	Italy	French Polynesia	2013-2014	19	0-20	22-58	>22
	Early 30s	Male	Italy	French Polynesia	2013-2014	19	0-20	22-56	<23

^a Days to viral clearance in sera^b Volunteer inoculation of Zika virus^c Viral shedding determined from mouse inoculation^d Equivocal result counted as positive^e Serum was positive by PCR and negative by culture^f Probable sexual transmission^g Serum was negative by PCR; urine was positive by PCR at later visit^h Serum was negative by PCR; urine was positive by PCRⁱ Possible transmission from monkey bite or mosquito^j Serum and swab of monkey bite site were negative by PCR; nasopharyngeal swab was positive by PCR^k No sera tested; plasma and urine positive by PCR; plasma was negative by PCR and urine and saliva were positive by PCR at a later visit

Figure 1: Systematic review process.

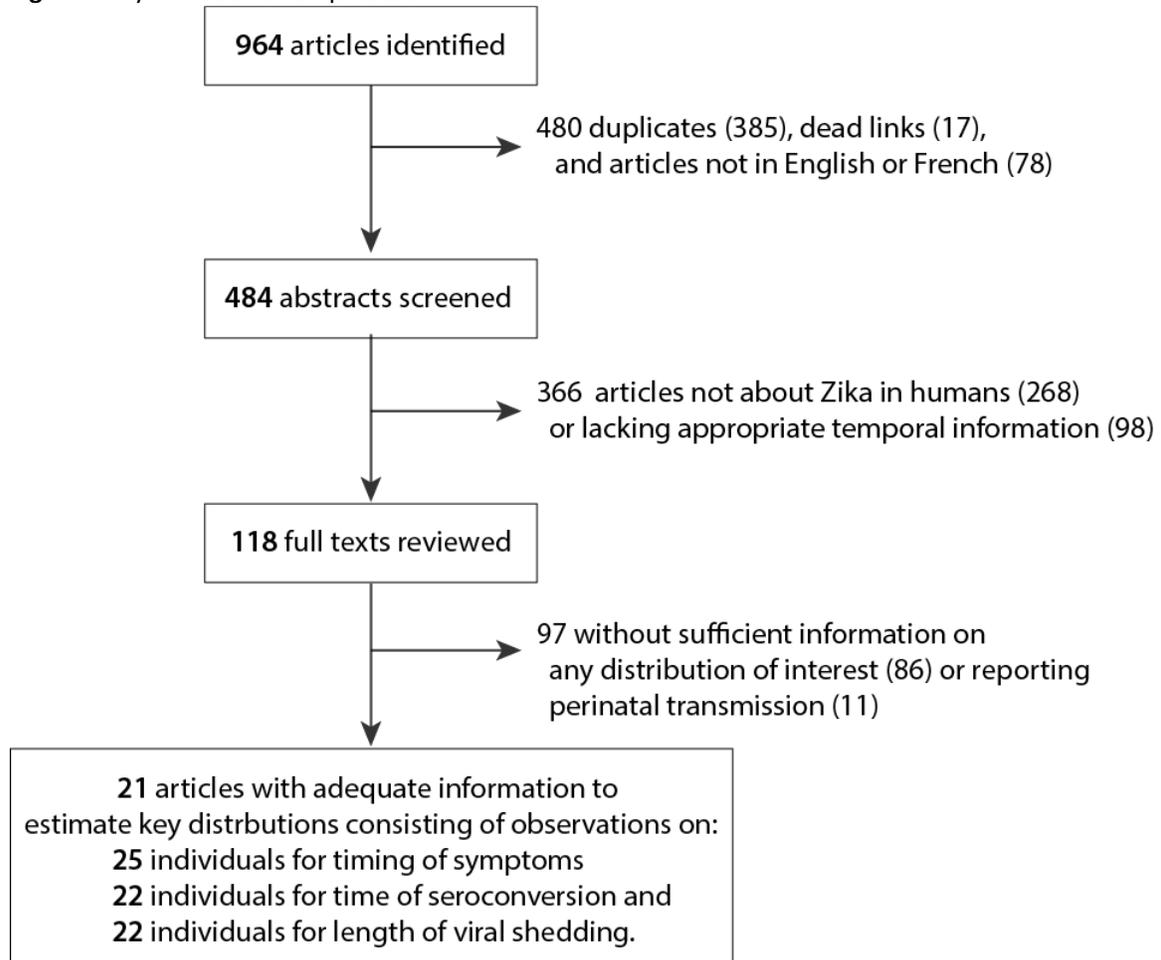


Figure 2: Percentage of the population with **(A)** symptom onset after a given time, **(B)** still shedding at a given day, and **(C)** testing seropositive as of a given day. Shaded regions indicate 95% credible intervals.

