Prevalence of asymptomatic Zika virus infection: a systematic review
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Objective To conduct a systematic review to estimate the prevalence of asymptomatic Zika virus infection in the general population and in specific population groups.

Methods We searched PubMed®, Embase® and LILACS online databases from inception to 26 January 2018. We included observational epidemiological studies where laboratory testing was used to confirm positive exposure of participants to Zika virus and in which Zika virus symptom status was also recorded. We excluded studies in which having symptoms of Zika virus was a criterion for inclusion. The main outcome assessed was percentage of all Zika virus-positive participants who were asymptomatic. We used a quality-effects approach and the double arcsine transformation for the meta-analysis.

Findings We assessed 753 studies for inclusion, of which 23 were included in the meta-analysis, totalling 11,305 Zika virus-positive participants. The high degree of heterogeneity in the studies ($I^2 = 99\%$) suggests that the pooled prevalence of asymptomatic Zika virus-positive participants was probably not a robust estimate. Analysis based on subgroups of the population (general population, returned travellers, blood donors, adults with Guillain–Barré syndrome, pregnant women and babies with microcephaly) was not able to explain the heterogeneity. Funnel and Doi plots showed major asymmetry, suggesting selection bias or true heterogeneity.

Conclusion Better-quality research is needed, using standardized methods, to determine the true prevalence of asymptomatic Zika virus and whether it varies between populations or over time.

Abstract in Arabic, Chinese, Français, Русский and Español at the end of each article.
and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement for reporting.11

Inclusion criteria

We included general or specific population-based studies of participants of all ages and from any country; pregnant women, newborns and infants, children, adults, newborns with congenital abnormalities, and adults with Guillain–Barré syndrome and other neurological diseases.

We included studies if exposure to Zika virus was identified, using molecular or serological methods. We used the Pan American Health Organization (PAHO), World Health Organization (WHO) guidelines for laboratory testing wherever possible.12 For a confirmed case these guidelines require: (i) presence of ribonucleic acid or Zika virus antigen in any specimen (serum, urine, saliva, tissue or whole blood) tested by reverse-transcriptase polymerase chain reaction method; or (ii) positive anti-Zika virus IgM antibodies and plaque reduction neutralization test for Zika virus titres ≥ 20 and four or more times higher than for other flaviviruses; or (iii) in autopsy specimens, detection of the viral genome (in fresh or paraffin tissue) by molecular techniques, or detection by immunohistochemistry. In practice, this definition was often not used in studies, especially in earlier research. We therefore included studies using alternative definitions for positive laboratory testing if the definition was clearly stated. One alternative definition was the PAHO-WHO guideline for probable cases: presence of Zika IgM antibodies, with no evidence of infection with other flaviviruses.12

We defined the primary outcome measure as percentage of all Zika virus-positive participants who were asymptomatic at the time of laboratory testing, or within 7 to 10 days of testing. The denominator was all participants who were Zika virus-positive. For the numerator, the PAHO-WHO guidelines for signs and symptoms were used wherever possible, which require patients to have rash (usually pruritic and maculopapular) with two or more of the following signs or symptoms: fever, usually < 38.5 °C; conjunctivitis (non-purulent/hyperemic); arthralgia; myalgia; and/or periarticular oedema.12 In practice, not all studies used the PAHO-WHO definition and we included studies using alternative definitions for symptoms if a clear definition was provided. Asymptomatic Zika virus-positive participants were those with no symptoms or with symptoms that did not meet the definition used for the particular study.

We included cross-sectional seroprevalence studies, cohort studies of pregnant women, cohort studies of newborns and infants, case-control studies of Guillain–Barré syndrome and other neurological diseases, case-control studies of microcephaly and case series with at least 20 participants. The cut-off value of 20 participants for case series was chosen as a reasonable minimum number for which prevalence data can be reported. A cross-sectional seroprevalence study in the general population is the most appropriate design to determine the prevalence of asymptomatic Zika virus infection. However, to make use of the limited information that was available, we chose to include other study designs and other populations. Published and completed unpublished studies were eligible for inclusion. Data from ongoing studies were also eligible for inclusion when results from a representative sample were available.

Publications in English, French, Spanish or Portuguese were included. There was no restriction on year of publication.

We excluded studies in which having symptoms of Zika virus was a criterion for inclusion of participants in the study. This is because it would give a biased value for percentage asymptomatic of 100% solely due to the inclusion criteria. We also excluded studies where the percentage of participants who were asymptomatic could not be determined.

Search strategy

The search strategy and keywords used are shown in Box 1. The titles and abstracts of these references were checked by one author against the inclusion criteria. Additional published articles were also identified through separate manual searches of PubMed® and revision of Zika virus article alerts by another author. The full text of any potentially relevant papers were checked by a second author and disagreements resolved by discussion and consultation with a third author. Papers excluded after review by a second reviewer and discussions between reviewers were detailed in a table, together with the reason for their exclusion. We also made contact (by email or in-person at key Zika virus meetings) with known research groups conducting cross-sectional studies of Zika virus. These groups were identified through the PAHO-WHO Zika virus research platform, which includes research protocols that detail ongoing research related to the virus.14

Data extraction

We extracted qualitative information into a Word version 14 table and quantitative data into an Excel version 14 spreadsheet (Microsoft Corporation, Redmond, USA). One author extracted the data and another author checked it; disagreements were resolved by discussion and consultation with a third author where necessary. We extracted the following data: country of study; region within the country; study design (cross-sectional, cohort, case-control, case series); population (all ages, pregnant women, newborns and infants, newborns with congenital abnormalities; adults, adults with Guillain–Barré syndrome); age range; period of study; definition of Zika virus positive according to laboratory tests; definition of symptomatic and asymptomatic Zika virus; preferential recruitment of participants with symptoms (yes/no); sample size calculation; and comments. Quantitative data extracted included: response rate; total number of participants; total number classified as Zika virus positive; number of Zika
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virus-positive participants classified as symptomatic and as asymptomatic; and percentage of the total sample who were symptomatic at time of recruitment. For the cohort studies we used Zika virus-positive status at any time during the pregnancy (for studies of pregnant women) or any time during the study (for studies of newborns and infants). We extracted quantitative data for relevant subgroups where the data and sample size allowed, including for population subgroups and different definitions of Zika virus exposure.

Quality assessment

The quality of the included studies was assessed independently by two authors using the critical appraisal checklist for prevalence studies, developed by The Joanna Briggs Institute.8 This tool includes the same dimensions as the Assessing Risk of Bias in Prevalence Studies tool,15 but was considered more useful for this review as it is applicable to a variety of study designs. The Joanna Briggs Institute tool also includes extra items related to sample size and subgroups. Disagreements were resolved by discussion and consultation with a third author where necessary.

Analysis

We summarized the findings from the included studies in numerical and narrative tables. We conducted quality-effects meta-analysis using MetaXL version 5.3 (Ersatz, EpiGear International, Sunrise Beach, Australia) and the double arcsine transformation of prevalence.16–18 We assessed heterogeneity using the Q and I² statistics. We used Doi plots and the Luis Furuya–Kanamori index to evaluate the presence of small-study effects, where asymmetry can indicate publication or non-publication of results.36 A symmetrical mountain-like plot with values of the Luis Furuya–Kanamori index within ±1 indicates no asymmetry; between ±1 and ±2 indicates minor asymmetry; and exceeding ±2 suggests major asymmetry.16

Due to the high degree of heterogeneity in the results, we also checked whether the heterogeneity could be explained by population subgroups. The number of included studies was insufficient for testing multiple subgroups. We also tested the sensitivity of the results to excluding the largest study and to using the actual sample figure, rather than the population estimate reported by the authors that accounts for symptoms not attributable to Zika virus infection.

