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SHORT REPORT
Role of *Chlamydia trachomatis* and emerging *Chlamydia*-related bacteria in ectopic pregnancy in Vietnam

S. HORNUNG[1], B. C. THUONG[2], J. GYGER[1], C. KEBBI-BEGHDADI[3], S. VASILEVSKY[1], G. GREUB[3,4] AND D. BAUD[1]*

[1] *Materno-fetal and Obstetrics Research Unit, Department of Obstetrics and Gynaecology, Maternity, University Hospital, Lausanne, Switzerland*
[2] *Tu Du Hospital, 284 Cong Quynh Pham Ngu Lao Ward, District 1, Ho Chi Minh City, Vietnam*
[3] *Center for Research on Intracellular Bacteria, Institute of Microbiology, Faculty of Biology and Medicine, University of Lausanne and University Hospital, Lausanne, Switzerland*
[4] *Infectious Disease Service, University Hospital, Lausanne, Switzerland*

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SUMMARY

In this case-control study, we investigated the seroprevalence and molecular evidence of *Chlamydia trachomatis* and *Waddlia chondrophila* in ectopic pregnancies (EP) and uneventful control pregnancies in 343 women from Vietnam. Whereas presence of *C. trachomatis* IgG was strongly associated with EP [adjusted odds ratio (aOR) 5·41, 95% confidence interval (CI) 2·58–11·32], its DNA remained undetected in all tubal lesions. We confirmed an independent association between antibodies against *Waddlia* and previous miscarriage (aOR 1·87, 95% CI 1·02–3·42). Further investigations are needed to understand the clinical significance of *Waddlia*'s high seroprevalence (25·9% in control pregnancies) in this urban population.

Key words: Adverse pregnancy outcome, *Chlamydia*-related bacteria, ectopic pregnancy, genital tract infection, intracellular bacteria.

Chlamydiae are obligate intracellular bacteria belonging to the order Chlamydiales [1]. *Chlamydia trachomatis* is the most common bacterial cause of sexually transmitted infections worldwide [2]. In women, 90% of *C. trachomatis* infections remain asymptomatic. However, if left untreated, chlamydial infection can lead to scarring of uterine tubes, pelvic inflammatory disease (PID), ectopic pregnancy (EP) and adverse pregnancy outcomes [2, 3]. *C. trachomatis* induced pathogenesis is largely a result of chronic immunopathological reactions, most likely caused by persistent infections [3].

Waddlia chondrophila, a *Chlamydia*-related bacterium, has recently been associated with both animal and human adverse pregnancy outcomes, such as miscarriage [2, 4–6]. Its mode of transmission and pathogenesis remains to be explored.

Since several *Chlamydia* spp. and *Chlamydia*-related bacteria colonize the cervico-vaginal mucosa [5–7], which may lead to tubal scarring and have been associated with adverse pregnancy outcomes in humans, we thus investigated their role in EPs. EP, a condition in which a fertilized egg settles and grows in a location other than the inner lining of the uterus, occurs in 2% of all pregnancies and remains the leading cause of pregnancy-related death in the first trimester of gestation [8, 9].

* Author for correspondence: D. Baud, MD, PhD, Materno-fetal & Obstetrics Research Unit, Department of Obstetrics and Gynecology, University Hospital, Centre Hospitalier Universitaire Vaudois (CHUV), 1011 Lausanne, Switzerland.
(Email: david.baud@chuv.ch)

Table 1. *Sociodemographical data and serologies according to pregnancy outcome*

Characteristics	Control ($n = 166$)	Ectopic pregnancy ($n = 177$)	P value
Age, years (±S.D.)	28 ± 5·2	30·3 ± 6·4	0·0 003
⩾40 years	6 (3·6%)	17 (9·6%)	0·031
Nulliparity	111 (66·9%)	72 (40·7%)	<0·001
Comorbidity	19 (11·5%)	7 (4%)	0·013
Pets at home	53 (31·93%)	54 (30·51%)	0·777
Lifetime sexual partners (⩾2)	2 (1·2%)	12 (6·8%)	0·012
Chlamydia trachomatis ELISA*			
Negative	153 (92·2%)	126 (71.2%)	<0·0 001
Positive	11 (6·6%)	44 (24.9%)	
Doubtful†	2 (1·2%)	7 (4%)	
Waddlia MIF			
Total Ig ⩾1/64	49 (29·5%)	71 (40.1%)	0·04
IgG ⩾1/64	43 (25·9%)	57 (32.2%)	0·2
IgM ⩾1/32	2 (1·2%)	3 (1.7%)	1
Waddlia ELISA OD	0·35 ± 0·097	0·371 ± 0·092	0·046
Parachlamydia IgG MIF	10 (6·0%)	19 (10.7%)	0·125
Estrella IgG MIF	21 (12·7%)	35 (19.8%)	0·081
Criblamydia MIF	4 (2·4%)	5 (2.8%)	1·0

