# Pregnancy and fertility-related adverse outcomes associated with *Chlamydia trachomatis* infection: a global systematic review and meta-analysis

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# Abstract

**Background**: Genital chlamydia infection in women is often asymptomatic, but may result in adverse outcomes before and during pregnancy. The purpose of this study was to examine the strength of the relationships between chlamydia infection and different reproductive health outcomes and to assess the certainty of the evidence.

**Methods**:This review was registered (CRD42017056818) and followed Cochrane guidelines. We searched three databases to quantitatively examine adverse outcomes associated with chlamydia infection. We included pregnancy and fertility-related outcomes. We performed meta-analyses on different study designs for various adverse outcomes using unadjusted and adjusted analyses.

**Results:** We identified 4,730 unique citations and included 107 studies reporting twelve pregnancy and fertility-related outcomes. Sixty-eight studies were conducted in high-income countries, 37 studies were conducted in low or middle-income countries, and two studies were conducted in both high and low-income countries. Chlamydia infection was positively associated with almost all of the 12 included pregnancy and fertility-related adverse outcomes in unadjusted analyses, including stillbirth (odds ratio = 5.05, 95% confidence interval (CI) 2.95-8.65 for case-control studies and risk ratio = 1.28, 95%CI 1.09-1.51 for cohort studies) and spontaneous abortion (odds ratio = 1.30, 95% CI 1.14-1.49 for case-control studies and risk ratio = 1.47, 95%CI 1.16-1.85 for cohort studies). However, there were biases in the design and conduct of individual studies, affecting the certainty of the overall body of evidence. The risk of adverse outcomes associated with chlamydia is higher in low- and middle-income countries compared to high-income countries.

**Conclusion**: Chlamydia is associated with an increased risk of several pregnancy and fertility-related adverse outcomes in unadjusted analyses, especially in low- and middle-income countries. Further research on how to prevent the sequelae of chlamydia in pregnant women is needed.

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**Keywords:** *Chlamydia trachomatis*; pregnancy; low and middle-income countries; infertility; adverse pregnancy outcomes

**Key messages:**

# *Chlamydia trachomatis* is associated with many adverse pregnancy and fertility-related outcomes in unadjusted analyses.

However, due to biases in the design and conduct of individual studies, the overall certainty of the evidence was low.

# There is a stronger association between chlamydia infection and adverse pregnancy outcomes in low- and middle- income countries compared to high-income countries.

# Further research on how to prevent the sequelae of chlamydia in pregnant women is needed.

# Background

*Chlamydia trachomatis* is the most common bacterial sexually transmitted infection (STI) in the world.1-3 In 2012, the global prevalence of chlamydia infection was 4.2% in women.4 Untreated chlamydia infection can cause pelvic inflammatory disease, mucopurulent cervicitis, and endometritis.5-7 Chlamydia infection has been linked to spontaneous abortion, chorioamnionitis, pre-term premature rupture of membranes, ectopic pregnancy, premature delivery, and stillbirth.8-10 In addition, chlamydia increases the risk of male and female infertility. Understanding the strength of associations between chlamydia infection and adverse outcomes before and during pregnancy is essential for improving maternal and child health programs.

Guidelines on chlamydia screening during pregnancy vary widely. The World Health Organization recommends a syndromic approach, which has been shown to miss 20-70% of cases.11 While United States Centers for Diseases Control and Prevention guidelines advocate for universal chlamydia screening in pregnant women,12 United Kingdom guidelines do not recommend routine chlamydia screening in pregnant women.13 There are limited data on the cost-effectiveness of prenatal chlamydia screening and treatment programs.14 Modeling studies suggest that the effectiveness of chlamydia screening largely depends on the likelihood of developing adverse outcomes.15-17

