

Syphilis in Pregnancy in Tanzania. II. The Effectiveness of Antenatal Syphilis Screening and Single-Dose Benzathine Penicillin Treatment for the Prevention of Adverse Pregnancy Outcomes

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Treatment for maternal syphilis with single-dose benzathine penicillin (2.4 million units intramuscularly) is being implemented in many parts of sub-Saharan Africa. To examine the effectiveness of this regimen, a prospective cohort of 1688 pregnant women was recruited in Tanzania. Birth outcomes were compared among women treated for high-titer ($n = 133$; rapid plasma reagin [RPR] titer $\geq 1:8$ and *Treponema pallidum* hemagglutination assay [TPHA]/fluorescent treponemal antibody [FTA] positive) and low-titer ($n = 249$; RPR titer $< 1:8$ and TPHA/FTA positive) active syphilis and 950 uninfected women. Stillbirth or low-birth-weight live births were observed in 2.3% and 6.3%, respectively, of women treated for high-titer active syphilis and in 2.5% and 9.2%, respectively, of seronegative women. There was no increased risk for adverse pregnancy outcome for women treated for high-titer active syphilis (odds ratio [OR], 0.76; 95% confidence interval [CI], 0.4–1.4) or low-titer active syphilis (OR, 0.95; 95% CI, 0.6–1.5), compared with seronegative women. Single-dose treatment is effective in preventing adverse pregnancy outcomes attributable to maternal syphilis.

Maternal syphilis remains an important cause of adverse pregnancy outcome in sub-Saharan Africa [1–5]. A recent study in the Mwanza region of Tanzania, discussed in a companion paper [6], has shown that 49% of women who had high-titer active syphilis (defined as a rapid plasma reagin [RPR] titer $\geq 1:8$ and a positive *Treponema pallidum* hemagglutination assay [TPHA] or fluorescent treponemal antibody [FTA] test result), experienced an adverse pregnancy outcome, compared with 11% of seronegative women. Antenatal screening and treatment of syphilis in pregnant women is a priority in regions with high syphilis seroprevalence and has been recommended as a potentially feasible and cost-effective intervention [7–10].

Guidelines for the treatment of maternal infection recommend a single intramuscular (im) dose of 2.4 million units (MU) of benzathine penicillin for the treatment of primary, secondary, and early latent syphilis and 3 doses of benzathine penicillin for late latent syphilis or syphilis of unknown duration [7, 8, 11]. However, many developing countries have implemented the single-dose regimen as their standard treatment for all stages of syphilis. When given as part of decentralized on-site screening, single-dose therapy has the advantage that patients are treated on the same day that they are tested. This should, theoretically, increase the number of detected case patients who are successfully treated [1, 10, 12, 13].

Several studies have attempted to measure the effectiveness of single-dose treatment in sub-Saharan Africa. However, most have failed to control for potential confounding effects of other factors affecting pregnancy outcome. In Zambia, 28% of pregnancies in women who attended intervention clinics that implemented health education, syphilis screening, and single-dose treatment resulted in stillbirth, miscarriage, low birth weight (LBW), preterm birth, or congenital syphilis, compared with 72% of pregnancies in women who attended control clinics [14]. However, only 46% of syphilis-seropositive women were actually treated. Furthermore, there was a one-third reduction in adverse pregnancy outcomes among mothers who did not have syphilis, suggesting that part of the observed effect on pregnancy outcome may have been due to improved health education. In South Africa, where triple-dose therapy for syphilis is recommended, 13%

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of pregnancies in women who did not receive treatment and 11% of pregnancies in women who received only 1 dose of benzathine penicillin (2.4 MU) ended in a perinatal death, compared with 2% of pregnancies in women who received 3 doses [15]. However, those receiving 2 or 3 doses of penicillin generally presented at the antenatal clinic (ANC) and received their first dose earlier in pregnancy than women receiving no treatment or only 1 injection. The timing of treatment in pregnancy or confounding factors related to earlier ANC attendance may, therefore, have explained the observed effect. This study also demonstrated the difficulty in implementing a 3-dose schedule. Only 45% of women who were syphilis seropositive had the full 3 doses, and 30% received no treatment at all. A higher risk of stillbirth was also observed in pregnant women given no or only 1 dose of benzathine penicillin for a positive RPR test result, compared with those receiving 3 doses in another South African study (odds ratio [OR], 19.4; 95% confidence interval [CI], 1.7–944.6) [16]. However, the numbers in this study were very small, and only 3 women received 1 dose of benzathine penicillin, making it difficult to draw any definitive conclusions about treatment efficacy.

There is some evidence that the treatment of syphilis in pregnancy may not always prevent adverse outcomes attributable to the infection [17, 18]. Possible reasons include reinfection, inadequate treatment, and gestational age at treatment. The risk of transmission to the infant appears to be greater if women are treated during the last trimester, compared with the first trimester [19, 20]. In practice, the most likely time to attend ANCs in sub-Saharan Africa is during the second or third trimester [10, 15], when treatment may be given too late to prevent infection of the fetus. We examined whether single-dose benzathine penicillin treatment is adequate to prevent adverse pregnancy outcomes in the Mwanza region of northwestern Tanzania, where syphilis screening is now being implemented using the single-dose benzathine penicillin regimen and where most pregnant women attend ANCs after 20 weeks of gestation.

