

Times to Key Events in the Course of Zika Infection and their Implications for Surveillance: A Systematic Review and Pooled Analysis

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Words: 2,619 text, 250 abstract.

ABSTRACT

Background

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.

Results

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.

1 INTRODUCTION

2 The explosion of Zika cases in Central and South America, combined with growing evidence that the
3 virus is responsible for an epidemic of microcephaly in Brazil, has prompted the World Health
4 Organization (WHO) to declare a Public Health Emergency of International Concern.¹ As of February 29,
5 2016, there have been at least a half-million Zika virus infections in the Americas.^{2,3} Although clinical
6 disease is generally mild or asymptomatic,⁴ there is increasing evidence of a link between Zika virus
7 infection and severe microcephaly in infants born to women infected during pregnancy, including a 10-
8 fold increase in microcephaly cases in Brazil in the wake of the 2015 Zika epidemic⁵. Zika virus infection
9 has been linked to Guillain-Barre in adults.^{5,6}

10

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

24 infectious period, are essential to designing evidence based surveillance systems and informing public
25 health policy.^{11,12}

26

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

31

32

33 **METHODS**

34 The systematic review was conducted according to the Meta-analysis of Observational Studies in
35 Epidemiology (MOOSE) guidelines¹³ and the Preferred Reporting Items for Systematic Review and Meta-
36 Analyses (PRISMA) guidelines¹⁴ where applicable (see Supplemental Material).

37

38 *Search strategy and selection criteria*

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

42

43 We included publications that provided information on time of exposure to Zika virus and 1)
44 time of symptom onset, 2) time of sample collection for virologic Zika virus testing (e.g. PCR or culture)
45 and test results (positive/negative), and/or 3) time of collection of samples for antibody testing and test
46 results (positive/negative). We excluded publications if they did not provide sufficient information to

47 determine a bounded time of exposure to Zika virus, contained no data from humans, were not in
48 English or French, or reported only perinatal transmission of Zika virus.

49

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

56

57 *Data abstraction*

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

69

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

84

85 *Statistical Methods - Pooled Analysis*

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

94

95 *Statistical Methods - Blood Supply Safety*

96 The impact of key distributions on blood supply safety was calculated assuming a constant incidence
97 rate. The number of possibly infected donors per 100,000 if no screening occurs was calculated as: (daily
98 incidence rate per 100,000) \times (mean time to viral clearance). This estimate was adjusted for symptom
99 based screening based on mean time to symptom onset, assuming that 80% of the population remains
100 asymptomatic. The effect of serological based screening was calculated based on the mean time to the
101 first of seroconversion or viral clearance (assuming independence), as the former cases would be
102 successfully screened, and the latter would no longer be infectious. We assumed that any screening
103 protocol would treat equivocal IgM ELISA results as being seropositive. Full equations are provided in
104 the supplement.

105

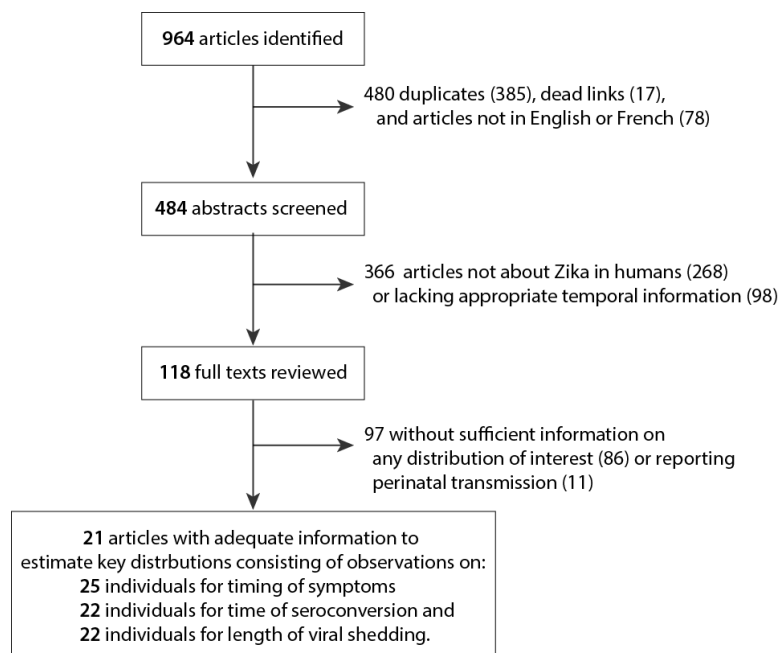
106 Sensitivity to key model assumptions was assessed (see Supplement for results).