MIF, Microimmunofluorescence; OD, optical density.
* MOMP-R, CT pELISA (R-Biopharm, Germany)
† Similar P values when doubtful were excluded.

A total of 343 patients were recruited at Tu Du Hospital, Hô Chi Minh City (Vietnam). The EP group included 177 women with an EP treated by laparoscopic salpingectomy. The control group included 166 women without any history of previous EP and who experienced an uneventful pregnancy. Blood samples, fallopian tubes or placental biopsies were collected for each EP and control patient. Local ethical committees of both hospitals (clinical part in Vietnam and experimental part in Switzerland) approved the study protocol and all patients included in the study gave their written consent.

Serological status and epidemiological data were compared between patients with and without EPs, or between patients with and without *Waddlia*-positive serology by Pearson's χ^2 test (or Fisher's exact test when indicated) for categorical variables. For continuous variables, medians were compared by the Wilcoxon–Mann–Whitney test. Multivariate logistic regressions were performed to identify factors independently associated with EPs and miscarriages. All statistical analyses were performed using Stata v. 13.0 (StataCorp., USA).

Sociodemographical data are presented in Table 1. All sera were tested for antibodies against *C. trachomatis* (Table 1), as described previously [1, 4, 6, 7, 10, 11]. *C. trachomatis* IgG seroprevalence was 6·6% in the present Asian control population. Similar prevalence has been described by other studies [7, 12, 13]. *C. trachomatis* seroprevalence was higher for women who experienced an EP (24·9%) than for women with an uneventful pregnancy (6·6%, $P < 0·001$).

For *Waddlia* and other *Chlamydia*-related bacteria microimmunofluorescence (MIF) tests were performed as described previously [1, 4, 6]. All immunofluorescence samples were read by two independent observers and only congruent results were considered positive. Sera that exhibited total immunoglobulin (Ig) titre ⩾1:64 were tested for IgG and IgM reactivity using corresponding anti-human Fluorescein-labelled Ig (FluolineG or FluolineM, bioMérieux, France) and serial twofold dilutions of sera. *Waddlia* IgG and IgM positivity cut-offs were ⩾1:64 and ⩾1:32, respectively [1]. There was a significant association between total anti-*Waddlia* antibodies detected by MIF and EP ($P = 0·04$). However, there was no statistical association with EP when anti-*Waddlia* IgG, or anti-*Waddlia* IgM, were considered. *Waddlia* ELISA was performed as described previously [14] and confirmed the association between *Waddlia* seropositivity and EP ($P = 0·046$). Serological evidence of human exposure to other *Chlamydia*-related bacteria, such as *Parachlamydia acanthamoebae, Estrella lausannensis* and *Criblamydia sequanensis* were not associated

Table 2. *Patient`s characteristics according to their* Waddlia *serological status*

Characteristics	*Waddlia* IgG negative ($n = 243$)	*Waddlia* IgG positive ($n = 100$)	*P* value
Age, years (±s.d.)	28·7 ± 5·7	30·3 ± 6·3	0·02
⩾40 years	10 (4·1%)	13 (13%)	0·007
Nulliparity	137 (56·4%)	46 (46%)	0·08
Previous miscarriage	34 (14%)	24 (24%)	0·005
Comorbidity	18 (7·4%)	7 (7%)	1
Pets at home	166 (68·31%)	70 (70%)	0·759
Lifetime sexual partners (⩾2)	11 (4·5%)	3 (3%)	0·765
Chlamydia trachomatis ELISA	37 (15·2%)	18 (18%)	0·513
Parachlamydia IgG MIF	19 (7·8%)	10 (10%)	0·525
Estrella IgG MIF	38 (15·6%)	18 (18%)	0·63
Criblamydia MIF	5 (2·1%)	4 (4%)	0·292

MIF, Microimmunofluorescence.

with EP (Table 1). When all variables from Table 1 were considered (stepwise logistic regression analysis), the only three independent factors associated with EP were a positive *C. trachomatis* serology [adjusted odds ratio (aOR) 5·41, 95% confidence interval (CI) 2·58–11·31], number of sexual partners (aOR 9·34, 95% CI 1·95–44·66) and parity (aOR 2·69, 95% CI 1·94–3·75), which are well known risk factors for EP [8, 9]. Patients' characteristics according to their *C. trachomatis* serological status are given in Supplementary Table S1.