Methods

Recruitment of the cohort. Women were enrolled in a prospective cohort at the main ANC in Mwanza, which serves ~9000 new ANC attenders each year. On-site syphilis screening began in February 1997. A 10-mL venous blood sample is routinely taken from all new attenders at the clinic for an RPR test. RPR-positive women are treated on the same day with a single dose of benzathine penicillin G (2.4 MU im). From September 1997 through November 1999, all eligible RPR-positive women screened and treated with single-dose benzathine penicillin, followed by the next 2 eligible RPR-negative women attending the ANC, were recruited. Inclusion criteria included informed consent, residence in Mwanza Municipality for at least 1 month, and an ultrasound-proven viable pregnancy. Women with a multiple gestation (i.e., >1 fetus), penicillin allergy, medical complications of pregnancy (hypertension, diabetes, or prior vaginal bleeding), or congenital fetal abnormalities were excluded. Participants were interviewed confidentially in

Swahili, using a structured, pretested questionnaire. Data were collected on sociodemographic characteristics, obstetric history, the date of the last menstrual period (LMP), and recent use of antibiotics. Serum samples from all study women were transported to the National Institute for Medical Research reference laboratory in Mwanza for further syphilis serologic testing.

Clinical procedures. An ultrasound examination was performed to identify the number of fetuses and obvious congenital deformities and to date the pregnancy. If no fetus was seen, a urine specimen was tested for human chorionic gonadotrophin (Clearview; Unipath) to confirm the pregnancy.

A first-void urine specimen was collected and placed in a cool-box. Women had a clinical examination for signs of reproductive tract infections (RTIs), and specimens were taken from the vagina and cervix. Vaginal wet preparations were examined by light microscopy in the clinic. The cohort was provided with iron and folate supplements and with chloroquine (300-mg base) as antimalarial prophylaxis, in accordance with Tanzanian national guidelines at the time of the study. Women with *Trichomonas vaginalis* or *Candida albicans* detected on their vaginal wet preparations were treated before they left the clinic. Participants were given an appointment to return to the clinic 2 weeks later and were treated for any RTI detected by reference laboratory tests, according to Tanzanian national guidelines. Recommended treatments at that time included metronidazole for the treatment of *T. vaginalis* and bacterial vaginosis, cotrimoxazole for the treatment of gonorrhoea and genital ulceration, erythromycin for the treatment of *Chlamydia trachomatis*, and clotrimazole pessaries for the treatment of vaginal candidiasis. Women who were seronegative for syphilis at the initial ANC screening who were later found to be RPR positive after retesting of serum samples at the reference laboratory were treated for syphilis with benzathine penicillin G (2.4 MU im). Those who failed to attend the ANC were contacted for treatment whenever possible.

Women recruited before 32 weeks of gestation had a second RTI screening and ultrasound examination during the third trimester. Women with proven RTIs at either visit were given contact slips for their partners to attend the clinic for free treatment. If the partner was a contact of an RPR-positive woman, he was presumptively treated with a single dose of benzathine penicillin G (2.4 MU im).

Recruited women were monitored at the time of delivery in 1 of the 2 main government hospitals in Mwanza. At admission, a 10-mL venous blood sample and a fingerprick sample for a malaria thick blood film and hematocrit were taken. After delivery, a placental blood smear and a 1-cm placental biopsy specimen from the maternal placental surface were collected.

After delivery, the infant was examined and weighed. Stillbirth was defined as a fetal death at >22 weeks of gestation, and intrauterine fetal death (IUFD) was defined as fetal death at ≤22 weeks of gestation. LBW was defined as birth weight of <2500 grams, preterm birth was defined as delivery at <37 weeks of gestation, and intrauterine growth retardation (IUGR) was defined as LBW after ≥37 weeks of gestation [21–23]. Gestational age at birth was estimated by 2 methods, ultrasound/LMP dating or a standard Dubovitz examination [24] performed on live-born infants within 5 days of birth. Analysis of pregnancy outcome was done separately for preterm birth as determined by the 2 different methods, because infants who were

delivered at home did not usually have a Dubovitz examination, and 59% of the cohort members were recruited after 24 weeks of gestation, when ultrasound dating becomes less reliable [25].

Infants born to women who were RPR positive at recruitment were treated after birth with benzathine penicillin G (0.5 mg/kg im) to treat a possible congenital infection. Women were contacted at home if they did not attend the hospital for delivery, to document the infant's gestational age and outcome of pregnancy. RPR-negative women who had become RPR positive by the time of delivery were contacted whenever possible and advised to attend the ANC with their infants to receive treatment.

Laboratory methods. The initial serum samples taken from all new attenders at the ANC were tested on site, using a qualitative RPR test (Syfacard-R; Murex Diagnostics), as described by the manufacturer. Serum samples from all recruited women were tested at the reference laboratory by TPHA (Microsyph™-TP 1000; Porton Cambridge). A quantitative RPR test was also done, using the same commercial RPR kit, to evaluate the clinic results and measure RPR titers. Serum samples were tested by an FTA assay (Trepo-Spot IF; bioMérieux) only if the RPR test was positive and the TPHA results were negative or indeterminate. The stages of serological syphilis were defined as high-titer active syphilis (RPR titer $\geq 1:8$ and TPHA or FTA positive), low-titer active syphilis (RPR titer $< 1:8$ and TPHA or FTA positive), past or previously treated infection (RPR negative and TPHA positive), and biological false positive (BFP; RPR positive but negative by both FTA and TPHA tests). Those who were seronegative by both the RPR and TPHA tests were considered to be uninfected. A quantitative RPR test was also performed on maternal serum samples collected at delivery.

