

ORIGINAL ARTICLE

Roles of bovine *Waddlia chondrophila* and *Chlamydia trachomatis* in human preterm birth

D. Baud^{1,2}, G. Goy¹, S. Vasilevsky^{1,2}, M.-C. Osterheld³, M. Roth-Kleiner⁴, A. Croxatto¹ and G. Greub^{1,5}

1) Center for Research on Intracellular Bacteria, Institute of Microbiology, Faculty of Biology and Medicine, University of Lausanne and University Hospital, 2) Materno-fetal and Obstetrics Research Unit, Department of Obstetrics and Gynaecology, Maternity, University Hospital, 3) Institute of Pathology, University Hospital, 4) Clinic of Neonatology, University Hospital and 5) Infectious Disease Service, University Hospital, Lausanne, Switzerland

Abstract

Waddlia chondrophila and *Chlamydia trachomatis* are intracellular bacteria associated with human miscarriage. We investigated their role in human preterm birth. Whereas presence of *Chlamydia trachomatis* DNA in genital tract was associated with human preterm birth, *Waddlia* was not, despite being present in women's genital tracts.

New Microbes and New Infections © 2014 The Authors. Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: Adverse pregnancy outcome, *Chlamydia*-like, genital tract infection, intracellular bacteria, preterm birth

Original Submission: 7 August 2014; **Revised Submission:** 13 October 2014; **Accepted:** 11 November 2014

Available online 29 November 2014

Corresponding author: G. Greub, Center for Research on Intracellular Bacteria (CRIB), Institute of Microbiology, University of Lausanne, Bugnon 48, 1011 Lausanne, Switzerland

E-mail: gilbert.greub@chuv.ch

Introduction

In 2010, approximately 15 million babies were born preterm worldwide, and more than one million died due to complications from preterm births (PTB). Neonates that survive PTB exhibit an increased risk of neurodevelopmental impairments and respiratory complications [1]. The proportion of spontaneous PTB attributed to infection is approximately 50% [2]. However, a pathogen is identified in only one third of the cases, despite evidence of infection. Obligate intracellular bacteria, which do not grow on media used routinely to isolate human pathogens from clinical samples, might represent possible agents of PTB.

Chlamydia trachomatis, an obligate intracellular bacterium, is considered the world's most common sexually transmitted bacterial pathogen. *Waddlia chondrophila* is another member of the Chlamydiales order that has been shown to cause abortions in bovines [3]. Both of these intracellular bacteria have also

been implicated in human adverse pregnancy outcomes [4–7]. In addition, *C. trachomatis* is known to cause premature rupture of the membranes and premature uterine activity, and growing evidence suggests a role for *C. trachomatis* in PTB [8]. In this study, we investigated the role of *Waddlia* and *Chlamydia* as emerging agents of PTB. We studied 407 women with PTBs or uneventful term pregnancies attending the University Hospital of Lausanne, Lausanne, Switzerland. In addition to serology, we also performed PCR to detect *Waddlia* and *Chlamydia* in the placenta and vaginal samples taken from these women, as well as histology on the placenta.

From 2006 to 2009, 407 women were enrolled into this study at the obstetrical ward of the University Hospital of Lausanne. The PTB group ($n = 146$) included women who spontaneously delivered before 37 weeks' gestation. The control group ($n = 261$) included women attending a labour ward with uneventful term pregnancies and no history of miscarriages, stillbirths or preterm labour. We compared demographic data and risk factors of patients with and without PTB or *C. trachomatis* infection by the Pearson χ^2 test (or the Fisher exact test when indicated) for categorical variables. For continuous variables, medians were compared by the Wilcoxon-Mann-Whitney test. Multivariable analyses were performed to control for covariates. Statistical analyses were performed using the Stata software, version 13.0 (StataCorp, College Station, TX, USA).

Only positive urine cultures, gestational and maternal age were significantly different between control and PTB groups (Table 1). Other infectious causes were investigated in the PTB group, showing that a positive culture was found in the vagina, maternal or fetal side of the placenta in 32%, 37% and 17% of the PTB patients, respectively (Supplementary Table 1). Among all these bacterial species, genital mycoplasma and *Gardnerella vaginalis*, which have been previously associated with PTB [9], were recovered in some subjects from the vagina only ($n = 25$ and 3, respectively), never from the placenta.