Results

We identified a total of 960 records from database searches and another 12 records through other sources (Fig. 1). No unpublished or in-process studies were identified. After screening, we assessed 64 full-text articles for eligibility (Fig. 1) and excluded 36 articles19–34 for various reasons (Table 1). No studies were excluded due to language restrictions. A total of 23 studies from 28 articles met the inclusion criteria for the review (Table 2; available at: http://www.who.int/bulletin/volumes/96/6/17-201541).1,4,63–80

We found only three cross-sectional seroprevalence studies of the general population, which are considered to be the most appropriate design to measure prevalence. These included the original study of Yap State residents, Federated States of Micronesia, conducted in 2007,4 a study of the general population and schoolchildren in French Polynesia conducted in 2014–201579 and a study in 2016 of the general population living near 19 index cases in San Juan, Puerto Rico.71 The majority of the studies were case series from population health surveillance programmes.72,74,75,79–80 Systematic screenings of an at-risk population46–48 or hospital-based screenings of an at-risk population62,64,66,72,76,78,79 A cohort design was used in four studies,51,70,77,79 a case-control design in two studies,58,59 and a cross-sectional study of blood donors in one study66 (Table 2).

There was considerable variation in the methods of laboratory testing and the definitions of Zika virus positivity used in the studies (Table 2). Also, few studies offered a definition for symptomatic or asymptomatic. Sample sizes in studies varied from 30 to over 9000 (Table 3).

The risk of bias scores ranged from 1 to 9 out of a possible total of 10, with a mean score of 5.8 (Table 2). The most common limitations were: sample not clearly representative of the population (18 studies); response rate not reported, or large number of non-responders (19 studies); and not accounting for confounding factors or failure to identify subgroup differences (17 studies). The three cross-sectional seroprevalence studies of the general population had risk of bias scores between 6 and 8.

The 23 studies included a pooled number of 11 305 participants positive for Zika virus, 6921 of whom were asymptomatic. Meta-analysis showed a combined prevalence of asymptomatic Zika virus of 61.8% (95% CI: 33.0–87.1%). However, there was substantial...
| Study                     | Exclusion category | Reason for exclusion                                                                 |
|--------------------------|-------------------|--------------------------------------------------------------------------------------|
| Alvim et al., 2016      | Outcome measure   | Percentage of participants with or without symptoms not reported                     |
| Brasil et al., 2016     | Exclusion criteria | Having symptoms was criterion for inclusion of participants                           |
| Brasil et al., 2016     | Exclusion criteria | Having symptoms was criterion for inclusion of participants                           |
| Carvalho et al., 2016   | Study type        | Case series with < 20 cases (19 only)                                               |
| De Paula-Freitas et al., 2016 | Exposure | No laboratory confirmation of exposure to Zika virus                                 |
| Dirlukow et al., 2016   | Outcome measure   | Percentage of participants asymptomatic not reported                                  |
| Ferreira da Silva et al., 2016 | Exposure | No laboratory or molecular testing for Zika virus                                      |
| Figueiredo et al., 2016 | Exclusion criteria | Having Zika virus symptoms was an inclusion criteria                                 |
| Franca et al., 2016     | Study type        | Very few participants tested for Zika virus either using PCR or serology (from email communication with corresponding author on 28 March 2017) |
| Hamer et al., 2016      | Outcome measure   | Percentage of participants with or without symptoms not reported                     |
| Mani, 2016              | Study type        | Summary of another study33 that was excluded due to very few participants undergoing laboratory testing |
| Melo et al., 2016       | Study type        | Case series with < 20 cases (11 only)                                               |
| Nah et al., 2016        | Outcome measure   | Participants' symptoms not reported. Modelling study                                  |
| Sarno et al., 2016      | Exposure          | No laboratory testing for Zika virus                                                 |
| Torres et al., 2016     | Outcome measure   | Percentage of participants asymptomatic could not be measured as all Zika virus-positive participants had symptoms |
| Yakob et al., 2016      | Study type        | No primary data presented                                                            |
| Araujo et al., 2017     | Outcome measure   | Percentage of participants with or without symptoms not reported                     |
| Bierlaire et al., 2017  | Study type        | Case series with < 20 cases (12 only)                                               |
| Chow et al., 2017       | Outcome measure   | Percentage of participants asymptomatic could not be determined as all enrolled participants were symptomatic |
| Eppes et al., 2017      | Exposure          | Only 8 women had positive test results for Zika virus. Insufficient information to calculate percentage of participants with or without symptoms |
| Gonzalez et al., 2017   | Outcome measure   | Percentage of participants with or without symptoms not reported                     |
| Griffin et al., 2017    | Exclusion criteria | Majority of children were selected for testing for Zika virus on the basis of having symptoms |
| Hancock et al., 2017    | Exposure          | Exposure data reported for a period where all cases tested positive for Zika virus by real-time reverse transcription-PCR |
| Huits et al., 2017      | Study type        | Only 6 of 31 travellers had confirmed Zika virus infection                             |
| Lee et al., 2017        | Outcome measure   | Percentage of participants with or without symptoms not measured or reported         |
| Marban-Castro et al., 2017 | Outcome measure | Insufficient information to decide whether study met inclusion criteria or to calculate percentage of participants with or without symptoms |
| Moreira et al., 2017    | Study type        | Systematic review                                                                   |
| Rac et al., 2017        | Outcome measure   | Percentage of Zika virus-positive participants with or without symptoms not reported. |
| Salinas et al., 2017    | Outcome measure   | Percentage of participants Zika virus-positive with or without symptoms not reported. |
| Schaub et al., 2017     | Study type        | Case series with < 20 cases (8 only)                                                |
| Syczynski et al., 2017  | Outcome measure   | Percentage of Zika virus-positive participants with or without symptoms not reported. |
| Tse et al., 2017        | Outcome measure   | Percentage of participants with or without symptoms not reported. Likely that they were selected based on having symptoms |
| Uncini et al., 2017     | Outcome measure   | Percentage of participants asymptomatic could not be measured as all Zika virus-positive participants had symptoms |
| Zambrano et al., 2017   | All asymptomatic  | Data on symptoms not recorded at time of laboratory testing. All women were asymptomatic at enrolment |
| Delaney et al., 2018    | Exposure          | Exposure to Zika virus tested in only a small proportion of participants              |

PCR: polymerase chain reaction.
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Table 3. Results of the systematic review of the prevalence of asymptomatic Zika virus infection