To better inform modeling studies and policy, more evidence is needed examining the associations between chlamydia infection and adverse pregnancy outcomes. Previous studies estimating the global burden of chlamydia infection,18 19 suggested the cost-effectiveness of chlamydia screening among women20 and identified morbidity associated with chlamydia infection in pregnancy as a significant global health problem, especially in low and middle-income countries.11 21 In addition, a few studies have confirmed the association between chlamydia infection and pregnancy and fertility-related adverse outcomes.22 23 However, limited studies have evaluated the bias in the design and conduct of individual studies that investigated those associations, and almost no study has summarized the certainty of the overall body of evidence. The purpose of our study was to examine the strength of the relationships between chlamydia infection and different reproductive health outcomes and to assess the certainty of the evidence.

# Methods

The protocol for this study was registered in PROSPERO, the International Prospective Register of Systematic Reviews (CRD42017056818, Supplementary 1 protocol). We followed the PRISMA checklist in writing and reporting this systematic review (Supplementary checklist 2).24

## Search Strategy and Selection Criteria

We searched three electronic databases (Medline via PubMed, EMBASE, and Cochrane) for studies on the relationship between chlamydia infection and any adverse outcomes related to pregnancy. The search terms are provided in Supplementary Data 3.

We included studies published in English before 31 May 2018 if they were quantitative and published in a peer-reviewed journal, including randomized controlled trials, prospective cohorts, retrospective cohorts, case-control studies, and cross-sectional studies. We excluded dissertations, conference abstracts, non-English language publications, and studies that did not quantitatively report data on adverse outcomes. Two independent reviewers (JM and WT) evaluated titles and abstracts for relevance. Full-text articles were gathered with the assistance of a global health librarian experienced in search algorithm development. Each full-text article was also assessed by two of four independent reviewers (JM, WT, RF, or WC). Discrepancies were brought to a third independent reviewer (JT) for discussion and resolution. We did not obtain original data sets from authors. Detailed study screening methods and a PRISMA flow diagram are provided in Fig 1.

We extracted the following variables for each publication: author, journal, year, study design, study period, population demographics, sample size, study country, chlamydia diagnostic, history of chlamydia treatment, human immunodeficiency virus infection, and adverse pregnancy outcomes. For case-control studies and cross-sectional studies, we extracted the numbers of patients with and without chlamydia infection among cases (women with adverse pregnancy outcomes) and among controls (women without adverse pregnancy outcomes). For cohort studies, we extracted cases and non-cases in the exposed group (women with chlamydia infection) and the unexposed group (women without chlamydia infection). In addition, we extracted the adjusted odds ratio (OR) or risk ratio (RR) from the included studies, if reported. We defined study as a single publication which may include multiple findings relevant to several study results.

## Assessment of risk of bias and study quality

For each study, we assessed the risk of bias using the Quality Assessment Tool for Quantitative Studies.25 26 Two reviewers assessed the risk of bias in six categories: selection bias, study design, confounders, blinding, data collection methods, withdrawals, and drop-outs. Each component was given a rating of one (strong quality) to three (weak quality).26 The risk of bias in each study is reported in Supplementary data 4A. We then assessed the certainty of the evidence for each outcome using the GRADE working group criteria.27 The summary of the findings and the level of certainty of the overall body of evidence were assessed using GRADEpro version 3.6 for Windows. Definitions for the included adverse outcomes are provided in the GRADE table (supplementary data 6). We compared findings from higher quality and lower quality studies, with the overall quality based on an assessment of the flaws in different domains and the seriousness of the flaws.

## Analysis and Synthesis

We examined a total of nine pregnancy-related adverse outcomes: stillbirth, infant death, spontaneous abortion, preterm labor (< 37 weeks), low birth weight, small for gestational age, premature rupture of membranes, postpartum endometritis, and preeclampsia. We examined three fertility-related outcomes: infertility, tubal factor infertility, and ectopic pregnancy. Tubal factor infertility was defined in women having proven infertility due to tubal occlusion (bilateral or unilateral) and confirmed by laparoscopy. For each case-control study and cross-sectional study, we calculated odds ratios from raw published study data. For each cohort study, we calculated relative effects from raw published study data. For the included studies, we included all women diagnosed with chlamydia as cases or exposures regardless of whether they were treated. For infertility, we included tubal factor infertility as a subgroup. We also extracted the adjusted estimates available in the literature and compared them with the unadjusted estimates. A Z-test was used to compare the log-transformed pooled crude estimates and the pooled adjusted estimates.