Women seropositive for *Waddlia* ($n = 100$, 29·2%) were older ($P = 0·007$) and experienced previous miscarriages more frequently ($P = 0·005$) than *Waddlia*-negative women (Table 2). The association between *Waddlia* seropositivity and miscarriage remained significant (aOR 1·87, 95% CI 1·02–3·42) even after adjustment for age, parity, comorbidity and other serologies including *C. trachomatis*. There was no statistical association between *Waddlia*-positive serology and medical comorbidities, gynaecological complaints during pregnancy, work status, number of lifetime sexual partners or presence of pets at home.

There was no cross-reaction between *Waddlia* and *C. trachomatis* serologies, since 77 patients (23·1%) were positive only for *Waddlia* IgG and 37 (11·1%) were positive only for *C. trachomatis* IgG (Table 2). Only 18 patient (5·4%) were positive for both bacteria ($P = 0·513$).

Presence of *Waddlia* [15, 16] and/or *C. trachomatis* [7] DNA was tested in IgG-positive patients. DNA extraction was performed from a 2-cm piece of fallopian tube (EP) or placental (C) tissue using Wizard SV genomic DNA purification kit (Promega Corporation, USA), and a pan-Chlamydiales PCR was performed as described previously [17]. This Pan-Chlamydiales PCR is able to detect up to five DNA copies per reaction and demonstrated similar performance compared to specific Chlamydiales PCRs. Neither the 50 fallopian tubes nor the 43 placental samples with a positive *Waddlia* and/or *C. trachomatis* serology were positive for *Waddlia* or *C. trachomatis* DNA. All 20 control patients with a negative serology (10 from the 'EP' group and 10 from the 'C' group) were also negative by PCR.

In summary, our data showed a strong association between *C. trachomatis* seropositivity and EP. However, neither the fallopian tubes nor placenta of women with positive *Chlamydia* or *Waddlia* serologies demonstrated presence of respective bacteria, which has also been shown by others [12]. Moreover, IgG but not IgM antibodies were detected during EPs. Thus, these results suggest that the persistence of the bacteria is not necessary to induce tubal damage, and reinforces the role of an immunopathological process due to a previous chlamydial infection [18, 19]. However, the physiopathology mechanism by which tubal scarring occurs without the presence of bacteria is not yet fully understood [12, 19].

Waddlia IgG seroprevalence in the control group (25·9%) was higher than previously described in other asymptomatic patients: 14·6% in Switzerland [6], and 7·1% in London [4]. This difference could be explained as a result of higher genetic susceptibility of the Vietnamese population to *Waddlia* infection or greater exposure to the yet unknown source of *Waddlia* infection [2, 4, 6].

Whereas our study only identified a limited association of *Waddlia* with EP ($P = 0·04$), we observed a strong correlation between previous history of miscarriage and positive *Waddlia* serology ($P = 0·005$). This

was expected since *Waddlia* was previously reported as an abortigenic agent in both animal and human populations [2, 4–6, 19].

A major limitation of the study was the absence of data concerning other potential confounding factors for EP (i.e. other infectious agents) and miscarriage (i.e. chromosomal anomalies).

In conclusion, this study confirmed the serological association of *C. trachomatis* with EP [8] and of *Waddlia* with miscarriage [4, 6]. Moreover, we showed an association between anti-*Waddlia* antibodies and EP using both immunofluorescence and ELISA. Absence of *C. trachomatis* and *W. chondrophila* DNA in the fallopian tubes or placental tissues suggests that immunopathological mechanisms rather than bacterial infection are involved in EP. Further investigations are needed to understand the high prevalence of *Waddlia* in this Asian population and to precise its role in EP.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268814003616.

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DECLARATION OF INTEREST

None.

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