107

108 Analyses were performed using JAGS and the R Statistical Language.^{17,18} All data and code is
109 available on GitHub (<https://github.com/HopkinsIDD/ZikaLitReview>).

110

111



112

113 **Figure 1:** Systematic review process.

114

115 **RESULTS**

116 *Systematic Review Results* We identified 964 articles discussing Zika indexed by Pubmed, Scopus and/or

117 Web of Science as of February 25, 2016 (Figure 1). After abstract and title screening, 846 articles were

118 excluded based on predetermined criteria (480 duplicates, dead links, or non-English or French; 366 not

119 about Zika in humans or lacking appropriate data). Among 118 articles selected for full text review and

120 possible data abstraction, 86 did not have sufficient exposure information or dates of onset to estimate

121 key distributions, and 11 reported suspected perinatal transmission. Authors were contacted for 4

122 articles lacking sufficient information on one or more cases; additional information was returned on one

123 case, but we were still unable to bound the time of exposure. We extracted data from 21 articles that

124 provided information on 25 unique Zika cases (Table 1). The analytic data set included: 25 individuals

125 with a bounded time of symptom onset, 49 virologic tests on 22 individuals, and 62 serologic tests on 22

126 individuals.

127

128 Of the cases in the final data set, 23 were infected while traveling in endemic areas, one via
129 sexual transmission, and one through experimental infection. The vast majority of cases occurred after
130 2008 and were among residents of the United States or Europe (Table 1). None of the reported
131 infections were among children, and there were roughly equal numbers of males and females (14/25
132 male).

133

134

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 ^l
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 PCR

ⁱ 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

^l Serum was positive by PCR and negative by culture

135

136 *Key Distributions*

137 We estimate the median incubation period of Zika virus to be 5.9 days (95% CI: 4.4-7.6), with a
138 dispersion of 1.46 (95% CI: 1.23-1.94). Hence, 5% of cases will develop symptoms by 3.2 days after
139 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
140 95% by 11.2 days (95% CI: 7.6-18.0) (Figure 2A).

141

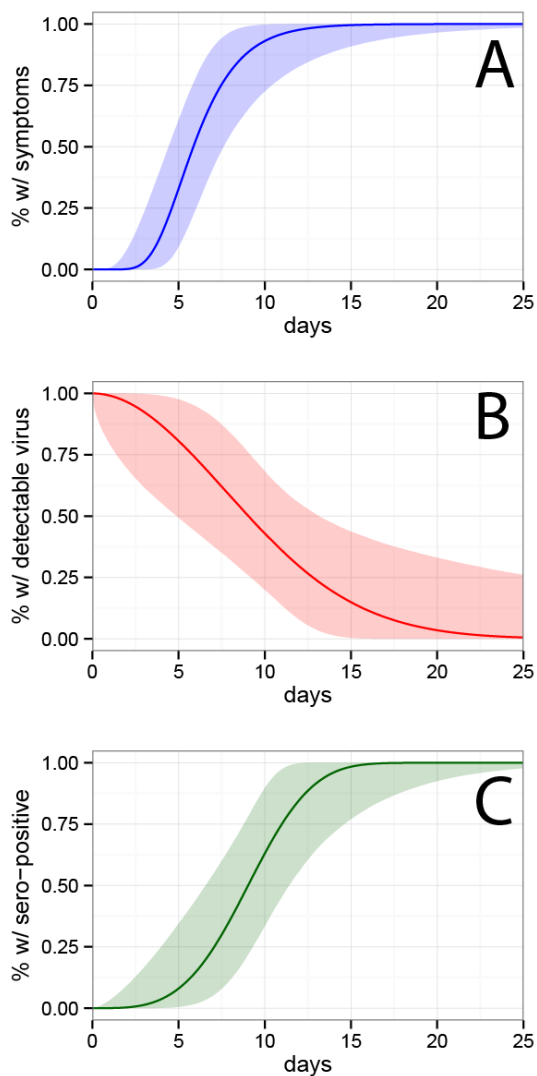
142 We estimate the mean time to viral clearance, defined as having no detectable virus in the
143 blood, to be 9.9 days (95% CI: 6.9-21.4). We estimate that 5% of cases will have no detectable virus by
144 2.4 days after infection (95% CI: 0.09-5.9), 25% by 5.8 days (95% CI: 1.4, 9.2), 75% by 12.7 days (95% CI:
145 9.2-25.9), and 95% by 18.9 days (95% CI: 13.6-79.4) (Figure 2B).