| Study, primary reference | Population or subgroup | Total no. of participants | No. classified as Zika virus positive | % asymptomatic (95% CI) | Comments |
|--------------------------|------------------------|---------------------------|--------------------------------------|------------------------|----------|
| Duffy et al., 2009        | General population: adjusted figures | 6 892 | 5 005 | 4 086 | 82 (81–83) | Figures adjusted for the percentage of symptoms unlikely to be attributable to Zika virus infection and adjusted to total Yap State population (3+ years of age) |
| Musso et al., 2014        | General population: actual figures | (557)<sup>a</sup> | (414)<sup>a</sup> | (258)<sup>a</sup> | (62 (58–67))<sup>a</sup> | Actual figures from tested sample |
| Adams et al., 2016        | Pregnant women | 9 343 | 426 | 43 | 10 (7–13) | Confirmed cases only |
| Asaujo et al., 2016       | Cases: babies with microcephaly Controls: babies without microcephaly or birth abnormalities | 32 | 13 | 6 | 46 (20–74) | Symptoms were measured in mothers |
|                          |                        | 62 | 0 | 0 | 0 | Not included in meta-analysis because no babies were Zika virus positive |
| Cao Lormeau et al., 2016  | Adults with Guillain–Barré syndrome | 42 | 42 | 4 | 10 (2–21) | NA |
| Dasgupta et al., 2016     | Travellers | 1 199 | 169 | 0 | 0 (0–1) | Bias towards asymptomatic participants |
|                          | Pregnant women travellers | 3 335 | 28 | 7 | 25 (10–43) | Bias towards asymptomatic patients |
| de Laval et al., 2016     | Travellers | 136 | 10 | 3 | 30 (5–62) | All co-travellers were screened |
| Diaz-Menéndez et al., 2016 | Travellers | 185 | 13 | 2 | 15 (0–41) | Bias towards symptomatic patients. World Health Organization definition of symptoms was applied to data |
| Leal et al., 2016         | Babies with microcephaly | 70 | 63 | 9 | 14 (7–24) | NA |
| Pacheco et al., 2016      | Babies with microcephaly | 50 | 4 | 4 | 100 (61–100) | NA |
| Parra et al., 2016        | Adults with Guillain–Barré syndrome | 42 | 17 | 0 | 0 (0–10) | Authors reported two definitions of Zika virus-positive: definite and probable. We used results from the definite definition |
| Adhikari et al., 2017     | Pregnant women travellers | 547 | 29 | 24 | 83 (67–95) | All pregnant women who had recently travelled were screened |
| Aubry et al., 2017        | General population: schoolchildren | 476 | 312 | 91 | 29 (24–34) | NA |
| Flamand et al., 2017      | General population: Pregnant women | 896 | 251 | 123 | 49 (43–55) | NA |
| Lozier et al., 2017       | General population | 367 | 114 | 65 | 57 (48–66) | Household-based cluster investigation around 19 index cases |
| Meneses et al., 2017      | Babies with congenital Zika virus syndrome | 87 | 87 | 21 | 24 (16–34) | Symptoms were measured in mothers during pregnancy |
| Pomar et al., 2017        | Babies with congenital Zika virus syndrome | 124 | 9 | 3 | 33 (6–68) | Symptoms were measured in mothers during pregnancy |
|                          | Pregnant women | 1 690 | 301 | 249 | 83 (78–87) | Tried to recruit a representative sample of all pregnant women |

(continues... )
heterogeneity \( (Q = 3291, P < 0.001, I^2 = 99\%) \), suggesting that the pooled prevalence is probably not a robust estimate. Analysis based on subgroups of the population (general population, returned travellers, blood donors, adults with Guillain–Barré syndrome, pregnant women or babies with microcephaly) was not able to explain the heterogeneity (Fig. 2). There was also significant heterogeneity within all subgroups.

Both the funnel plot (Fig. 3) and Doi plot (Fig. 4) showed major asymmetry. The most likely explanations for the asymmetry are selection bias, including publication bias, or true heterogeneity in the included studies. The largest study (population-adjusted sample: 6892; actual sample: 557) had a weight of 40.7% in the meta-analysis. Excluding this study completely removed the asymmetry (Luis Furuya-Kanamori index: \(-0.57\)).

### Discussion

Although we found 23 studies for this review, the high degree of heterogeneity in the studies made it difficult to form clear conclusions as to the true prevalence of asymptomatic Zika virus infection. Furthermore, subgroup analysis by population group was unable to explain the heterogeneity. While the prevalence of asymptomatic Zika virus infection appeared to be lower in returned travellers and adults with Guillain–Barré syndrome, this could be due to the lack of representativeness of the samples, as those with symptoms are more likely to be tested.

The large variation in prevalence of asymptomatic Zika virus infection in the general population, which ranged from 29% (95% CI: 24–24%) in schoolchildren from French Polynesia to 82% (95% CI: 81–83%) in the general population of Yap State, could be due to several reasons. One possibility could be the lack of representativeness of the French Polynesia sample as the response rate was not reported. A second possibility is that the population prevalence in Yap State was underestimated due to the method of assessing symptom status, which was done retrospectively and then adjusted for the percentage unlikely to be attributable to Zika virus infection. The high degree of sensitivity of the results to the removal of this study lends support to this possibility. A third possibility is that differences in definitions of symptoms and criteria for Zika virus infection (including the diagnostic test used) could have led to differences in prevalence estimates. This possibility is supported by the lower prevalence of asymptomatic Zika virus infection in pregnant women with confirmed recent infection than in those with possible recent infection (42% versus 63%; Table 3) in the United States. Finally, the difference could be real.

The authors of a systematic review and meta-analysis of 55 influenza virus infection studies also found considerable heterogeneity in the proportion of asymptomatic infected persons. Despite the large number of studies, the heterogeneity could not be explained by the type of influenza, the laboratory tests used to detect the virus, the year of the study, or the location of the study.
Fig. 2. Prevalence of asymptomatic Zika virus infection in the systematic review of the literature

| Study or subgroup                                    | Prevalence (95% CI)     | % Weight |
|------------------------------------------------------|-------------------------|----------|
| **General population**                               |                         |          |
| Duffy et al., 2009                                   | 0.82 (0.81–0.83)        | 40.7     |
| Aubry et al., 2017                                   | 0.29 (0.24–0.34)        | 2.9      |
| Aubry et al., 2017                                   | 0.49 (0.43–0.55)        | 2.5      |
| Luzier et al., 2017                                  | 0.57 (0.48–0.66)        | 2.0      |
| **Microcephaly-babies subgroup**                     |                         |          |
| Shapiro-Mendoza et al., 2017                         | 0.34 (0.25–0.42)        | 1.4      |
| Pomar et al., 2017                                   | 0.33 (0.06–0.68)        | 1.1      |
| Meneses et al., 2017                                 | 0.24 (0.16–0.34)        | 1.0      |
| **Returned travelers subgroup**                      |                         |          |
| Díaz Menéndez et al., 2016                          | 0.15 (0.00–0.41)        | 1.1      |
| Leal et al., 2016                                    | 0.14 (0.07–0.24)        | 0.9      |
| Araujo et al., 2016                                  | 0.46 (0.20–0.74)        | 1.5      |
| **Blood donors subgroup**                            |                         |          |
| Musso et al., 2014                                   | 0.74 (0.59–0.86)        | 1.5      |
| Stone et al., 2017                                   | 0.44 (0.30–0.58)        | 0.4      |
| **Guillain-Barré syndrome subgroup**                 |                         |          |
| Cao Lomeau et al., 2016                              | 0.19 (0.02–0.21)        | 1.9      |
| Parra et al., 2016                                   | 0.10 (0.00–0.10)        | 1.1      |
| Ruiz et al., 2017                                   | 0.10 (0.03–0.51)        | 1.2      |
| **Pregnant women subgroup**                          |                         |          |
| Adams et al., 2016                                   | 0.10 (0.07–0.13)        | 2.9      |
| Diasquita et al., 2016                               | 0.25 (0.10–0.43)        | 1.0      |
| Adhikari et al., 2017                                | 0.83 (0.67–0.95)        | 1.6      |
| Flammant et al., 2017                                | 0.77 (0.73–0.80)        | 6.6      |
| Pome rate et al., 2017                               | 0.83 (0.78–0.87)        | 2.8      |
| Reynolds et al., 2017                                | 0.63 (0.40–0.96)        | 5.5      |
| Roda et al., 2017                                    | 0.56 (0.40–0.72)        | 0.2      |
| Shapiro-Mendoza et al., 2017                         | 0.38 (0.36–0.40)        | 13.4     |
| Shua et al., 2018                                    | 0.79 (0.68–0.88)        | 1.7      |
| **Guillain-Barré syndrome subgroup**                 |                         |          |
| Aubry et al., 2017                                   | 0.92 (0.84–1.00)        | 31.4     |
| **Pregnant women subgroup**                          |                         |          |
| Adams et al., 2016                                   | 0.10 (0.07–0.13)        | 2.9      |
| Diasquita et al., 2016                               | 0.25 (0.10–0.43)        | 1.0      |
| Adhikari et al., 2017                                | 0.83 (0.67–0.95)        | 1.6      |
| Flammant et al., 2017                                | 0.77 (0.73–0.80)        | 6.6      |
| Pome rate et al., 2017                               | 0.83 (0.78–0.87)        | 2.8      |
| Reynolds et al., 2017                                | 0.63 (0.40–0.96)        | 5.5      |
| Roda et al., 2017                                    | 0.56 (0.40–0.72)        | 0.2      |
| Shapiro-Mendoza et al., 2017                         | 0.38 (0.36–0.40)        | 13.4     |
| Shua et al., 2018                                    | 0.79 (0.68–0.88)        | 1.7      |
| **Blood donors subgroup**                            |                         |          |
| Musso et al., 2014                                   | 0.74 (0.59–0.86)        | 1.5      |
| Stone et al., 2017                                   | 0.44 (0.30–0.58)        | 0.4      |
| **Guillain-Barré syndrome subgroup**                 |                         |          |
| Cao Lomeau et al., 2016                              | 0.10 (0.02–0.21)        | 1.9      |
| Parra et al., 2016                                   | 0.00 (0.00–0.10)        | 1.1      |
| Ruiz et al., 2017                                   | 0.10 (0.03–0.51)        | 1.2      |
| **Pregnant women subgroup**                          |                         |          |
| Adams et al., 2016                                   | 0.10 (0.07–0.13)        | 2.9      |
| Diasquita et al., 2016                               | 0.25 (0.10–0.43)        | 1.0      |
| Adhikari et al., 2017                                | 0.83 (0.67–0.95)        | 1.6      |
| Flammant et al., 2017                                | 0.77 (0.73–0.80)        | 6.6      |
| Pome rate et al., 2017                               | 0.83 (0.78–0.87)        | 2.8      |
| Reynolds et al., 2017                                | 0.63 (0.40–0.96)        | 5.5      |
| Roda et al., 2017                                    | 0.56 (0.40–0.72)        | 0.2      |
| Shapiro-Mendoza et al., 2017                         | 0.38 (0.36–0.40)        | 13.4     |
| Shua et al., 2018                                    | 0.79 (0.68–0.88)        | 1.7      |