We conducted thirty-seven meta-analyses to determine the pooled odds ratio or risk ratio of chlamydia in women with adverse outcomes compared to women without chlamydia (Table 1). Meta-analyses were done according to outcomes and study designs. As there were limited studies with adjusted outcomes, we reported unadjusted relative effect in the forest plots. Meta-analyses were performed using STATA 15.0 (StataCorp LP). Heterogeneity was assessed with an *I*-squared statistic.28 We used a random-effects model. In comparison to a fixed-effects model, a random-effects model has a wider confidence interval for the underlying parameter and better anticipates the possibility that other unpublished studies may report different outcomes from those included in the meta-analysis.29 To detect publication bias, we visually inspected funnel plots for asymmetry for each meta-analysis with ten or more studies. An Egger test was also used to detect publication bias.

To investigate potential causes of heterogeneity, we conducted subgroup analyses among all outcomes with 10 or more case-control studies based on World Bank income classification (high versus low-middle income countries), year of publication (before or after 2000), and category of specimen type as a surrogate for diagnostic accuracy (serum versus urine or reproductive tract). If a study could not be classified into either sub-group, we excluded it from the subgroup analysis. Adjusted risk ratios were used in subgroup analyses.

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The funders had no role in the decision to publish or in any of the analysis or writing associated with this manuscript.

# Results

## Study selection

A total of 6429 studies were identified and 1699 duplicates were removed. Each of the 4730 unique titles was examined and 4506 studies were excluded. The resulting 224 full-text studies were examined and 117 of these were excluded: 104 full-text articles did not meet inclusion criteria; the full-texts for 13 studies were unable to be located. A total of 107 studies reporting a total of 160 observations were included in the analysis. We focused on reporting twelve study outcomes (Fig 1, Supplementary data 4B).

Of the 107 included studies, 36 studies analyzed more than one outcome. Thirty-seven studies were conducted in low-middle income countries and 68 were conducted in high-income countries. Two studies were conducted in both low-middle and high-income countries. Forty-eight studies were conducted before 2000 and 59 studies were conducted in 2000 or later (Supplementary data 4B). All studies included in this review were case-control studies, cross-sectional studies, or cohort studies. Only two studies specified whether patients received treatment for chlamydia. The sample size for the included studies ranged from 55 to 354,127, with a median of 326 participants. Diagnostic methods varied widely across studies. Fifty-six studies used serum samples for diagnosis and 51 studies used urine or reproductive tract samples.

All included studies investigated the relationship between chlamydia and adverse pregnancy outcomes (see Supplementary data 4B). Among the 107 included studies, they examined the impact of chlamydia on stillbirth (seven studies),30-36 infant death (three studies),37-39 infertility (30 studies),5 40-60 w1-w8 (see Supplementary data 11), tubal factor infertility (12 studies),5 41 42 44 48 50 54 57 58 w1 w3 w5 spontaneous abortion (23 studies),59 60 w2 w6 w9-w25 preterm birth (25 studies),31 32 34 35 37 78 w24 w26-w44 ectopic pregnancy (29 studies),5 30 45 48 w5 w8 w15 w18 w45-w65 low birth weight (11 studies),34 37 38 w24 w26 w30 w32 w34 w36 w43 w66 small for gestational age (six studies),32 34 35 38 w39 w66 premature rupture of membranes (eight studies),34 37 w23 w32 w42 w67-w69 postpartum endometritis (four studies),34 w30 w70 w71 and preeclampsia (two studies).w72 w73 Nine studies contained outcomes for both tubal infertility and infertility. As a results, we stratified the results within each of the nine studies provided two effect estimates in the forest plot. We conducted a total of 37 meta-analyses, including one for each outcome for each study design. This included 34 for case-control and cohort studies (Table 1) and three for cross-sectional studies (supplementary data 7). All included studies for each of these meta-analyses are listed in Supplementary data 4B.