Sera were tested for antibodies directed against *C. trachomatis* and *W. chondrophila*, respectively, by using the MOMP-R, CT pELISA (R-biopharm, Darmstadt, Germany) [10] and *Waddlia*-specific immunofluorescence as described

elsewhere [6]. Isolated IgG mainly reflects past or chronic infection, whereas IgM and/or IgA reflect acute infection. Briefly, *W. chondrophila* strain ATCC VR-1470 was used as antigen, whereas Fluoline G or M (bioMérieux, Marcy l'Étoile, France) were used as secondary antibodies. An antibody titre of $\geq 1/64$ for IgG and $\geq 1/32$ for IgM were considered as positive, respectively [6]. There was no difference between control and PTB groups in terms of anti-*Chlamydia* IgG and IgA and anti-*Waddlia* IgG and IgM titres (Table 1). A total of 54 patients tested positive only for *Waddlia* IgG and 26 only for *Chlamydia* IgG, indicating the absence of serologic cross-reaction between both pathogens. Only six patients tested positive for both *Waddlia* and *Chlamydia* IgG ($p = 0.446$).

C. trachomatis IgG seropositivity (Table 2) was associated with civil status (divorced vs. married, odds ratio (OR) 7.85; 95% confidence interval (CI) 2.61–23.62), education (OR 0.28; 95% CI 0.10–0.81) and number of previous sexual partners (>6 vs. 1: OR 13.12, 95% CI 1.53–112.32; “not answered” vs. 1: OR 13.55, 95% CI 1.76–104.09). Patients who used condoms as a previous contraceptive method show less *C. trachomatis* positive serologies, although this was not statistically significant. Of note, only six (55%) and one (9%) of the 11 patients positive for *C. trachomatis* DNA were also positive for *C. trachomatis* IgG and IgA, respectively. However, *C. trachomatis* IgG-positive patients exhibited significantly more histologic chorioamnionitis (50%) than *C. trachomatis* IgG-negative patients (28.3%, $p = 0.015$).

TABLE 1. Characteristics of patients according to term history

Characteristic	Control (n = 261)	PTB (n = 146)	p
Gestational age at birth, weeks, \pm SD	39.6 \pm 1.1	32.6 \pm 3.3	<0.001
Age, years, \pm SD	31.5 \pm 5.0	32.4 \pm 5.9	0.057
<35 years	194 (74.3%)	94 (64.4%)	0.041
≥ 35 years	67 (25.7%)	52 (35.6%)	
Parity, \pm SD	0.5 \pm 0.8	0.5 \pm 0.8	0.950
0	160 (61.3%)	95 (65.1%)	0.228
1	72 (27.6%)	30 (20.6%)	
>1	29 (11.1%)	21 (14.4%)	
Origin			0.156
European	217 (83.1%)	113 (77.4%)	
Non-European	44 (16.9%)	33 (22.6%)	
Civil status			0.739
Married	201 (77.0%)	109 (74.7%)	
Single	49 (18.8%)	32 (21.9%)	
Divorced	11 (4.2%)	5 (3.4%)	
Education			0.150
Nonuniversity studies	170 (65.1%)	106 (72.6%)	
University studies	91 (34.9%)	40 (27.4%)	
No. of lifelong sexual partners			0.393
1	58 (22.2%)	37 (25.3%)	
2–3	43 (16.5%)	29 (19.9%)	
4–6	45 (17.2%)	19 (13.0%)	
>6	36 (13.8%)	13 (8.9%)	
Not answered	79 (30.3%)	48 (32.9%)	0.407
Condom as previous contraceptive method	69 (26.4%)	33 (22.6%)	
Smoking status			0.543
Nonsmoker	224 (85.8%)	129 (88.4%)	
Smoker	37 (14.2%)	17 (11.6%)	
Pets at home	82 (31.4%)	39 (26.7%)	0.366
Vegetarian	5 (1.9%)	5 (3.4%)	0.341
<i>Chlamydia trachomatis</i> serology			0.569
IgG positive	19 (7.3%)	13 (8.9%)	
IgA positive	10 (3.8%)	9 (6.2%)	0.330
Both IgG and IgA positive	7 (2.7%)	7 (4.8%)	0.270
<i>C. trachomatis</i> PCR			0.012
Cervicovaginal swab	2 (0.7%)	7 (4.8%)	0.012
Placenta	2 (0.7%)	7 (4.8%)	0.012
At least one PCR positive	2 (0.7%)	9 (6.2%)	0.002
<i>Waddlia</i> serology			0.428
Total Ig $\geq 1/64$	47 (18.0%)	31 (21.2%)	0.889
IgG $\geq 1/64$	38 (14.6%)	22 (15.1%)	0.092
IgM $\geq 1/16$	9 (3.5%)	11 (7.5%)	
<i>Waddlia</i> PCR			0.252
Cervico-vaginal swab	11 (4.2%)	10 (6.9%)	0.587
Placenta	11 (4.2%)	4 (2.7%)	
Other infections			<0.001
Positive urine culture	7 (2.7%)	38 (26%)	1.000
<i>Streptococcus agalactiae</i>	45 (18.0%)	22 (17.5%)	1.000
<i>Brucella abortus</i>	19 (7.3%)	11 (7.5%)	0.539
<i>Parachlamydia acanthamoebae</i>	2 (0.8%)	0 (0%)	1.000
<i>Simkania negevensis</i>	3 (1.2%)	1 (0.7%)	