* schoolchildren

Notes: We searched for studies published from inception of the databases until 26 January 2018. The forest plot shows percentage of participants who tested positive for Zika virus and were asymptomatic. Prevalence was estimated from the quality effects model and using the double arcsine transformation of prevalence. The dotted line represents the combined prevalence found in the meta-analysis (0.62).

Zika virus the amount and quality of the available evidence is insufficient to provide a single estimate of the prevalence of asymptomatic infection or to determine whether the heterogeneity found in this review is real.

In relation to the heterogeneity in prevalence, comparing two included studies that presented data on completed pregnancies from the United States Zika pregnancy registry and used similar surveillance methods is important.75,76 One study in the USA found an asymptomatic Zika virus infection prevalence of 63%,76 this is consistent with an earlier report of 61% from the same population,3 suggesting little variation over time. The other study was of completed pregnancies in United States Territories (American Samoa, Puerto Rico and United States Virgin Islands) and the Federated States of Micronesia and Marshall Islands76 and found a prevalence of asymptomatic Zika virus infection of 38%.24 If the difference is real or a result of differences in ascertainment of asymptomatic Zika virus infection is difficult to know. The registry is based on surveillance systems, which depend on testing in clinical practice and which can be affected by the care-seeking behaviour of the population. This raises the issue of the ability of surveillance systems to provide unbiased results for Zika virus research questions.83,84

Although we included population subgroups in our meta-analysis there were insufficient data to study the effect of demographic variables on the prevalence of asymptomatic Zika virus. While three of the included studies reported on age, sex or geographical differences in symptomatic infection,69–71 clear conclusions were not possible to make.

A key strength of this review was the use of high-quality systematic review methods.7 Limitations of the review include the small number of studies found, especially cross-sectional seroprevalence studies, and the heterogeneity in the methods used across studies. The majority of studies included in the review were based on population health surveillance or screening programmes, rather than good-quality research studies. Furthermore, the included studies used various definitions of Zika virus positivity and rarely offered a definition for Zika virus symptom status. A variety of laboratory tests were used with varying degrees of validity, which can lead to potential misclassification error.83 A
particular issue for Zika virus infection is the serological cross-reactivity of current IgM antibody assays with dengue virus, among other flaviviruses. The potential effect on the results is not known. In several studies there was also a bias towards inclusion of participants with symptoms due to the criteria for population surveillance or because symptomatic people are more likely to seek health care (e.g. travellers returning from Zika virus-endemic areas).

One clear finding from this review is that, given the current state of the evidence, it is not possible to give an accurate figure for the prevalence of asymptomatic Zika virus. Nor is it known whether the prevalence varies between populations or over time. Better-quality research is needed to estimate prevalence in the general population and in specific population groups. The use of standardized protocols developed by WHO and partners, particularly the protocol for the cross-sectional seroprevalence study of Zika virus infection in the general population, will be important in this regard. The protocol aims to standardize the diagnostic tests and definitions used, as well as encouraging consistent reporting. Use of the protocol will ensure results can be compared across regions and countries and help to improve the quality of the studies by minimizing bias. In this way the results of studies will better inform future public health surveillance and interventions.

Acknowledgements
Michelle Haby was contracted by the Pan American Health Organization to work on the Zika virus research platform and support Zika virus research efforts during the initial stages of this review, including study selection.

Competing interests: None declared.

Fig. 3. Funnel plot of publication bias in the systematic review of the prevalence of asymptomatic Zika virus infection

Note: The vertical line represents the combined effect size from the fixed effect meta-analysis

Fig. 4. Doi plot of publication bias in the systematic review of the prevalence of asymptomatic Zika virus infection

LFK index: -5.47 (major asymmetry)

Note: The vertical line represents the combined effect size from the quality effects meta-analysis.
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Abstract

Asymptomatic Zika virus prevalence: systematic review

Objectif

Prévalence des infections à virus Zika asymptomatiques: revue systématique

Méthodes

Nous avons fait des recherches en ligne dans PubMed®, Embase® et LILACS afin de trouver des références parues depuis la date de création de ces bases de données jusqu’au 26 janvier 2018. Nous avons inclus des études épidémiologiques observationnelles dans lesquelles des tests en laboratoire ont été utilisés pour confirmer l’exposition des participants au virus Zika et dans lesquelles le statut symptomatique/asymptomatique de l’infection à virus Zika a été consigné. Nous avons écarté les études pour lesquelles l’existence de symptômes d’infection à virus Zika a été utilisée comme critère d’inclusion. Le principal résultat évalué a été le pourcentage de participants asymptomatiques infectés par le virus Zika. Pour notre méta-analyse, nous avons employé une approche qualité-effets et réalisé une transformation à double arc-sinus.

Résultats

Pour l’inclusion dans notre méta-analyse, nous avons évalué 753 études, 23 d’entre elles ont été retenues, ce qui représente 11 305 participants infectés par le virus Zika. Compte tenu de la très grande hétérogénéité des études (I² = 99%), la prévalence groupée des participants asymptomatiques infectés par le virus Zika ne constitue probablement pas une estimation fiable. L’analyse portant sur des sous-groupes de population (population générale, personnes revenant de voyages, donneurs de sang, adultes atteints du syndrome de Guillain-Barré, femmes enceintes, nouveau-nés présentant une microcéphalie) n’a pas permis d’expliquer cette hétérogénéité. Les courbes de biais (funnel plot et Doi plot) ont révélé une asymétrie majeure, suggérant ainsi un biais de sélection ou une vraie hétérogénéité.

Conclusion

Des études de meilleure qualité doivent être réalisées en utilisant des méthodes standardisées afin de déterminer la véritable prévalence des infections asymptomatiques à virus Zika et de déterminer si elle varie entre les populations ou au fil du temps.

Résumé

Распространенность бессимптомной инфекции, вызываемой вирусом Зика: систематический обзор

Цель

Распространенность бессимптомной инфекции, вызываемой вирусом Зика, в общей популяции и в определенных группах населения.