Trichomoniasis was diagnosed by vaginal wet preparations and culture using commercial culture media (InPouchJ TV; BioMed Diagnostics). After 7 days of incubation at 35°C, these preparations were read by light microscopy for the presence of motile trichomonads. Vaginal smears were heat-fixed, Gram-stained, and examined for both bacterial vaginosis, using the Nugent method [26], and candidiasis. Cervical specimens taken for *Neisseria gonorrhoeae* detection were inoculated onto modified Thayer-Martin medium, incubated for 48 h at 35°C, and identified by colony morphology, Gram-staining, phadebact testing (Boule Diagnostics AB), and oxidase and catalase reactivity. A second cervical swab was taken for the detection of *C. trachomatis* by a commercial immunoassay (IDEIA PCE Chlamydia; DAKO Diagnostics), to allow for early treatment of infected women. First-void urine samples were frozen at -20°C and later tested by polymerase chain reaction (AMPLICOR CT/NG; Roche Diagnostics Systems) at the University of Montreal and Hôpital du St. Sacrement du CHA (Montreal, Québec, Canada) for *C. trachomatis* and *N. gonorrhoeae*. Positivity for *N. gonorrhoeae* was confirmed using a second primer (16S rRNA).

At delivery, maternal blood was collected into a heparinized capillary tube. After centrifugation at 11,800 rpm for 5 min, the percentage packed cell volume was read against a microhematocrit reader. Peripheral and placental blood smears were stained with Giemsa stain and examined by light microscopy for asexual malaria parasites. Placental biopsy specimens were stored in a 10% solution of neutral buffered formalin, processed, and examined for malaria pigment, indicating past or chronic infection in the second half of

the pregnancy, and for the parasitized red blood cells of active infection at delivery [27, 28].

An anonymous human immunodeficiency virus (HIV) test was done on stored maternal serum samples collected at delivery. HIV infection was diagnosed if both screening (Vironostika HIV Uni-Form II; Organon Teknika) and confirmatory ELISA results (Enzygnost Anti-HIV 1/2 Plus; Behring) were positive. Women were not informed about their results, since these were only linked to the main results at data entry using the unique study number and could not be traced back to individual study participants. At the time of the study, voluntary counseling and HIV testing services for pregnant women were not established at ANCs or labor wards in Mwanza, but women could attend the regional hospital for HIV testing if they wished to know their HIV status.

Data analysis and sample size. Data were entered in dBase IV (Ashton-Tate) and analyzed with STATA 6 software (StataCorp). Pregnancy outcome analyses were based on the syphilis serologic test results from the reference laboratory. Syphilis-positive and -negative women were compared for sociodemographic and maternal factors and differences in birth outcome. Proportions were compared using the χ^2 and Fisher's exact tests, as appropriate. Means of normally distributed continuous variables were compared using the Student's *t* test. Crude and adjusted ORs for the association between serological syphilis and adverse pregnancy outcomes were obtained using multiple logistic regression analysis. Confounding variables were variables that had a 10% effect on the OR between syphilis and the specific pregnancy outcome. Further analysis was conducted to compare outcomes when treatment was given at different gestational ages.

Results

Cohort recruitment and characteristics. Over the course of 26 months, 19,878 women were screened for syphilis by RPR testing at the ANC, and 1522 (7.7%) were found to be seropositive. In total, 1688 women (556 RPR positive and 1132 RPR negative) were recruited into the cohort. Eight hundred fifty RPR-positive women (55.8%) were not eligible, predominantly because they were not residents of Mwanza or were planning to leave prior to delivery. Eight RPR-positive women had already been screened for syphilis during the current pregnancy, and 9 women refused or left the clinic before completing enrollment procedures. Other reasons for exclusion of RPR-positive women included penicillin allergy ($n = 1$), having twins or triplets ($n = 38$), a congenital fetal abnormality ($n = 4$), no identified pregnancy ($n = 47$), an ectopic pregnancy ($n = 2$), IUFD ($n = 5$), and being recruited previously during that pregnancy ($n = 2$). An RTI screen in the third trimester was performed for 76.1% of women.

Between recruitment and delivery, 150 participants (8.9%) were lost to follow-up (9.9% of RPR-positive women and 8.4% of RPR-negative women; $P = .31$). Women lost to follow-up before delivery were younger than those followed up (mean age, 22.7 vs. 23.8 years; $P = .01$) and were more likely to be primigravidae (41.3% vs. 28.3%; $P = .03$) and single (24.7% vs. 14.2%; $P =$

.001). Overall, 1538 women (91.1%) were monitored up to the time of delivery, 78.5% of whom gave birth in a hospital. Live birth or stillbirth was recorded for all these women, but 234 live infants born at home did not have their birth weight recorded, and 258 infants did not have a Dubovitz examination [24].

Two women had incomplete serologic test results at the reference laboratory and were dropped from further analysis. The reference laboratory results for the remaining 1686 women were used for all further analyses. After reference laboratory RPR testing, 559 women were classified as RPR positive, and 1127 were classified as RPR negative. Among RPR-positive women, 153 (27.4%) had high-titer active syphilis, and 277 (49.2%) had low-titer active syphilis (figure 1). A further 129 (23%) RPR-positive women had a BFP RPR test result, of whom 124 were treated with benzathine penicillin. Twenty-seven (2.4%) of the 1127 RPR-negative women had past or treated syphilis, and the remainder were syphilis seronegative. Forty-four seronegative women who were treated for a false-positive ANC RPR test result, 27 women with past or treated syphilis, 7 untreated RPR-positive women, and 23 initially seronegative women who were RPR positive at delivery were excluded from the analysis of penicillin treatment and pregnancy outcomes.