PTB, preterm birth.

TABLE 2. Characteristics of patients according to *Chlamydia trachomatis* serologic status

Characteristic	IgG negative (n = 375, 92.1%)	IgG positive (n = 32, 7.9%)	p
Age, years, \pm SD	31.9 \pm 5.3	30.9 \pm 6.5	0.298
<35 years	263 (91.3%)	25 (8.7%)	0.421
≥ 35 years	112 (94.1%)	7 (5.9%)	
Parity, \pm SD	0.5 \pm 0.8	0.5 \pm 0.7	0.997
0	233 (91.4%)	22 (8.6%)	0.38
1	97 (95.1%)	5 (4.9%)	
>1	45 (90%)	5 (10.0%)	
Origin			0.164
European	307 (93.0%)	23 (7.0%)	
Non-European	68 (88.3%)	9 (11.7%)	
Civil status			>0.001
Married	288 (92.9%)	22 (7.1%)	
Single	77 (95.1%)	4 (4.9%)	
Divorced	10 (62.5%)	6 (37.5%)	
Education			0.017
Nonuniversity studies	248 (89.9%)	28 (10.1%)	
University studies	127 (97.0%)	4 (3.1%)	
No. of lifelong sexual partners			0.006
1	94 (99.0%)	1 (1.0%)	
2–3	66 (91.7%)	6 (8.3%)	
4–6	61 (95.3%)	3 (4.7%)	
>6	43 (87.8%)	6 (12.2%)	
Not answered	111 (87.4%)	16 (12.6%)	
Place of residence			0.699
Rural	126 (91.3%)	12 (8.7%)	
City	249 (92.6%)	20 (7.4%)	
Condom as previous contraceptive method			0.094
No	277 (90.8%)	28 (9.2%)	
Yes	98 (96.1%)	4 (3.9%)	
Smoking status			0.114
Nonsmoker	209 (95.0%)	11 (5.0%)	
Smoker	48 (88.9%)	6 (11.1%)	

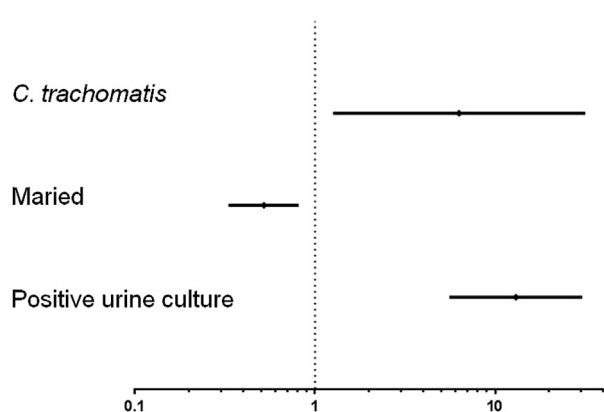


FIG. 1. Stepwise logistic regression showing independent association of positive *Chlamydia trachomatis* PCR with preterm birth.