Методы

Авторы провели поиск в онлайн-базах данных PubMed®, Embase® и LILACS с момента их создания до 26 января 2018 года. В обзор были включены наблюдательные эпидемиологические исследования, в которых использовалось лабораторное тестирование для подтверждения инфицирования участников вирусом Зика, а также те, в которых было указано наличие или отсутствие проявления симптомов этой инфекции. Авторы исключили исследования, в которых наличие симптомов этой инфекции было критерием для включения. Основной результат оценивался как процентный доля участников с положительным результатом обследования на вирус Зика, у которых инфекция, определенная по отношению к общему числу участников, протекала бессимптомно. Для метаанализа авторы использовали подход, основанный на качественном эффекте, и двойное арксинус-преобразование.

Результаты

Авторы провели оценку 753 исследований, из которых 23 были включены в метаанализ, т. е. в общей сложности 11 305 участников, инфицированных вирусом Зика. Высокая степень гетерогенности в исследованиях (I² = 99%) свидетельствует о том, что общая распространенность участников, инфицированных вирусом Зика, у которых отсутствовали проявления симптомов инфекции, по-видимому, являлась недостоверной оценкой. Анализ, основанный на подгруппах населения (общая популяция, вернувшиеся из поездок путешественники, доноры крови, взрослые с синдромом Гийена — Барре, беременные женщины и дети с микроцефалией), не смог объяснить эту гетерогенность. Воронкообразная диаграмма и диаграмма ЦИО показали большую асимметрию, что свидетельствует о систематической ошибке отбора или истинной гетерогенности.

Вывод

Необходимо провести более качественные исследования с использованием стандартизированных методов для определения истинной распространенности бессимптомной инфекции, вызываемой вирусом Зика, а также выяснить, изменяется ли она среди популяций или с течением времени.
Prevalencia de la infección asintomática del virus de Zika: una revisión sistemática

Objetivo
Llevar a cabo una revisión sistemática para estimar la prevalencia de una infección asintomática del virus de Zika en la población general y en grupos de población específicos.

Métodos
Se realizaron búsquedas en las bases de datos en línea de PubMed®, Embase® y LILACS desde el origen hasta el 26 de enero de 2018. Se incluyeron estudios epidemiológicos observacionales en los que se usaron pruebas de laboratorio para confirmar la exposición positiva de los participantes al virus de Zika y en las que también se registró el estado de los síntomas del virus de Zika. Se excluyeron los estudios en los que mostraron síntomas del virus de Zika fue un criterio de inclusión. El principal resultado evaluado fue el porcentaje de todos los participantes que resultaron positivos al virus de Zika y no presentaban síntomas. Se siguió un enfoque calidad-efectos y la transformación de acoseno doble para el metanálisis.

Resultados
Se evaluaron 753 estudios para su inclusión, de los cuales 23 se incluyeron en el metanálisis, sumando un total de 11 305 participantes positivos para el virus de Zika. El alto grado de heterogeneidad en los estudios (I² = 99%) sugiere que la prevalencia combinada de participantes asintomáticos con el virus de Zika probablemente no era una estimación robusta. Los análisis basados en subgrupos de población (población general, viajeros de vuelta, donantes de sangre, adultos con síndrome de Guillain-Barré, mujeres embarazadas y bebés con microcefalia) no pudieron explicar la heterogeneidad. Los gráficos de Funnel y Doi mostraron una simetría importante, lo que sugiere un sesgo de selección o una verdadera heterogeneidad.

Conclusion
Se necesita una investigación de mejor calidad, que use métodos estandarizados, para determinar la verdadera prevalencia del virus de Zika asintomático y si varía entre las poblaciones o con el tiempo.

Referencias

1. Regional Zika epidemiological update (Americas) May 25, 2017. Pan American Health Organization, 2017. Available from: http://www.paho.org/hq/index.php?option=com_content&view=article&id=11159&Itemid=41691&lang=en [cited 2017 Jul 12].
2. Krauer F, Riesen M, Reveiz L, Oladapo OT, Martinez-Vega R, Pongo TV, et al.; WHO Zika Causality Working Group. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barre syndrome: systematic review. PLoS Med. 2017 01 5;14(1):e1002203. doi: http://dx.doi.org/10.1371/journal.pmed.1002203 PMID: 28045901
3. Honein MA, Dawson AL, Petersen EE, Jones AM, Lee EH, Yazdy MM, et al.; WHO Zika Causality Working Group. Zika virus and Guillain-Barre syndrome: is there sufficient evidence for causality? Front Neuro. 2016 09 30;7:170. doi: http://dx.doi.org/10.3389/fneur.2016.00170 PMID: 27476763
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 Jun 11;360(24):2536–43. doi: http://dx.doi.org/10.1056/NEJMoa0902460 PMID: 19503582
5. Honein MA, Dawson AL, Petersen EE, Jones AM, Lee EH, Yazdy MM, et al.; US Zika Pregnancy Registry Collaborators. Birth defects among fetuses and infants of US women with evidence of possible Zika virus infection during pregnancy. JAMA. 2017 01 31;317(5):59–68. doi: http://dx.doi.org/10.1001/jama.2016.19006 PMID: 27860197
6. Guidelines for surveillance of Zika virus disease and its complications. Washington: Pan American Health Organization, 2016
7. Lozzer M, Adams L, Febo MG, Torres-Apone J, Bello-Pagan M, Ryff KR, et al. Zika virus disease in pregnancy and sex—Puerto Rico, November 1, 2015–October 25, 2016. MMWR Morb Mortal Wkly Rep. 2016 11 11;65(44):1219–23. doi: http://dx.doi.org/10.15585/mmwr.mm6544a4 PMID: 27832051
8. Joanna Briggs Institute reviewers’ manual: 2014 edition: supplement. The Joanna Briggs Institute; 2014
9. Higgins JPT, Green S. editors. Cochrane handbook for systematic reviews of interventions, version 5.1.0 [updated March 2011]. London: The Cochrane Collaboration, 2011. Available from: www.hansbook.cochrane.org [cited 2017 Jul 12].
10. Haby M, Pinart M, Biasi V, Reveiz L. Prevalence of asymptomatic Zika Virus infection. PROSPERO 2017: CRD42017059342. York: Centre for Reviews and Dissemination, University of York, 2017. Available from: http://www.cdruyork.ac.uk/PROSPERO/display_record.php?ID=CRD42017059342 [cited 2017 Jul 12].
11. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009 Jul 21;6(7):e1000097. doi: http://dx.doi.org/10.1371/journal.pmed.1000097 PMID: 19621072
12. Systematic reviews