The mean age of the cohort was 23.7 years (SD, 5.0 years; range, 13–44 years). Women with low-titer active syphilis had the highest mean age (table 1). Most women (84.8%) were married, 87.8% had lived in Mwanza for ≥ 1 year, and 35.4% were from the Sukuma ethnic group. Only 11.4% had received secondary level education, and 20.5% said they were unable to read. The mean number of pregnancies was 2.8 (SD, 1.8 pregnancy; range, 1–14 pregnancies), and 29.4% were primigravidae. Only 18.0% attended the ANC before 20 weeks of gestation. The mean gestational age at recruitment was 25.3 weeks (SD, 5.8 weeks; range, 6.4–40.9 weeks) and was lower among primigravidae, compared with multigravidae (24.3 vs. 25.7 weeks; $P < .001$). Bacterial vaginosis and *C. albicans* were the most prevalent infections in the cohort but were not associated with syphilis serostatus. Trichomoniasis at recruitment was positively associated with high- and low-titer active syphilis ($P < .001$), and *N. gonorrhoeae* was positively associated with high-titer syphilis ($P < .0001$). At delivery, 103 (10.3%) of 1003 seronegative women were HIV positive, compared with 15 (11.3%) of 133 women with high-titer active syphilis, 41 (16.8%) of 246 women with low-titer active syphilis, and 17 (14.4%) of 118 women with BFP RPR test results.

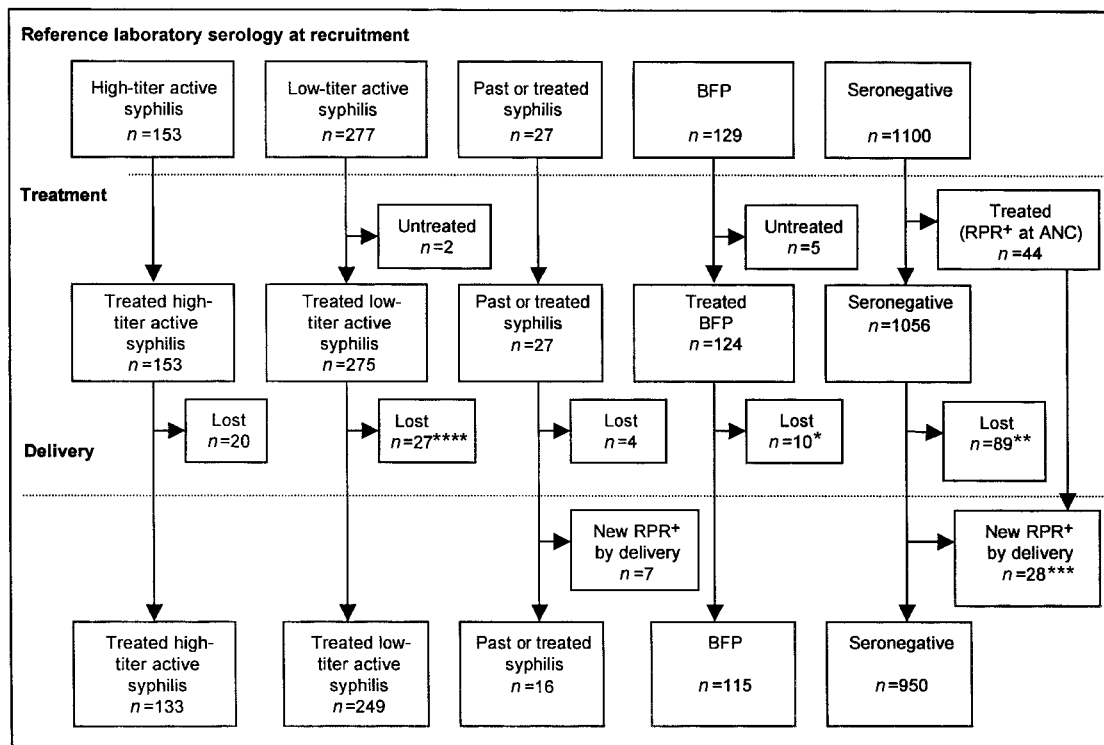


Figure 1. Flow of cohort to delivery, by syphilis serostatus at recruitment. “High-titer active syphilis” is defined as a rapid plasma reagin (RPR) titer $\geq 1:8$ and positive *Treponema pallidum* hemagglutination assay (TPHA)/fluorescent treponemal antibody (FTA) test results. “Low-titer active syphilis” is defined as an RPR titer $< 1:8$ and positive TPHA/FTA test results. “Past or treated syphilis” is defined as a negative RPR test result but positive TPHA/FTA test results. “BFP” (biological false-positive) is defined as a positive RPR test result but negative TPHA/FTA test results. *Includes 9 treated women and 1 untreated woman with a BFP test result; **includes 6 treated and 83 untreated seronegative women; ***includes 5 treated seronegative women; ****includes 1 untreated woman with low-titer active syphilis. ANC, antenatal clinic; +, positive.