## Risk of bias in individual studies and overall certainty of the evidence

We examined the risk of bias in individual studies based on the six domains. The overall risk of bias analysis suggests that the original quality of the included studies is low. Those findings are presented in Supplementary data 4A.

We examined biases associated with small studies. In order to assess biases associated with small studies, we examined funnel plots for case-control studies of infertility, case-control studies of spontaneous abortion, case-control studies of preterm birth, cohort studies of preterm birth, and case-control studies of ectopic pregnancy (Supplementary data 5). Most of the funnel plots were roughly symmetrical except for case-control studies of preterm birth (Bias [95%CI] =2.67 [0.39,4.95], *P*=0.027) and case-control studies of spontaneous abortion (Bias [95%CI] =3.00 [0.70,5.31], *P*=0.015). In these two funnel plots, the gap in the lower left of the funnel suggests that studies with low precision and small odds ratios have not been published.

We examined the overall certainty associated with the evidence. The overall certainty for each of the 12 pregnancy and fertility-related adverse outcomes was either low or very low (Table 1). Those results are further detailed in Supplementary data 6. We also present the results for each relevant outcome from cross-sectional studies in Supplementary data 7. However, we could not obtain adjusted odds ratios for cross-sectional studies because of the small number of cross-sectional studies and the fact that few controlled for confounders.