Waddlia seropositivity was not associated with age, number of lifelong sexual partners, place of residence (rural vs. urban), smoking, pet ownership or meat consumption (data not shown). Interestingly, 4.9% of women of European heritage vs. 14.3% of those of non-European heritage had positive *Waddlia* IgG serologies of $\geq 1/256$ ($p 0.008$). This correlation was also observed for *Waddlia* IgM of $\geq 1/32$ (3.6% of European vs. 10.4% of non-European, $p 0.034$). Among non-European women, the highest *Waddlia* IgG titres ($\geq 1/128$ or $\geq 1/256$) were more frequent for black ethnicity (20% and 14.3%, respectively). When total immunoglobulin ($lg \geq 1/64$) against *Waddlia* was considered, condoms as a previous contraceptive method protected against

Waddlia infection (12.1% condom users vs. 21.4% of the non-condom users exhibited anti-*Waddlia* Ig reactivity, $p 0.041$).

After DNA extraction with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), all vaginal swabs and placenta samples were screened for *C. trachomatis* DNA by a TaqMan real-time PCR targeting the cryptic plasmid of *C. trachomatis*, as described earlier [11]. Samples were also tested by a 16S rRNA *Waddlia*-specific real-time PCR, as described previously [12]. No PCR inhibition was observed for either pathogen. Vaginal and placental samples from the PTB group were significantly more often *C. trachomatis* DNA positive than the control group ($p 0.002$, unadjusted OR 8.51; 95% CI 1.81–39.93). This association reminded significant even after adjustment for maternal age, origin, civil status, education, number of sexual partners and positive urine cultures (OR 7.93; 95% CI 1.34–46.76). Stepwise logistic regression allowed us to confirm that the presence of *C. trachomatis* DNA is an independent factor associated with PTB (Fig. 1). There was no difference between the control and PTB groups regarding *Waddlia* PCR results. Thirty-four patients were positive only for *W. chondrophila*, whereas ten other subjects were positive only for *C. trachomatis* by PCR. Only one patient was positive for both, demonstrating the absence of cross-amplification of these two obligate intracellular bacteria. Presence of *Waddlia* DNA was demonstrated in the genital tract or placenta of 13 PTB subjects (Table 3). Seven of them had more than one miscarriage in their medical history. Of these 13 patients, four exhibited both a positive

TABLE 3. Clinical history, serology, PCR and placental histology of preterm patients with samples positive for *Waddlia* by real-time PCR

Patient no.	Maternal age, years	No. pregnancies	Parity	Gestational age at birth, weeks	Birth weight, g	Country of origin	<i>Waddlia</i>			Placental histology	Other possible etiologies ^a
							IgG	IgM	PCR positive		
17	37	2	0	35.3	2520	Switzerland	—	—	P	Calcifications	
28	35	10	4	31.5	1870	Angola	—	—	P and VS	Subchorial fibrin, necrosis, lymphocytes in chorion and amnion, vasculitis	
45	27	1	0	34.1	2340	Switzerland	—	—	VS	—	
66	29	1	0	25.6	890	Portugal	—	—	VS	Subchorial fibrin, lymphocytes in chorion and amnion, oedema	
133	40	2	1	30.5	1270	Togo	1/128	—	VS	Villous oedema	
185	33	2	0	35.1	2060	Switzerland	—	—	VS	Villous oedema	
223	32	2	0	36.3	2370	Portugal	—	—	VS	—	CT IgG and IgA positive ^b
261	39	4	0	34.4	2540	Italy	1/128	—	P	Decidual fibrin	
283	34	4	1	36.4	2560	Congo	1/256	—	VS	Subchorial fibrin	
314	36	1	0	31.3	1310	Switzerland	—	—	VS	—	
351	33	2	0	35.3	2280	Italy	1/128	1/32	VS	Marginal hemorrhage	
476	35	2	1	30	1530	Italy	—	—	VS	Lymphocytes in decidua	
572	26	1	0	35	1920	Switzerland	—	—	P and U	Intervillous hemorrhage	CT PCR positive placenta ^c

Haematoxylin and eosin-stained histologic sections of all placenta specimens were examined for the type and degree of placentitis, endometritis and/or vasculitis by a pedopathologist.

P, placenta; VS, vaginal swab; U, urine; CT, *C. trachomatis*.