13. Standardized protocol: cross-sectional seroprevalence study of Zika virus infection in the general population Geneva: World Health Organization and Institut Pasteur, 2016. Available from: http://origin.who.int/reproductivehealth/zika/zika-virus-research-agenda/en/ [cited 2016 Jul 22].
14. Published primary research studies and protocols. Washington: Pan American Health Organization, 2017. Available from: http://www.paho.org/zika-research/ [cited 2017 Feb 6].
15. Hoy D, Brooks P, Woff A, Blyth F, March L, Bain C, et al. Assessing risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement. J Clin Epidemiol. 2012 Sep;65(9):934–9. doi: http://dx.doi.org/10.1016/j.jclinepi.2011.11.014 PMID: 22742910
16. Barendregt JJ, Doi SA. MetaXL User Guide. Version 5.3. Sunrise Beach: EpiGear International Pty Ltd, 2011.
17. Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. Meta-analysis of prevalence. J Epidemiol Community Health. 2013 Nov;67(11):974–8. doi: http://dx.doi.org/10.1136/jech-2013-203104 PMID: 23963506
18. Doi SA, Barendregt JJ, Khan S, Thalib L, Williams GM. Advances in the meta-analysis of heterogeneous clinical trials II: The quality effects model. Contemp Clin Trials. 2015 Nov 45 Pt A:123–9. doi: http://dx.doi.org/10.1016/j.cct.2015.02.010 PMID: 26093432
19. Alvim LB, Romano OSO, Mateu E, Ferreira ACS, Zauli D. Zika virus in Brazil: a prevalence study. Proceedings of the 68th Annual Scientific Meeting of the American Association for Clinical Chemistry, 2016, United States. Clin Chem. 2016;62(10) Supplement 1:S144–5.
20. de Araújo TVB, Ximenes RAA, Miranda-Filho DB, Souza WV, Montaneroy LR, de Melo APL, et al.; investigators from the Microcephaly Epidemic Research Group; Brazilian Ministry of Health; Pan American Health Organization; Instituto de Medicina Integral Professor Fernando Figueira; State Health Department of Pernambuco; Association between microcephaly, Zika virus infection, and other risk factors in Brazil: Final report of a case-control study. Lancet Infect Dis. 2018 Mar 18;18(3):328–36. doi: http://dx.doi.org/10.1016/S1473-3099(17)30727-2 PMID: 29240991
21. Bierlaire D, Maquén S, Broult J, Musso D. Zika virus and blood transfusion: the experience of French Polynesia. Transfusion. 2017 03;57 3pt2:729–33. doi: http://dx.doi.org/10.1111/trf.14028 PMID: 28185278
22. Brasil P, Calvet GA, Sequeira AM, Wakimoto M, de Sequeira PC, Nobre A, et al. Zika virus outbreak in Rio de Janeiro, Brazil: clinical characterization, epidemiological and virological aspects. PLoS Negl Trop Dis. 2016 04 12;10(4):e0004636. doi: http://dx.doi.org/10.1371/journal.pntd.0004636 PMID: 27070912
23. Brasil P, Pereira JP, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. Zika virus infection in pregnant women in Rio de Janeiro N Engl J Med. 2016 12 15;375(24):2321–34. doi: http://dx.doi.org/10.1056/NEJMoa1602412 PMID: 26943629
24. Brasil P, Pereira JP, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. Zika virus infection in pregnant women in Rio de Janeiro N Engl J Med. 2016 12 15;375(24):2321–34. doi: http://dx.doi.org/10.1056/NEJMoa1602412 PMID: 26943629

Bull World Health Organ 2018;96:402–413 doi: http://dx.doi.org/10.2471/BLT.17.201541
58. de Araújo TVB, Rodrigues LC, de Alencar Ximenes RA, de Barros Miranda-Arsuaga M, et al. Prospective Zika virus disease cohort: systematic screening. Lancet. 2016 Aug 27;388(10054):868. doi: 10.1016/S0140-6736(16)30562-6. PMID: 27853484

59. de Araújo TVB, Rodrigues LC, de Alencar Ximenes RA, de Barros Miranda-Arsuaga M, et al. Zika virus seroprevalence, French Polynesia, 2014–2015. Emerg Infect Dis. 2017 Sep;23(9):1634–8. doi: 10.3201/eid2309.161416 PMID: 28078739

60. Leal MC, Muniz LF, Ferreira TS, Santos CM, Almeida LC, Van Der Linden V, et al. Zika virus infection in Spain. Enferm Infecc Microbiol Clin. 2018 Jan;36(1):4–11. doi: 10.1016/j.eimc.2016.10.003 PMID: 27743483

61. Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, Gonzalez-Manrique G, Prado MV, et al.; Zika Pregnancy and Infant Registries Working Group. Pregnancy outcomes after maternal Zika virus infection during pregnancy. B US Territories, January 1, 2016–April 25, 2017. MMWR Morb Mortal Wkly Rep. 2017 Oct 16;66(16):1615–21. doi: 10.15585/mmwr.mm6616a1 PMID: 28766231

62. Stone M, Bakkour S, Lee TH, Lantier M, Simmons G, Brambilla D, et al. Zika RNA persistence in blood and body fluids and clinical outcomes in infected blood donors. Proceedings of the AABB Annual Meeting 2017, United States. Transfusion. 2017;57 Supplement 3:A4.

63. Shiu C, Starker R, Kwal J, Bartlett M, Crane A, Greissman S, et al. Zika virus testing and outcomes during pregnancy. Florida, USA, 2016. Emerg Infect Dis. 2018 Jan;24(1):1–8. doi: 10.3201/eid2401.170979 PMID: 29260671

64. Siqueira E, Prado MV, et al.; Zika Pregnancy and Infant Registries Working Group. Patterns in Zika virus testing and infection, by report of symptoms and pregnancy status: a population-based study, Brazil. J Infect Dis. 2017 Oct 16;65(9):1462–8. doi: 10.1093/cid/cix588 PMID: 29020245

65. Rodo C, Sonnino-Aranedes A, Suay A, Sulieño E, García I, Frick A, et al. Zika virus disease cohort: systematic screening. Lancet. 2016 Apr 9;387(10027):1531–9. doi: 10.1016/S0140-6736(16)30562-6. PMID: 27853484

66. Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, Gonzalez-Manrique G, Prado MV, et al.; Zika Pregnancy and Infant Registries Working Group. Pregnancy outcomes after maternal Zika virus infection during pregnancy. B US Territories, January 1, 2016–April 25, 2017. MMWR Morb Mortal Wkly Rep. 2017 Oct 16;66(16):1615–21. doi: 10.15585/mmwr.mm6616a1 PMID: 28766231

67. Shiu C, Starker R, Kwal J, Bartlett M, Crane A, Greissman S, et al. Zika virus testing and outcomes during pregnancy. Florida, USA, 2016. Emerg Infect Dis. 2018 Jan;24(1):1–8. doi: 10.3201/eid2401.170979 PMID: 29260671

68. Adhikari EH, Jacoby SO, Rogers VL, Roberts SW, Nelson DB, et al. Infant outcomes among women with Zika virus infection during pregnancy: results of a large prenatal Zika screening program. Am J Obstet Gynecol. 2017;216(1 Supplement 1):S345. doi: 10.1016/j.ajog.2017.01.018 PMID: 28536655

69. Leal MC, Muniz LF, Ferreira TS, Santos CM, Almeida LC, Van Der Linden V, et al. Zika virus infection in Spain. Enferm Infecc Microbiol Clin. 2018 Jan;36(1):4–11. doi: 10.1016/j.eimc.2016.10.003 PMID: 27743483

70. Meneses JDA, Ishigami AC, de Mello LM, de Albuquerque LL, de Brito CA, Cordeiro MT, et al. Lessons learned at the epicenter of Brazil’s congenital Zika epidemic: evidence from 978 confirmed cases. Clin Infect Dis. 2017 May 15;64(10):1302–8. doi: 10.1093/cid/cix690 PMID: 28432957

71. Menezes JDA, Ishigami AC, de Mello LM, de Albuquerque LL, de Brito CA, Cordeiro MT, et al. Lessons learned at the epicenter of Brazil’s congenital Zika epidemic: evidence from 978 confirmed cases. Clin Infect Dis. 2017 May 15;64(10):1302–8. doi: 10.1093/cid/cix690 PMID: 28432957

72. Van Kerkhove MD, Reveiz L, Souza JP, Jaenisch T, Carson G, Broutet N; Zika Virus Response Epidemiology and Laboratory Team. Patterns in Zika virus testing and infection, by report of symptoms and pregnancy status: a population-based study, Brazil. J Infect Dis. 2017 Oct 16;65(9):1462–8. doi: 10.1093/cid/cix588 PMID: 29020245

73. Stone M, Bakkour S, Lee TH, Lantier M, Simmons G, Brambilla D, et al. Zika RNA persistence in blood and body fluids and clinical outcomes in infected blood donors. Proceedings of the AABB Annual Meeting 2017, United States. Transfusion. 2017;57 Supplement 3:A4.