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| **Table 1: Summary of findings for quantitative analysis from the case-control and cohort studies, with unadjusted and adjusted results** |  |
| **Outcome** | Overall Sample | Case-Control Study (Unadjusted effect size) | Case-Control Study (Adjusted effect size) | Cohort Study (Unadjusted effect size) | Cohort Study (Adjusted effect size) |  |
| **No. of Outcomes** | **Sample Size** | **No. of Studies**  | **Sample Size** | **OR** | **I2** | **No. of Studies**  | **Sample Size** | **aOR** | **I2** | **No. of Studies**  | **Sample Size** | **RR** | **I2** | **No. of Studies** | **Sample Size** | **aRR** | **I2** | **Certainty of Evidence** |
| All adverse pregnancy-related outcomes# | 160 | 2876075 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Still birth | 8 | 415704 | 4 | 610 | 5.05[2.95,8.65] | 33.70% | N/A | N/A | N/A | N/A | 4 | 415094 | 1.28[1.09,1.51] | 19% | 3 | 408152 | 0.95[0.87,1.03] | 64.20% | low  |
| Infant death | 3 | 10494 | 2 | 6239 | 1.86[1.11,3.10] | N/A | 1 | 6156 | 1.80[1.14,2.90] | N/A | 1 | 4255 | 1.87[0.76,4.56] | N/A | 1 | 4255 | 1.02[0.37,2.81] | N/A | Very low |
| Infertility | 39 | 581309 | 24 | 8049 | 2.72[2.37,3.12] | 82.70% | 2 | 732 | 1.91[1.29,2.83] | 61.70% | 5 | 573250 | 1.84[1.77,1.90] | 90.90% | 2 | 572446 | 1.37[1.28,1.47] | 0.00% | low |
| Tubal infertility | 14 | 574323 | 7 | 1872 | 4.20[3.24,5.44] | 89.70% | 1 | 244 | 3.16[1.53,6.53] | N/A | 2 | 572446 | 1.86[1.79,1.93] | 96.50% | 2 | 572446 | 1.37[1.28,1.47] | 0.00% | low |
| Spontaneous abortion | 23 | 13429 | 14 | 11694 | 1.30[1.14,1.49] | 81.10% | 2 | 2858 | 0.97[0.79,1.19] | 0% | 4 | 1529 | 1.47[1.16,1.85] | 50.60% | 1 | 615 | 0.70[0.40,1.22] | N/A | Very low |
| Preterm labor  | 25 | 606793 | 11 | 7792 | 1.29[1.11,1.50] | 81.70% | 4 | 4106 | 1.15[0.95,1.39] | 84.40% | 13 | 599000 | 1.54[1.48,1.60] | 98.00% | 6 | 586317 | 1.09[1.03,1.15] | 34.10% | low |
| Ectopic pregnancy | 31 | 619271 | 25 | 14573 | 3.24[2.95, 3.55] | 87.000% | 10 | 10480 | 2.82[2.52,3.16] | 83.20% | 5 | 604697 | 1.67[1.64,1.70] | 89.40% | 4 | 604578 | 1.31[1.27,1.35] | 94.30% | low |
| Low birth weight  | 11 | 211515 | 3 | 1296 | 1.80[1.20,2.71] | 35% | 2 | 1213 | 2.36[1.41,3.94] | 0% | 7 | 210218 | 1.33[1.27,1.40] | 49% | 4 | 202151 | 1.13[0.94,1.36] | 26.2% | low |
| Small for gestational age | 6 | 400895 | 2 | 426 | 1.64[0.81,3.31] | 59% | N/A | N/A | N/A | N/A | 4 | 400670 | 1.19[1.12,1.27] | 0.00% | 2 | 393600 | 0.97[1.13,1.31] | 0.00% | low |
| Premature rupture of membranes | 8 | 6882 | 2 | 718 | 2.34[1.19,4.60] | 0% | 1 | 534 | 2.40[1.10,5.23] | N/A | 5 | 6163 | 1.53[1.20,1.96] | 51.30% | 1 | 4227 | 1.50[0.90,1.05] | N/A | Very low |
| Postpartum endometritis | 4 | 8310 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | 4 | 8310 | 1.61[1.24,2.08] | 58.90% | N/A | N/A | N/A | N/A | Very low |
| Preeclampsia | 2 | 1473 | 2 | 1473 | 1.56[0.9,2.98] | 0% | 2 | 1473 | 1.73[0.88,.3.38] | 88% | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | Very low |
| Note:#: Include stillbirth, infant death, spontaneous abortion, preterm labor, low birth weight, small for gestational age, premature rupture of membranes, postpartum endometritis, and Preeclampsia; N/A: Not available |  |

Supplementary data eight shows forest plots for the three meta-analyses related to stillbirth, spontaneous abortion, and infertility in some, but not all study designs. The odds ratios reported in supplementary data eight are only from case-control studies.

For stillbirth, in the meta-analyses of case-control studies, the odds of chlamydia were higher among women with stillbirth than women without stillbirth (pooled unadjusted OR=5.05, 95% CI 2.95-8.65, *I2*=33.7%, n=610, Supplementary data 8). In the meta-analysis of cohort studies, the risk ratio of stillbirth was higher among women with chlamydia than women without chlamydia (pooled unadjusted RR=1.28, 95%CI 1.09-1.51, I2=19%, n=415,094, Supplementary data 8).

For spontaneous abortion, the odds of chlamydia infection in case-control studies were higher among women with spontaneous abortion compared to women without spontaneous abortion (pooled unadjusted OR = 1.30, 95% CI 1.14-1.49], *I2* = 81.1%, n = 11,694, Supplementary data 8). The risk ratio of spontaneous abortion in cohort studies was higher among women with chlamydia compared to women without chlamydia (pooled unadjusted RR=1.47, 95%CI 1.16-1.85, *I*2=50.6%, n=1,592).

