ORIGINAL ARTICLE

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

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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.

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

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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 ≥1/64 for IgG and ≥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, ±SD	39.6 ± 1.1	32.6 ± 3.3	<0.001
Age, years, ±SD	31.5 ± 5.0	32.4 ± 5.9	0.057
<35 years	194 (74.3%)	94 (64.4%)	0.041
≥35 years	67 (25.7%)	52 (35.6%)	
Parity, ±SD	0.5 ± 0.8	0.5 ± 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			
European	217 (83.1%)	113 (77.4%)	0.156
Non-European	44 (16.9%)	33 (22.6%)	
Civil status			
Married	201 (77.0%)	109 (74.7%)	0.739
Single	49 (18.8%)	32 (21.9%)	
Divorced	11 (4.2%)	5 (3.4%)	
Education			
Nonuniversity studies	170 (65.1%)	106 (72.6%)	0.150
University studies	91 (34.9%)	40 (27.4%)	
No. of lifelong sexual partners			
1	58 (22.2%)	37 (25.3%)	0.393
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%)	
Condom as previous contraceptive method	69 (26.4%)	33 (22.6%)	0.407
Smoking status			
Nonsmoker	224 (85.8%)	129 (88.4%)	0.543
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
Chlamydia trachomatis serology			
IgG positive	19 (7.3%)	13 (8.9%)	0.569
IgA positive	10 (3.8%)	9 (6.2%)	0.330
Both IgG and IgA positive	7 (2.7%)	7 (4.8%)	0.270
C. trachomatis PCR			
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
Waddlia serology			
Total Ig ≥1/64	47 (18.0%)	31 (21.2%)	0.428
IgG ≥1/64	38 (14.6%)	22 (15.1%)	0.889
IgM ≥1/16	9 (3.5%)	11 (7.5%)	0.092
Waddlia PCR			
Cervico-vaginal swab	11 (4.2%)	10 (6.9%)	0.252
Placenta	11 (4.2%)	4 (2.7%)	0.587
Other infections			
Positive urine culture	7 (2.7%)	38 (26%)	<0.001
Streptococcus agalactiae	45 (18.0%)	22 (17.5%)	1.000
Brucella abortus	19 (7.3%)	11 (7.5%)	1.000
Parachlamydia acanthamoebae	2 (0.8%)	0 (0%)	0.539
Simkania negevensis	3 (1.2%)	1 (0.7%)	1.000

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, ±SD	31.9 ± 5.3	30.9 ± 6.5	0.298
<35 years	263 (91.3%)	25 (8.7%)	0.421
≥35 years	112 (94.1%)	7 (5.9%)	
Parity, ±SD	0.5 ± 0.8	0.5 ± 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			
European	307 (93.0%)	23 (7.0%)	0.164
Non-European	68 (88.3%)	9 (11.7%)	
Civil status			
Married	288 (92.9%)	22 (7.1%)	>0.001
Single	77 (95.1%)	4 (4.9%)	
Divorced	10 (62.5%)	6 (37.5%)	
Education			
Nonuniversity studies	248 (89.9%)	28 (10.1%)	0.017
University studies	127 (97.0%)	4 (3.1%)	
No. of lifelong sexual partners			
1	94 (99.0%)	1 (1.0%)	0.006
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			
Rural	126 (91.3%)	12 (8.7%)	0.699
City	249 (92.6%)	20 (7.4%)	
Condom as previous contraceptive method			
No	277 (90.8%)	28 (9.2%)	0.094
Yes	98 (96.1%)	4 (3.9%)	
Smoking status			
Nonsmoker	209 (95.0%)	11 (5.0%)	0.114
Smoker	48 (88.9%)	6 (11.1%)	