^aAll patients were also tested for *C. trachomatis*, *Brucella abortus*, *Streptococcus agalactiae*, *Parachlamydia acanthamoebae* and *Simkania negevensis*.

^bPositive serology for *C. trachomatis* observed in this case reflects a possible coinfection because there is no serologic cross-reaction between *C. trachomatis* and *Waddlia chondrophila*, and because the *W. chondrophila* serology was negative.

^c*C. trachomatis* positive PCR reflects a likely coinfection because there is no cross-amplification with the PCRs we used.

Waddlia serology and presence of *Waddlia* DNA. Two had presence of *Waddlia* in multiple samples, including vaginal swab, urine and placenta. Only two of these 13 patients had either a positive *C. trachomatis* serology or PCR. Ten of these 13 patients had abnormal placenta histologies.

Discussion

Both *C. trachomatis* and *W. chondrophila* have been implicated in adverse pregnancy outcomes [4,5,7,8]. However, there have been contradictory findings pertaining to the role of *C. trachomatis* infection in PTB. A few small studies have failed to demonstrate a risk of PTB associated with *C. trachomatis* infections [13,14], although several large, well-conducted studies have supported a role for *C. trachomatis* in PTB [15–17]. Thus, in a large study including 4055 subjects, about 15% of PTB was attributed to *C. trachomatis* [16].

We have previously confirmed an association between *Waddlia* antibodies and human miscarriage and have demonstrated its presence in placenta and/or genital tract [4–7]. In the present study, *W. chondrophila* was not associated with PTB despite inflammation, and abnormal histology of the placenta was observed among patients infected by *Waddlia* (positive PCR). Women of African descent were more likely to have positive *Waddlia* serology. Moreover, condom use was inversely associated with *Waddlia* seropositivity.

In contrast to *Waddlia*, presence of *Chlamydia* DNA in the genital tract and/or in the placenta was strongly associated with PTB. However, *C. trachomatis* seropositivity did not correlate with PTB, suggesting that acute rather than chronic *C. trachomatis* infection is associated with PTB. Interestingly, these results are opposite to our previous findings, in which we demonstrated that both chronic and acute *C. trachomatis* infections correlate with miscarriages [18]. Our results confirm those of a population-based prospective study recently published in the Generation R cohort in the Netherlands [16]. In this large study (>4000 pregnant women), *Chlamydia* DNA was strongly associated with PTB but not with miscarriage or perinatal death. Conflicting data between *Chlamydia* DNA, serology and pregnancy outcomes may reflect different pathophysiologic mechanisms, in which *Chlamydia*-induced miscarriages are the result of an immunologic process, whereas the direct impact of the bacteria results in *Chlamydia*-induced PTB.

A limitation of our study was the absence of investigation of other infectious etiology of PTB in all patients (PTB and control subjects). Some pathogens can reach the placenta by haematogenous spread or by an ascending route from the cervix. Among bacterial infections, *Ureaplasma urealyticum*, *Mycoplasma hominis* and bacterial vaginosis have been associated with PTB,

but controversies regarding their true role during pregnancy persist [9,19].

Overall, these results strongly suggest a role of acute *C. trachomatis* infection in PTB, and we strongly recommend systematically testing for *C. trachomatis* in any woman at risk for or after preterm delivery.

Conflict of interest

None declared.

Acknowledgements

This work was supported by an interdisciplinary grant from the University of Lausanne, and partially by grants from the Swiss National Science Foundation (32C0B0-116445), by the State Secretary for Education and Research, Bern, Switzerland (project number C05.0141), by the COST Action 855 (Animal Chlamydiosis and Zoonotic Implications) and by the Department of Obstetrics and Gynecology. D. Baud was supported by the “Société Académique Vaudoise” through the “Paul Blanc” grant, the SICPA Foundation, “Air Canada” travel grant and the “Fondation Leenaards” through the “Bourse pour la relève académique.” We thank all midwives and doctors who participated in the sampling for this study. Their involvement was essential to the whole process, and they enthusiastically gave their time to provide information and samples. We thank S. Aeby for technical help, and F. Damnon, K. Lepigeon and A. Baud for computer assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.nmni.2014.11.004>.