For infertility, the odds of chlamydia in case-control studies were higher among women with infertility compared to women without infertility (pooled unadjusted OR=2.72, 95% CI 2.37-3.12, *I2*=82.7%, n=8,049, Supplementary data 8). The risk ratio of infertility in cohort studies was higher among women with chlamydia compared to women without chlamydia (pooled unadjusted RR=1.84, 95% CI 1.77-1.90, *I*2=90.9%, n=573,250).

Our meta-analysis also suggests that chlamydia is associated with infant death (unadjusted OR = 1.86, 95% CI 1.12-3.10, I2 = 0%; unadjusted RR=1.87, 95%CI 0.76-4.56, *I*2=N/A), preterm labor (unadjusted OR =1.29, 95% CI 1.11-1.50, *I2* = 81.7%; unadjusted RR=1.54, 95%CI 1.48-1.60, *I2=*98%), ectopic pregnancy (unadjusted OR =3.24, 95% CI 2.95-3.55, *I2* = 87.0%; unadjusted RR=1.67, 95%CI 1.64-1.70, *I2=*89.4%), low birth weight (unadjusted OR = 1.80, 95% CI 1.20-2.71, *I2* = 61.1%; unadjusted RR=1.33, 95%CI 1.27-1.40, *I2=*49%) and premature rupture of membranes (unadjusted OR = 2.34, 95% CI 1.19-4.60, *I2* = 0%; unadjusted RR=1.53, 95%CI 1.20-1.96, *I2=*51.3%). We also observed an association between chlamydia and small for gestational age in case control studies (unadjusted OR = 1.64, 95% CI 0.81-3.31, *I2* = 59%) and cohort studies (unadjusted RR=1.19, 95%CI 1.13-1.29, *I2=*0%). In cohort studies, we also observed an association between chlamydia and postpartum endometritis (unadjusted RR=1.61, 95%CI 1.24-2.08, *I2=*58.9%). Forest plots for these outcomes are provided in Supplementary data 9. For both case-control and cohort studies, six out of 11 meta-analyses of these study designs had *I*2 > 50%. The overall certainty of evidence associating with each of these findings is presented in Supplementary data 6.

## Comparison of unadjusted odds ratios with adjusted odds ratios

Within each study design, we compared the pooled unadjusted estimates with the pooled adjusted estimates. Results showed that the point estimate is lower for the adjusted estimate compared to the unadjusted estimate for several outcomes (spontaneous abortion, infertility, tubal infertility, stillbirth, infant death, preterm labor), suggesting that controlling for confounding reduces the strength of association (Table 1).

## Subgroup meta-analyses

We also conducted subgroup meta-analyses on case-control studies by country income level (low versus middle or high), year of publication (before or after 2000), and diagnostic sample (serum versus reproductive tract or urine) for spontaneous abortion, preterm labor, low birth weight, infertility, tubal factor infertility, and ectopic pregnancy (Supplementary data 10). We did not conduct subgroup meta-analyses for cohort studies because most cohort studies were conducted in high-income countries, implemented after 2000, and used serum testing. We did not include low birth weight in the subgroup analyses because there were insufficient data for the subgroups of low-income countries, before 2000, and serum testing. We did not include tubal factor infertility in the subgroup analyses by diagnostic sample because there were insufficient data from a reproductive tract sample.