Table 1. Sociodemographic characteristics of cohort at recruitment.

Characteristic	Total (n = 1686)	Seronegative (n = 1100)	High-titer ^a active syphilis (n = 153)	Low-titer ^b active syphilis (n = 277)	BFP RPR test result (n = 129)	Past or treated syphilis (n = 27)
Mean age, years	23.7	23.7	22.8	24.2	23.8	24.9
Age group						
<20 years	20.2	20.6	26.8	13	23.3	22.2
20–24 years	41.9	42.5	39.2	45.1	36.4	29.6
25–29 years	24	22.3	23.5	29.6	24.8	33.3
≥30 years	13.9	14.6	10.5	12.3	15.5	14.8
Gravidity						
1	29.4	33.1	25.5	14.1	36.4	25.9
2	25.7	24.6	34.6	30.3	19.4	7.4
3–5	35.8	33.3	32	45.5	37.2	51.9
≥6	9.1	9.1	7.8	10.1	7	14.8
No education	13.8	9.9	24.2	20.6	17.8	22.2
Sukuma ethnic group	35.4	30.2	45.7	51.1	33.3	25.9
Married	84.8	84.7	78.4	89.9	81.4	88.9
≥2 Sex partners in past year	10.7	8.6	19.6	14.1	10.9	7.4
History of genital ulceration	12.9	7	32.7	22.4	17.1	22.2
Other reproductive tract infections						
<i>Chlamydia trachomatis</i> ^c	7.6	6.6	13.1	7.6	8.5	11.1
<i>Neisseria gonorrhoeae</i> ^c	2.4	2	7.8	1.8	0.8	0
Bacterial vaginosis ^c	30.2	29.1	32	33.9	29.5	22.2
<i>Candida albicans</i> ^d	29.2	30.3	30.7	24.9	27.1	29.6
<i>Trichomonas vaginalis</i>	21.4	17.8	37.9	28.5	19.4	7.4

NOTE. Data are percentage of women, except for mean age. BFP, biological false positive.

^a Rapid plasma reagin (RPR) titer ≥1:8.

^b RPR titer <1:8.

^c One sample each was missing for *C. trachomatis*, *N. gonorrhoeae*, and bacterial vaginosis at recruitment.

^d Three samples were missing for *C. albicans*.

Syphilis serostatus and pregnancy outcomes. There were no significant differences in adverse pregnancy outcome between seronegative women and women treated for syphilis. By univariate analysis, 143 (17.3%) of 826 seronegative women had an adverse pregnancy outcome (stillbirth, IUGR, or a preterm birth, as determined by ultrasound/LMP dating), compared with 15.2% of women with either high- or low-titer active syphilis (table 2). There were 2 cases of IUFD, one in a seronegative woman and one in a woman treated for a BFP RPR test result. IUFD was diagnosed at 19 weeks of gestation, and, for the purposes of analysis, these 2 cases were combined with stillbirths. Women treated for high-titer active syphilis had a similar proportion of stillbirths as seronegative women (2.3% vs. 2.5%; $P = 1.00$). There was a slightly higher proportion of stillbirths among women treated for low-titer active syphilis, compared with seronegative women (4.8% vs. 2.5%, respectively), but this difference was not significant ($P = .09$).

There was no difference in the mean birth weight of live-born infants between different serological groups ($P = .24$, analysis of variance), and the proportion of LBW live-born infants was lower in all groups of treated RPR-positive women, compared with seronegative women. The proportion of preterm births, as defined by ultrasound/LMP dating, was lower among women treated for high-titer (8.5%) and low-titer (9.3%) active syphilis than among seronegative women (11.8%; $P = .3$). Women with BFP RPR test results and seronegative mothers had a similar rate of preterm births. Preterm live birth, as determined by Du-

bovitz examination [24], occurred in 4.0% of women treated for high-titer active syphilis, 3.2% of women treated for low-titer active syphilis cases, and 4.2% of seronegative women. There were no significant differences in the proportion of infants with IUGR, by syphilis serostatus. Women treated for BFP RPR test results were not at increased risk of any adverse pregnancy outcome, compared with seronegative women.

Among women treated for active syphilis, those with an RPR titer of ≥1:32 at recruitment had the highest proportion of LBW, preterm birth, or other adverse outcome. After treatment, 5 (14.7%) of 34 had an LBW infant, 8 (16.7%) of 48 had a preterm live birth, and 10 (28.6%) of 35 had an adverse outcome. However, these proportions were not significantly different from those seen in seronegative women, although there was a borderline difference in the proportion of any adverse outcomes among women with an RPR titer ≥1:32, compared with seronegative women (28.6% vs. 17.3%; $P = .08$).

Adjusted ORs of the association between adverse pregnancy outcomes and treated serological syphilis are shown in table 3. Women treated for high-titer active syphilis were not at any increased risk of adverse pregnancy outcome (OR, 0.76; 95% CI, 0.4–1.4), stillbirth (OR, 0.68; 95% CI, 0.2–2.4), LBW (OR, 0.53; 95% CI, 0.2–1.3), or preterm birth, as determined by ultrasound/LMP dating (OR, 0.58; 95% CI, 0.3–1.1) or by Dubovitz examination [24] (OR, 0.70; 95% CI, 0.2–2.1), compared with uninfected women. Similar results were found for women treated for low-titer active syphilis or BFP RPR test results,

Table 2. Pregnancy outcomes in women treated with benzathine penicillin for syphilis, compared with seronegative women.