74. Asymptomatic Zika virus prevalenceMichelle M Haby et al.

75. Asymptomatic Zika virus prevalenceMichelle M Haby et al.
### Table 2. Characteristics of studies included in the systematic review of the prevalence of asymptomatic Zika virus infection

| Study, author and year of primary reference | Country or territory | Population | Study design | Definition of Zika virus positive | Definition of symptomatic Zika virus | Risk of bias score |
|-------------------------------------------|----------------------|------------|--------------|-----------------------------------|--------------------------------------|-------------------|
| Duffy et al., 2009⁴                       | Federated States of Micronesia (Yap State) | General population | Cross-sectional | Evidence of recent infection: positive for IgM antibody against Zika virus by ELISA in serum | Defined as acute onset of generalized macular or papular rash, arthritis or arthralgia, or non-purulent conjunctivitis | 8                 |
| Musso et al., 2014⁵                       | French Polynesia | Blood donors | Cross-sectional | Positive to Zika virus nucleic acid test in serum by real-time RT–PCR | Not defined. Blood donors who were Zika-virus positive were telephoned and asked about "Zika fever-like syndrome" (rash, conjunctivitis, arthralgia) after their donation | 7                 |
| Adams et al., 2016⁵                        | USA (Puerto Rico) | Pregnant women | Case series (surveillance) | Confirmed case: positive by RT–PCR in blood or urine. Presumptive case: positive Zika virus IgM by ELISA and negative dengue virus IgM by ELISA, or positive Zika virus by MAC-ELISA in a pregnant woman | Not defined | 5                 |
| Araujo et al., 2016⁶                       | Brazil (metropolitan region of Recife) | Cases: neonates with microcephaly. Controls: live neonates without microcephaly, with no brain abnormalities or birth defects | Case–control | Positive by RT–PCR or IgM serum test of mothers and neonates | Not defined. Presence of maternal rash was reported | 8                 |
| Gao-Lormeau et al., 2016⁷                  | French Polynesia | Cases: adults with Guillain–Barré syndrome. (Controls: excluded because no data on Zika symptoms were reported) | Case–control | Presence in serum of PRNT antibodies for Zika virus and anti-Zika virus IgG or IgM | Not defined. Described as recent history of viral syndrome before onset of neurological symptoms. Participants’ most commonly reported rash, arthralgia and fever | 9                 |
| Dasgupta et al., 2016⁸                     | USA | Travellers; pregnant women travellers | Case series (surveillance) | Confirmed case: detection of Zika virus RNA by RT–PCR or; anti-Zika IgM antibodies by ELISA with neutralizing antibody titres against Zika virus, at levels ≥4-fold higher than those against dengue virus | Defined as at least one of the following: fever, rash, arthralgia, or conjunctivitis | 5                 |
| de Laval et al., 2016⁹                     | French Guiana | Travellers | Cohort | Confirmed case: viral RNA detected by real-time PCR in blood or urine, or Zika virus IgM antibodies and neutralizing antibodies found in serum. Malaria excluded by thin and thick blood smears; dengue and chikungunya viruses excluded by blood real-time PCR | Not defined. All participants had cutaneous rash or other symptoms | 3                 |

(continues . .)
| Study, author and year of primary reference<sup>a</sup> | Country or territory | Population | Study design | Definition of Zika virus positive | Definition of symptomatic Zika virus | Risk of bias score<sup>b</sup> |
|---|---|---|---|---|---|---|
| Díaz-Menéndez et al., 2016<sup>62,63</sup> | Spain (Madrid; one hospital) | Travellers<sup>d</sup> | Case series | Confirmed case: positive microneutralization antibodies and/or positive RT–PCR for RNA in urine, blood, semen or amniotic fluid<sup>d</sup> | Not defined. Participants had one or more of: temperature > 38 °C, maculopapular rash, arthralgia, red eyes or headache | 6 |
| Leal et al., 2016<sup>64</sup> | Brazil (Pernambuco; one hospital) | Babies with microcephaly | Case series | Positive by Zika virus-specific IgM capture ELISA in cerebrospinal fluid | Not defined. Presence and timing of maternal rash during pregnancy was reported | 4 |
| Pacheco et al., 2016<sup>65</sup> | Colombia | Babies with possible microcephaly | Case series (surveillance) | Positive for Zika virus RNA in serum using RT–PCR and negative for syphilis, toxoplasmosis, other agents, rubella, cytomegalovirus and herpes virus tests, and normal karyotypes | Defined as fever and rash, plus at least one of the following symptoms: nonpurulent conjunctivitis, headache, pruritus, arthralgia, myalgia or malaise | 6 |
| Parra et al., 2016<sup>66</sup> | Colombia (Cucuta, Medellín, Neiva, Barranquilla and Cali; six hospitals) | Adults with Guillain–Barré syndrome | Case series | Definite case: positive for Zika virus RNA in blood, cerebrospinal fluid or urine by RT–PCR. Probable case: positive ELISA for antiflavivirus antibodies in cerebrospinal fluid, serum or both, but negative RT–PCR for Zika virus and for the four dengue virus serotypes | Defined as onset of systemic symptoms by Pan American Health Organization case definition | 6 |
| Adhikari et al., 2017<sup>67,68</sup> | USA (Dallas, Texas) | Pregnant women travellers<sup>d</sup> | Case series (screening)<sup>f</sup> | Probable case: positive by serum IgM test or real-time RT–PCR (serum or urine or both). Confirmation by serum PRNT<sup>g</sup> | Not defined. Participants’ symptoms included rash, fever, conjunctivitis and arthralgia | 8 |
| Aubry et al., 2017<sup>69</sup> | French Polynesia | General population, including schoolchildren | Cross-sectional | Positive for Zika virus IgG in blood by recombinant antigen-based indirect ELISA (schoolchildren) or in serum by microsphere immunoassay (general population) | Not defined. Participants were asked ‘whether they had clinical manifestations suggestive of past Zika infection’ | 6 |
| Flamand et al., 2017<sup>70</sup> | French Guiana | Pregnant women | Cohort | Zika virus-positive by real-time RT–PCR in at least one blood or urine sample, or positive for Zika virus IgM antibodies in serum, irrespective of IgG results<sup>h</sup> | Defined as a clinical illness compatible with Zika virus in the 7 days before confirmation by RT–PCR or between the beginning of the outbreak and the date of laboratory diagnosis for IgM-positive cases. A compatible clinical illness was defined as at least one of the following symptoms: fever, a macular or papular rash, myalgia, arthralgia or conjunctival hyperaemia | 9 |
| Lozier et al., 2017<sup>71</sup> | Puerto Rico | General population (within 100 m radius of the residences of 19 index cases) | Cross-sectional (household-based cluster investigations) | Current infection: detection of Zika virus nucleic acid by RT–PCR in any specimen (serum, urine or whole blood). Recent infection: detection of anti-Zika virus IgM antibody by ELISA in serum. Recent flavivirus infection: detection of both anti-Zika virus IgM and anti-dengue virus IgM antibodies by ELISA in a serum specimen, in the absence of Zika virus or dengue virus nucleic acid detection (results were a subset of recent Zika virus infection). Zika virus positivity: evidence of current or recent Zika virus or flavivirus infection | Defined as presence of rash or arthralgia | 7 |