References

- [1] Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet* 2012;379(9832):2162–72.
- [2] Lockwood CJ. Predicting premature delivery—no easy task. *N Engl J Med* 2002;346:282–4.
- [3] Borel N, Ruhl S, Casson N, Kaiser C, Pospischil A, Greub G. *Parachlamydia* spp. and related *Chlamydia*-like organisms and bovine abortion. *Emerg Infect Dis* 2007;13:1904–7.
- [4] Baud D, Goy G, Osterheld MC, Borel N, Vial Y, Pospischil A, et al. *Waddlia chondrophila*: from bovine abortion to human miscarriage. *Clin Infect Dis* 2011;52:1469–71.

- [5] Baud D, Regan L, Greub G. Emerging role of *Chlamydia* and *Chlamydia*-like organisms in adverse pregnancy outcomes. *Curr Opin Infect Dis* 2008;21:70–6.
- [6] Baud D, Thomas V, Arafa A, Regan L, Greub G. *Waddlia chondrophila*, a potential agent of human fetal death. *Emerg Infect Dis* 2007;13:1239–43.
- [7] Baud D, Goy G, Osterheld MC, Croxatto A, Borel N, Vial Y, et al. Role of *Waddlia chondrophila* placental infection in miscarriage. *Emerg Infect Dis* 2014;20:460–4.
- [8] Andrews WW, Goldenberg RL, Mercer B, Iams J, Meis P, Moawad A, et al. The Preterm Prediction Study: association of second-trimester genitourinary *Chlamydia* infection with subsequent spontaneous preterm birth. *Am J Obstet Gynecol* 2000;183:662–8.
- [9] Capoccia R, Greub G, Baud D. *Ureaplasma urealyticum*, *Mycoplasma hominis* and adverse pregnancy outcomes. *Curr Opin Infect Dis* 2013;26:231–40.
- [10] Baud D, Regan L, Greub G. Comparison of five commercial serological tests for the detection of anti-*Chlamydia trachomatis* antibodies. *Eur J Clin Microbiol Infect Dis* 2010;29:669–75.
- [11] Jaton K, Bille J, Greub G. A novel real-time PCR to detect *Chlamydia trachomatis* in first-void urine or genital swabs. *J Med Microbiol* 2006;55(pt 12):1667–74.
- [12] Goy G, Croxatto A, Posfay-Barbe KM, Gervaix A, Greub G. Development of a real-time PCR for the specific detection of *Waddlia chondrophila* in clinical samples. *Eur J Clin Microbiol Infect Dis* 2009;28:1483–6.
- [13] Choi SJ, Park SD, Jang IH, Uh Y, Lee A. The prevalence of vaginal microorganisms in pregnant women with preterm labor and preterm birth. *Ann Lab Med* 2012;32:194–200.
- [14] Silveira MF, Ghanem KG, Erbedding EJ, Burke AE, Johnson HL, Singh RH, et al. *Chlamydia trachomatis* infection during pregnancy and the risk of preterm birth: a case-control study. *Int J STD AIDS* 2009;20:465–9.
- [15] Cohen I, Veille JC, Calkins BM. Improved pregnancy outcome following successful treatment of chlamydial infection. *JAMA* 1990;263:3160–3.
- [16] Rours GI, Duijts L, Moll HA, Arends LR, de Groot R, Jaddoe VW, et al. *Chlamydia trachomatis* infection during pregnancy associated with preterm delivery: a population-based prospective cohort study. *Eur J Epidemiol* 2011;26:493–502.
- [17] Rours GI, de Krijger RR, Ott A, Willemse HF, de Groot R, Zimmermann LJ, et al. *Chlamydia trachomatis* and placental inflammation in early preterm delivery. *Eur J Epidemiol* 2011;26:421–8.
- [18] Baud D, Goy G, Jaton K, Osterheld MC, Blumer S, Borel N, et al. Role of *Chlamydia trachomatis* in miscarriage. *Emerg Infect Dis* 2011;17:1630–5.
- [19] Vouga MI, Greub G, Prod'homme G, Durussel C, Roth-Kleiner M, Vasilevsky S, et al. Treatment of genital mycoplasma in colonized pregnant women in late pregnancy is associated with a lower rate of premature labour and neonatal complications. *Clin Microbiol Infect* 2014;20:1074–9.