Outcome, syphilis serostatus	Total	No. (%) with outcome	Crude OR (95% CI)	P
All outcomes^a				
Seronegative	826	143 (17.3)	1	
High-titer active syphilis	99	15 (15.2)	0.85 (0.5–1.5)	.86
Low-titer active syphilis	204	31 (15.2)	0.86 (0.6–1.3)	
BFP	100	16 (16.0)	0.91 (0.5–1.6)	
Stillbirth				
Seronegative	950	24 (2.5)	1	
High-titer active syphilis	133	3 (2.3)	0.89 (0.3–3.0)	.12
Low-titer active syphilis	249	12 (4.8)	1.95 (0.96–4.0)	
BFP	115	1 (0.9)	0.34 (0.04–2.5)	
Live births				
LBW^b (mean g ± SD)^c				
Seronegative (3075 ± 490)	802	74 (9.2)	1	
High-titer active syphilis (3075 ± 453)	96	6 (6.3)	0.66 (0.3–1.6)	.25
Low-titer active syphilis (3024 ± 482)	192	10 (5.2)	0.54 (0.3–1.1)	
BFP (3144 ± 475)	99	7 (7.1)	0.75 (0.3–1.7)	
Preterm birth, by ultrasound/LMP dating				
Seronegative	926	109 (11.8)	1	
High-titer active syphilis	130	11 (8.5)	0.69 (0.4–1.3)	.54
Low-titer active syphilis	237	22 (9.3)	0.77 (0.5–1.4)	
BFP	114	13 (11.4)	0.94 (0.5–1.8)	
Preterm birth, by Dubovitz examination^d				
Seronegative	778	33 (4.2)	1	
High-titer active syphilis	100	4 (4.0)	0.9 (0.3–2.7)	.89
Low-titer active syphilis	189	6 (3.2)	0.74 (0.3–1.8)	
BFP	98	5 (5.1)	1.21 (0.5–3.2)	
IUGR in full-term births^b				
Seronegative	802	33 (4.1)	1	
High-titer active syphilis	96	3 (3.1)	0.75 (0.2–2.5)	.38
Low-titer active syphilis	192	3 (1.6)	0.37 (0.1–1.2)	
BFP	99	3 (3.0)	0.73 (0.2–2.4)	

NOTE. High-titer active syphilis is defined as a rapid plasma reagin (RPR) titer ≥1:8; low-titer active syphilis is defined as an RPR titer <1:8. BFP, biological false positive; CI, confidence interval; IUGR, intrauterine growth retardation; LBW, low birth weight; LMP, last menstrual period; OR, odds ratio.

^a Includes stillbirths, LBW infants, and preterm delivery for 1229 women with full outcome data.

^b No birth weight data were available for 218 live births.

^c P = .24, analysis of variance for comparison of means.

^d Data were missing for 242 live births (17.2%).

with the exception of women treated for low-titer active syphilis, who had a nonsignificant increased risk of stillbirth (OR, 1.81; 95% CI, 0.9–3.9), compared with seronegative women.

Effect of gestational age at treatment. Overall, 25% of RPR-positive women received benzathine penicillin at or after 30 weeks of gestation. Timing of treatment in pregnancy did not differ significantly by syphilis serostatus (P = .64). Outcomes were stratified by gestational age at ANC attendance or treatment for seronegative women and women with high- and low-titer active syphilis (table 4). With the exception of stillbirth, women with high-titer active syphilis treated at later gestational ages had adverse birth outcomes more frequently than did women treated before 20 weeks of gestation. Women treated for high-titer syphilis from 30 weeks of gestation had the highest proportion of adverse outcomes, although this was not significantly different from outcomes in seronegative women who had attended the ANC at the same gestational age (28.6% vs. 15.9%; P = .22).

There was a trend toward higher rates of preterm birth, as

determined by ultrasound/LMP dating, for all serological groups if the first ANC attendance was late in pregnancy. However, there was no significant difference in the proportion of preterm births among women treated for high-titer syphilis or seronegative women who attended the ANC at ≥30 weeks of gestation (16.7% vs. 15.4%; P = .79). Women with high-titer syphilis treated after 30 weeks of gestation also had a higher proportion of preterm births, as determined by Dubovitz examination [24], compared with seronegative women, but this difference was not significant (8.7% vs. 3.0%; P = .2), and the number of cases was small. Similar trends were observed in women treated for low-titer active syphilis, but these were also not statistically significant.

Newly RPR-positive women. By delivery, 35 (3.4%) of 1024 women who initially were RPR negative had become RPR positive. Seven such cases occurred in 22 women with past or treated syphilis. The remaining 28 new RPR-positive cases occurred in 1002 women who initially were syphilis seronegative.

Table 3. Multivariate analysis of birth outcomes, by syphilis status.

Pregnancy outcome	Seronegative	High-titer ^a active syphilis	Low-titer ^b active syphilis	BFP RPR test result
All outcomes ^c	1	0.76 (0.4–1.4)	0.95 (0.6–1.5)	0.86 (0.5–1.5)
Stillbirth ^d	1	0.68 (0.2–2.4)	1.81 (0.9–3.9)	0.31 (0.04–2.4)
LBW ^e	1	0.53 (0.2–1.3)	0.67 (0.3–1.4)	0.60 (0.3–1.4)
Preterm birth, by ultrasound/LMP dating ^f	1	0.58 (0.3–1.1)	0.76 (0.5–1.2)	0.86 (0.5–1.6)
Preterm birth, by Dubovitz score ^g	1	0.70 (0.2–2.1)	0.68 (0.2–1.7)	1.10 (0.4–3.1)
IUGR ^h	1	0.62 (0.2–2.1)	0.52 (0.2–1.8)	0.60 (0.2–2.1)

NOTE. Data are odds ratio (95% confidence interval). BFP, biological false positive; IUGR, intrauterine growth retardation; LBW, low birth weight; LMP, last menstrual period; RPR, rapid plasma reagin.