146

147 We estimate the mean time to seroconversion is 9.1 days (95% CI: 7.0-11.6). We estimate that
148 5% of cases will have detectable antibodies by 4.4 days after infection (95% CI: 1.3-7.0), 25% by 7.1 days
149 (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)
150 (Figure 2C).

151

152



153

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

156

157 *Implications for Surveillance and Blood Supply Safety*

158 The mean time to viral clearance from the blood is 9.9 days, hence, in settings with ongoing

159 transmission, if no screening of any type were performed, there would be a 9.9 per 100,000 donors

160 increase (95% CI: 6.9-21.4) in the risk of a blood donation being infected with Zika for every 1 in 100,000

161 increase in daily Zika incidence. Preventing those with recent symptoms of possible Zika infection from

162 donating would only decrease this risk by 7% (RR 0.93, 95% CI 0.89-0.99), as 80% of individuals with Zika
163 infection are asymptomatic, and even those who do develop symptoms will be infectious but
164 asymptomatic for an average of six days (assuming blood donations can transmit Zika virus from the
165 moment of infection). Serological screening is more effective, reducing the risk by 29% (RR 0.71, 95% CI:
166 0.28-0.88), but still only marginally improves blood supply safety.

167
168 Since it may not be practical to stop blood donations until the Zika epidemic has passed,
169 countries may consider virologic (i.e., nucleic acid) testing of particular lots of donated blood for
170 targeted use in pregnant women. Still, even nucleic acid testing is imperfect; we did find a single case of
171 a negative virologic blood test followed by a positive one, though this was in the context of a perinatal
172 transmission and not part of our main analysis.³⁸

173
174 In settings where the risk is solely from imported Zika cases, ensuring blood supply safety is far
175 easier. By 23.4 (95% CI, 14.3-154.3) days after infection, 99% of infections are expected to no longer
176 have detectable virus in their blood. While this number cannot be estimated with confidence given the
177 low number of observations it is based upon, it can serve as the basis for a risk averse donation rule (e.g.
178 no donation for 300 days after travel to a Zika endemic regions, over twice the upper limit of the
179 confidence interval for this estimate).

180
181 It is important to note that here we assume that not having detectable virus in blood implies
182 safe blood donation; however, risk to the blood supply when virus is present in other fluids cannot be
183 ruled out. We found four cases in which virus was no longer detectable in blood but a saliva, nasal, or
184 urine sample tested positive (Table 1). While we have inadequate data to estimate the time to viral
185 clearance in these fluids, we estimate the latest of these positive tests was 12.0 days after infection

186 (95% CI: 10.1-18.2) for the individuals in our dataset. Duration of viremia in other fluids may be relevant
187 to other public health recommendations (e.g., how long to abstain from sex with a potentially pregnant
188 partner).

189

190 **DISCUSSION**

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

197

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

204

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

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

219

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

224

225

226 **ACKNOWLEDGEMENTS**

227 We would like to thank Prof. Alfonso Javier Rodriguez-Morales for providing additional data on
228 reported cases. We also thank Joshua Sharfstein for helping to bring together the team to perform this
229 rapid review. We further thank the Department of Epidemiology and the Office of Public Health Practice
230 and Training at the Johns Hopkins Bloomberg School of Public Health for financially supporting this
231 work.

232

233

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