The association of spontaneous abortion with chlamydia was higher in low-middle income countries (adjusted OR = 3.10, 95% CI =2.13-4.52, *I2* = 79.3%, n = 1287) than in high-income countries (adjusted OR = 1.16, 95% CI = 1.01-1.51, *I2* = 62.7%, n = 10,407, *P<0.001*). We identified two studies that reported an adjusted association between spontaneous abortion and chlamydia infection in high-income countries (adjusted OR=0.97, 95%CI 0.79-1.19, *I*2=0%, n=2858). The association of infertility with chlamydia infection was higher in low-middle income countries (adjusted OR=3.73, 95%CI 2.89-4.82, *I*2=82.2%, n=1854) compared to high-income countries (adjusted OR=2.82, 95% CI 2.41-3.31, *I*2=86.1%, n=5,496, *P*=0.07). However, the association of tubal factor infertility with chlamydia infection was lower in low-middle income countries (adjusted OR = 4.45, 95% CI =2.84-6.99, *I2* = 96%, n = 412) compared to high-income countries (adjusted OR = 6.56, 95% CI =4.49-9.58, *I2* = 62.1%, n = 761). For ectopic pregnancy, the association with chlamydia was stronger in low-middle income countries (adjusted OR=3.45, 95%CI 2.99-3.98, *I*2=73, n=3,791) compared to high-income countries (adjusted OR=1.99, 95%CI 1.74-2.27, *I*2=62%, n=6,997, *P*<0.001) (Fig 2, Supplementary data 10).

We also compared pooled results for spontaneous abortion, preterm labor, low birth weight, infertility, tubal factor infertility, and ectopic pregnancy based on study quality. The association between spontaneous abortion and chlamydia was higher in lower quality studies (adjusted OR = 1.83, 95% CI =1.50-2.23, *I2* = 78.7%, n = 16547) than in higher quality studies (adjusted OR = 1.01, 95% CI = 0.84-1.21, *I2* = 37.6%, n = 5147 *P<0.001*). The association between infertility and chlamydia was higher in lower quality studies (adjusted OR=3.39, 95%CI 2.93-3.93, *I*2=84.6%, n=4,676) compared to higher quality studies (adjusted OR=1.60, 95% CI 1.18-2.16, *I*2=87.9%, n=3,373, *P*<0.001). In addition, the association between tubal factor infertility with chlamydia infection was higher in lower quality studies (adjusted OR = 4.27, 95% CI =4.18-7.47, *I2* = 86.8%, n = 1173) than in higher quality studies (adjusted OR =1.20, 95% CI =0.64-2.25, n = 699, *P*<0.001) (Supplementary data 10).

# Discussion

We conducted a global systematic review and meta-analysis on the association between chlamydia and adverse pregnancy and reproductive health outcomes among women. A previous review examined the prevalence of chlamydia infection in low-middle income countries.11 Our review extends previous work by quantifying the association between chlamydia and several pregnancy-related outcomes. Our unadjusted analyses suggested that chlamydia is moderately associated with ten of the twelve adverse pregnancy and reproductive health outcomes.

We found that chlamydia was associated with stillbirth and spontaneous abortion. That finding is consistent with two previous reviews which reported that chlamydia in pregnancy is positively associated with intrauterine fetal demise. However, those two studies did not disaggregate studies conducted in high-income countries and studies conducted in low-middle income countries.w74 w75,11 w76 Our review included more low-middle income country studies and more definitively addressed the link between chlamydia and stillbirth. Similarly, spontaneous abortion has not been a well-established adverse outcome of chlamydia infection in pregnancy,w77-w79 in part because causes of spontaneous abortion are difficult to ascertain.8 w78 w80 Our review provided additional evidence for the relationships between chlamydia and these two adverse outcomes.

Our results suggest that chlamydia infection is associated with infertility, especially tubal factor infertility. That is consistent with several studies that examined chlamydia infection and fertility.22 w81 w82 In low-middle income countries, infertility is a particularly challenging problem due to stigma and the lack of resources available for assisted reproductive technologies.w83 w84 Previous studies have identified bacterial reproductive tract infections as a major contributor to infertility in low-middle income countries.11 w83 w85

We found that the associations between chlamydia and spontaneous abortion, infertility, and ectopic pregnancy were stronger in low-middle income countries compared to high-income countries. This finding is consistent with previous work showing a higher prevalence and morbidity of chlamydia in low-middle income countries compared to high-income countries.11 18 Although research from low-middle income countries was limited, this difference may suggest disparities in the detection and treatment of chlamydia. Previous research has highlighted the challenges of managing STIs in low-middle income countries.11 w86 Resource-constrained settings may lack funds to procure diagnostics and often must rely on a syndromic approach to clinical management.w86 Furthermore, partner management can be difficult,w87 leading to high rates of re-infection.w88