^a RPR titer \geq 1:8.

^b RPR titer <1:8.

^c Adjusted for age, gravidity, and placental malaria.

^d Adjusted for age, gravidity, *Trichomonas vaginalis* infection, placental malaria, and maternal anemia.

^e Adjusted for age, gravidity, tribe, placental malaria, and maternal anemia.

^f Adjusted for age, placental malaria, and maternal anemia.

^g Adjusted for age, gravidity, maternal anemia and malaria, and placental malaria.

^h Adjusted for age, gravidity, ethnic group, no. of sex partners in past year, and placental malaria.

Nine of these women tested negative at delivery by both TPHA and FTA. A further 10 were negative by TPHA but were not tested by FTA. One woman had neither TPHA nor FTA tests performed on the delivery sample. The remaining 8 women were positive by at least 1 specific treponemal test, giving a minimum seroconversion rate for active syphilis among seronegative women of 0.8% (8/1001). There were no adverse outcomes documented in these women.

Discussion

Single-dose benzathine penicillin is being implemented as a treatment regimen in many countries, but there are few data on its effectiveness in preventing adverse pregnancy outcomes caused by maternal syphilis. A recent retrospective study in the Mwanza region of Tanzania has shown that high-titer active syphilis poses the greatest risk to the developing fetus and results in an 18-fold increased risk of stillbirth and a 4-fold increase in the risk of any adverse outcome [6]. The key finding in the present study is that women in Mwanza Municipality who received treatment for high-titer active syphilis with a simple regimen of single-dose benzathine penicillin had the same or lower risks of adverse pregnancy outcomes, compared with women who were syphilis seronegative.

The study was conducted in a population of ANC attenders who can be considered to be representative of pregnant women from many urban centers in sub-Saharan Africa, since RPR prevalence at the ANC and rates of stillbirth and preterm birth in the Mwanza region are comparable to those in other parts of the continent [14, 29–32]. Other studies on the effectiveness of single-dose benzathine penicillin treatment in sub-Saharan Africa have given conflicting results. Treated RPR-positive women had birth outcomes similar to those of RPR-negative women in a recent study in Nairobi [30]. However, there was no adjustment for other factors that might have influenced pregnancy outcome, and RPR-positive women may have included those with low

RPR titers or with BFP results who would not have been at risk of pregnancy outcomes attributable to maternal syphilis. Our data contrast with results from South Africa, where women treated with a single dose of benzathine penicillin had poorer birth outcomes than did those receiving 3 doses [15, 16]. Studies advocating triple-dose treatment have generally been small, non-randomized studies that have failed to measure the background rate of adverse outcomes among seronegative women [15] or to control for differences between women who only received 1 injection and those who received 3 doses [15, 16]. The current study is the first prospective study examining the effectiveness of syphilis screening and treatment while controlling for the effect of potentially confounding variables such as malaria and other RTIs. The observed lack of difference between infected and uninfected women suggests that single-dose benzathine penicillin does prevent adverse pregnancy outcomes attributable to maternal syphilis. Although the CIs for all outcomes crossed 1.0, so that a possible modest increased risk of adverse outcomes in the treated group cannot be excluded, the upper limits of the CIs are low and provide reassurance that the risk has been reduced by treatment to levels close to those among seronegative women.

It should be noted that the study did not pragmatically evaluate the effectiveness of on-site screening on pregnancy outcome. Recruited women who were RPR negative at the ANC but RPR positive according to reference laboratory testing were treated for ethical reasons. Seropositive women who were missed by ANC screening were included in the treated group for the study analysis, because they were given the same benzathine penicillin treatment as women who were RPR positive at the ANC screening. Comparisons of pregnancy outcome were analyzed according to the reference laboratory serologic test results. Misclassification of syphilis status should have been avoided by using the same serological classification of syphilis as the previous study of untreated maternal syphilis [6] and by stratifying outcomes by serological status. This allowed for adverse pregnancy outcomes to be identified in women who were most at risk because they

Table 4. Pregnancy outcomes by syphilis serostatus, stratified by gestational age at antenatal clinic (ANC) attendance or treatment.