(continues . . )
| Study, author and year of primary reference | Country or territory | Population | Study design | Definition of Zika virus positive | Definition of symptomatic Zika virus | Risk of bias score |
|-------------------------------------------|----------------------|------------|--------------|----------------------------------|-------------------------------------|------------------|
| Meneses et al., 2017 [72]                 | Brazil               | Babies with congenital Zika virus syndrome | Case series | Zika virus-specific IgM tested by MAC-ELISA in cerebrospinal fluid. Positive results were followed by PRNT to confirm specificity of IgM antibodies against Zika virus and rule out cross-reactivity against other flaviviruses, including dengue virus. | Defined as presence of symptoms related to a possible Zika virus infection during gestation: fever, maculopapular rash, arthralgia and conjunctivitis | 4                |
| Pomar et al., 2017 [73, 74]               | French Guiana (Western part) | Pregnant women, Babies with congenital Zika virus syndrome | Case series (screening) | Positive by RT–PCR (using the RealStar® Zika kit; Altona Diagnostics GmbH, Hamburg, Germany) in blood or urine or both, or by anti-Zika virus antibody detection using an in-house (National Referral Centre) IgM and IgG antibody-capture ELISA | Not defined. Participants' symptoms were fever, pruritus, erythema, conjunctivitis, arthralgia or myalgia | 6                |
| Reynolds et al., 2017 [5, 75]             | USA                  | Pregnant women | Case series (surveillance) | Recent possible infection: based on presence of Zika virus RNA by nucleic acid test (e.g. RT–PCR) on any maternal, placental, fetal, or infant specimen (serum, urine, blood, cerebrospinal fluid, cord serum and cord blood); or serological evidence of recent Zika virus infection or recent unspecified flavivirus infection from a maternal, fetal or infant specimen (i.e. Zika virus PRNT titre ≥ 10 with positive or negative Zika virus IgM, and regardless of dengue virus PRNT titre). Infants with positive or equivocal Zika virus IgM were included, provided a confirmatory PRNT was performed on a maternal or infant specimen | Not defined. Participants' symptoms were described as "preceding arbovirus-like syndrome," characterized by fever, headache, retro-orbital pain, nonpurulent conjunctivitis, maculopapular rash, arthralgia or myalgia | 5                |
| Rodo et al., 2017 [76]                    | Spain                | Pregnant women travellers | Case series | Not defined. Reported as confirmed by RT–PCR, or probable by positive Zika virus-IgM or positive Zika virus neutralization tests (specimen type not reported) | Not defined. 13/17 symptomatic pregnant women had a rash | 1                |
| Rozé et al., 2017 [77]                    | France, Martinique   | Adults with Guillain–Barre syndrome | Cohort | Recent infection: Zika virus nucleic acid detected by RT–PCR in any specimen (cerebrospinal fluid, urine and plasma); or serum antibodies to Zika virus detected by Zika virus MAC-ELISA, and negative IgM MAC-ELISA against dengue virus or positive for neutralizing antibodies against Zika virus | Not defined. Participants' symptoms were described as "preceding arbovirus-like syndrome," characterized by fever, headache, retro-orbital pain, nonpurulent conjunctivitis, maculopapular rash, arthralgia or myalgia | 6                |

(continues . . )
| Study, author and year of primary reference | Country or territory | Population | Study design | Definition of Zika virus positive | Definition of symptomatic Zika virus | Risk of bias score |
|-------------------------------------------|---------------------|------------|--------------|---------------------------------|-------------------------------------|-------------------|
| Shapiro-Mendoza et al., 201778            | United States Territories and freely associated States | Pregnant women. Babies with ≥ 1 birth defect | Case series (surveillance) | Recent possible infection: based on presence of Zika virus RNA by nucleic acid test (e.g. RT–PCR) on any maternal, placental, fetal, or infant specimen (serum, urine, blood, cerebrospinal fluid, cord serum and cord blood); or serological evidence of recent Zika virus infection or recent unspecified flavivirus infection (i.e. Zika virus PRNT titer ≥ 10 with positive or negative Zika virus IgM, and regardless of dengue virus PRNT titer). Infants with positive or equivocal Zika virus IgM were included, provided a confirmatory PRNT was performed on a maternal or infant specimen (serum, urine, and cerebrospinal fluid) | Defined as one or more signs or symptoms consistent with Zika virus disease: acute onset of fever, rash, arthralgia or conjunctivitis | 5 |
| Stone et al., 201779                      | USA                 | Zika virus RNA-positive blood donors | Cohort | Blood compartments and body fluids (whole blood, plasma, urine, saliva and semen) were tested for Zika virus RNA by real time RT–PCR. Plasma samples were tested for Zika virus IgM and IgG antibodies (specimen type not reported) | Not defined. Participants developed “multiple Zika virus-related symptoms” | 2 |
| Shiu et al., 201880                       | USA                 | Pregnant women | Case series (screening) | PRNT was performed if real-time RT–PCR or IgM in serum or urine was positive. Women with non-negative Zika virus IgM, Zika virus PRNT > 10 and dengue virus PRNT < 10 were considered to be infected with Zika virus. Women with IgM-positive tests, but with PRNT results not yet available were also included | Not defined. Participants had “documented symptoms suspicious for Zika virus infection” | 7 |

ELISA: enzyme-linked immunosorbent assay; Ig: immunoglobulin; MAC-ELISA: IgM antibody capture enzyme-linked immunosorbent assay; RNA: ribonucleic acid; PRNT: plaque reduction neutralization test; RT–PCR: reverse transcription-polymerase chain reaction; USA: United States of America.

1. If a study had more than one reference, we awarded one reference the status of primary reference.
2. The risk of bias was measured using the critical appraisal checklist for prevalence studies developed by the Joanna Briggs Institute, which has a maximum score of 10. The risk of bias scores ranged from 1 to 9, with a mean score of 5.8.
3. A sample was considered positive when amplification showed a cycle threshold value < 38.5. However, to avoid false-negative results due to the pooling, each minipool showing a cycle threshold value < 40 with at least one primer-probe set was controlled by individual RT–PCR. Even if the two primers-probe sets did not react with the four dengue virus serotypes, the specificity of the amplified product from two donors whose blood was Zika virus-positive by RT–PCR was controlled by sequencing.
4. Travelers were those with recent travel to or from a Zika-affected area.
5. A patient where the detection of RNA of Zika virus by means of a confirmed positive PCR (two positive PCRs designed with different genomic targets and similar sensitivity or in different aliquots of the same sample) was obtained, was considered as a confirmed case. The confirmation of positive cases by immunofluorescence tests requires positive results in microneutralization tests.
6. The study was actually a cohort study but only the baseline data are used here.
7. Serum IgM assay was performed by Dallas County Health and Human Services for specimens collected > 2 weeks after travel in asymptomatic and symptomatic pregnant women, up to 9 months after return from travel. Presumptive positive or equivocal serum IgM specimens were forwarded to the United States Centers for Disease Control and Prevention for confirmatory PRNT testing. Serum real-time RT–PCR for Zika virus RNA was performed by Dallas County Health and Human Services on any specimen collected within 4 weeks of symptom onset or within 6 weeks of return from travel. In August 2016, following release of the interim guidance for urine testing and evaluation of pregnant women, the authors implemented real-time RT–PCR testing of subsequent urine specimens for pregnant women with presumptive positive or equivocal serum IgM.26
8. Serology was done using an in-house MAC-ELISA (based on whole virus antigens obtained in cell culture and on hyperimmune ascitic fluid) at each trimester of pregnancy. The sensitivity of the test was evaluated in sera from 71 patients with Zika virus infection confirmed by real-time PCR between day 5 and day 20 after symptom onset, was 87% and increased to more than 98% for sera sampled after day 7 from symptoms onset. The specificity was very low in sera from people with confirmed acute dengue virus infection, but increased to more than 80% for a panel of serum-negative samples for all tested arboviruses.
9. The use of PRNT for confirmation of Zika virus infection is not routinely recommended in Puerto Rico; dengue virus is endemic and cross-reactivity is likely to occur in most cases. In Puerto Rico, detection of Zika virus IgM antibodies in a pregnant woman, fetus or infant (within 48 hours after delivery) was considered sufficient to indicate recent possible Zika virus infection.
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Title:
Prevalence of asymptomatic Zika virus infection: a systematic review

Date:
2018-06-01

Citation:
Haby, M. M., Pinart, M., Elias, V. & Reveiz, L. (2018). Prevalence of asymptomatic Zika virus infection: a systematic review. BULLETIN OF THE WORLD HEALTH ORGANIZATION, 96 (6), pp.402-+. https://doi.org/10.2471/BLT.17.201541.

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