However, there were biases in the design and conduct of individual studies that investigated causal associations. We found that pooled unadjusted estimates were slightly higher than pooled adjusted estimates. In addition, the pooled estimates in case-control studies were higher than in cohort studies. These findings indicate that the pooled estimates may be subject to bias related to study design and data analysis. When using data on associations to make aetiological inferences, we should take study design and adjustment for confounding into consideration. However, most of the included studies did not report an adjusted result. For example, for spontaneous abortion, only two studies, both from high-income countries, adjusted for confounders.

There are several potential mechanisms by which *C. trachomatis* could cause pregnancy and fertility-related adverse outcomes: ascending infection to the upper reproductive tract; persistent chlamydia infection; a pro-inflammatory response in the epithelium to either the bacteria or specific antigens; or cHSP60-induced delayed hypersensitivity.w89 w90 However, these mechanisms were not addressed in the included studies and require further investigation. We also found that observational study design, lack of blinding, and no adjustment for confounders contributed to bias. When designing future studies, researchers should take these biases into consideration.

Our review has some limitations. First, we observed substantial heterogeneity across studies, and many included studies only reported unadjusted estimates. That is likely due to differences in study populations and study design. All studies included in this review were observational studies, resulting in their overall quality being classified as low or very low. We acknowledge that the included studies are inevitably at higher risk of bias, especially from confounding variables such as previous medical history. For example, syphilis is more common among women with chlamydia infection and is also known to cause adverse pregnancy outcomes. Second, studies varied in diagnostic method, and some studies used serologic diagnostic methods. Serology may detect a previous rather than current infection.w91 However, our subanalysis did not show a consistent difference between serologically diagnosed chlamydia and chlamydia diagnosed based on urine or reproductive tract samples (Supplementary data 10). Third, 105 out of the 107 included studies did not provide information on previous chlamydia treatment. Some high-income countries have guidelines on screening and resources to implement these guidelines, which likely resulted in a high proportion of chlamydia-infected pregnant women receiving treatment. Studies in which patients were recently treated may cause our analysis to underestimate the strength of the association between chlamydia and adverse outcomes. In addition, the strengths of association were potentially underestimated in high-income countries when compared to low-middle income countries because treatment coverage may be higher in high-income countries. Fourth, we did not include grey literature and did not include non-English language publications, which may lead to selection bias. Finally, only a minority of studies came from low-middle income countries, despite the increased burden of infection in low-middle income countries.

# Conclusion

Our review demonstrates associations between chlamydia and several pregnancy and fertility-related adverse outcomes. These findings suggest a greater association between chlamydia and adverse outcomes in low-middle income countries compared to high-income countries. Our study further highlights the need for continued health systems strengthening to prevent, screen, diagnose, and treat chlamydia infection in pregnant women.

# Contributors

# JM, WT, and JDT drafted the protocol, JW performed the literature search, JM, WT, WC, and RF extracted the data. JT solved the inconsistency in the data extraction process. RC and WT evaluated the quality of the evidence. JDK, TD, RC, and JM contributed ideas for analysis. WT and KL did the statistical analysis and interpreted the results. KL and WT wrote the manuscript with input from JDT, JDK, TD, RC, and JM.

# Declaration of interests

Dr. Jeffrey D. Klausner has received research support and donated testing supplies from Cepheid and Hologic Incorporated.

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# Figures

# Fig 1. PRISMA flowchart

# Fig 2. Subgroup analysis of ectopic pregnancy, infertility, tubal infertility and spontaneous abortion by country income (case-control studies only)

Note: HICs means high-income countries; LMICs means low and middle-income countries.