Pregnancy outcome, syphilis serostatus	Gestational age, weeks			<i>P</i> ^a
	<20	20–29.9	≥30	
All outcomes				
High-titer active syphilis	1/21 (4.8)	8/57 (14.0)	6/21 (28.6)	.04
Low-titer active syphilis	4/44 (9.1)	18/110 (16.4)	9/50 (18.0)	.24
Seronegative	19/143 (13.3)	97/513 (18.9)	27/170 (15.9)	.62
Stillbirth				
High-titer active syphilis	1/22 (4.6)	1/80 (1.2)	1/31 (3.2)	.85
Low-titer active syphilis	2/52 (3.9)	8/129 (6.2)	2/68 (2.9)	.74
Seronegative	7/161 (4.4)	17/575 (3.0)	0/214 (0)	.01
Prematurity by ultrasound/LMP dating				
High-titer active syphilis	0/22 (0)	6/79 (7.6)	5/30 (16.7)	.04
Low-titer active syphilis	2/50 (4.0)	11/121 (9.1)	9/66 (13.6)	.08
Seronegative	12/154 (7.8)	64/558 (11.5)	33/214 (15.4)	.02
Prematurity by Dubovitz score				
High-titer active syphilis	0/19 (0)	2/58 (3.5)	2/23 (8.7)	.16
Low-titer active syphilis	1/42 (2.4)	3/101 (3.0)	2/46 (4.4)	.60
Seronegative	7/131 (5.3)	21/479 (4.4)	5/168 (3.0)	.31

NOTE. Data are no. of women with the designated outcome/total no. of women who were treated or first attended the ANC at that gestational age (%). High-titer active syphilis is defined as a rapid plasma reagin (RPR) titer $\geq 1:8$; low-titer active syphilis is defined as an RPR titer $< 1:8$.

^a *P* value for trend.

had high-titer active syphilis, whereas women who had BFP RPR test results or low-titer active syphilis could be examined separately. Misclassification of outcome status was considered to be unlikely for stillbirth and LBW but may have occurred for preterm birth and IUGR. Women attending an ANC late in pregnancy would have had less-accurate ultrasound gestational age measurements, and the Dubovitz examination may also have misclassified some preterm births since it is a proxy measure of gestational age [25]. However, it is reassuring that there was no increased risk of preterm birth using either definition of prematurity in women with active syphilis.

Most women attended the ANC for care late during their pregnancies. It was not possible to determine whether single-dose treatment would have prevented spontaneous abortion in women treated before 20 weeks of gestation. Spontaneous abortion is known to be associated with untreated maternal syphilis [4], but it is difficult to investigate unless women are recruited early in pregnancy. Adverse neonatal outcomes were also missed with this study design, since infants were treated at birth for ethical reasons. Although losses to follow-up in this study were low, there was a higher loss to follow-up among women who had high-titer active syphilis, compared with seronegative women (13.1% vs. 8.0%). If these women had a higher proportion of adverse pregnancy outcomes, then the study could have overestimated the effectiveness of syphilis screening.

The mean gestational age of the first ANC attendance, 24.1 weeks, was similar to that seen in other studies in sub-Saharan Africa [15] and is late in comparison with developed countries. In the United States, one of the main risks for congenital syphilis is believed to be treatment late in pregnancy [33]. In the present study, although overall numbers in each gestational age

group were small, there was a significant trend toward adverse pregnancy outcomes and preterm birth with late attendance at the ANC for women with high-titer active syphilis. This may be an effect of the length of time the fetus is exposed to penicillin in utero. A relatively lengthy duration of antibiotic exposure with a long-acting penicillin may be needed to ameliorate the processes by which syphilis initiates preterm birth. However, an increased risk of preterm birth and stillbirth with initial ANC attendance late in pregnancy was also seen among seronegative women. The observed trend toward adverse outcomes among women treated for syphilis later in pregnancy may, therefore, be partly explained by the early provision of other ANC interventions or by confounding with other risk factors for poor birth outcome. Pregnant women should be encouraged to attend ANCs earlier in their pregnancy for syphilis screening, earlier provision of vitamin and iron supplements, antimalarial prophylaxis, and treatment of other RTIs.

Treatment failure has also been reported in women treated early in pregnancy because of reinfection after treatment [34]. Some authors have argued for rescreening women for syphilis at delivery because of the risk of acquiring the infection after the initial screening [35–37]. These South African studies found seroconversion rates of 2.7%–3.0% during pregnancy, on the basis of a positive RPR (no titer cutoff) and TPHA/FTA test result. The seroconversion rate for RPR in Mwanza was similar (3.4%). However, the true seroconversion rate for syphilis is likely to be lower once BFP RPR test results are excluded. If the true incidence of syphilis is only 1% and the prevalence of RPR at the ANC is 8%, then, for every 1000 women screened at the ANC who are admitted for delivery, 920 initially RPR-negative women would need to be rescreened, which would

detect 9 true incident cases. Because women currently attend so late in pregnancy for screening, the incidence in the short period left prior to delivery is likely to be low in many settings. There is, however, evidence that women who have not had antenatal care are at a higher risk of maternal syphilis than ANC attenders [36]. A more effective approach might be to screen women at delivery who have not attended an ANC and have therefore not been screened during that pregnancy [37], which at least ensures that both the mother and her infant can be treated.

In conclusion, our data support the use of single-dose benzathine penicillin to prevent adverse pregnancy outcomes attributable to maternal syphilis in this population. Ideally, on-site ANC syphilis screening programs should be introduced where possible to provide same-day screening and treatment. Investigators in Nairobi have recently highlighted the difficulties in sustaining a syphilis screening program and have suggested that mass treatment in pregnancy might be an alternative to case finding [38]. Operational studies are needed to examine the cost-effectiveness of syphilis screening and treatment in resource-poor settings and the most appropriate strategy for the delivery of benzathine penicillin to prevent adverse pregnancy outcomes attributable to maternal syphilis. These should include studies on the effectiveness of contact tracing and whether the use of a rapid specific treponemal test to confirm syphilis infection in RPR-positive women would be cost-effective in areas where there are high rates of BFP RPR test results.

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