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

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Short Report - Epidemiology and Infection

ROLE OF *CHLAMYDIA TRACHOMATIS* AND EMERGING *CHLAMYDIA*-RELATED BACTERIA IN ECTOPIC PREGNANCY IN VIETNAM

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ABSTRACT

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 (C) pregnancies in 343 women from Vietnam. Whereas presence of *Chlamydia trachomatis* IgG was strongly associated with EP (adjusted Odds Ratio [aOR] 5.41; 95% Confidence Intervals [95%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* high seroprevalence (25.9% in C) in this urban population.

KEYWORDS

Chlamydia-related bacteria, adverse pregnancy outcome, genital tract infection, intracellular bacteria, ectopic pregnancy.

SHORT REPORT

Chlamydiae are obligate intracellular bacteria belonging to the *Chlamydiales* order [1]. *C. 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, PID (pelvic inflammatory disease), ectopic pregnancy 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 cervicovaginal 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 ectopic pregnancies. Ectopic pregnancy, 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].

A total of 347 patients were recruited at Tu Du Hospital, Hô Chi Minh City (Vietnam). The "Ectopic Pregnancy" group (EP) included 177 women with an ectopic pregnancy treated by laparoscopic salpingectomy. The "Control" group (C) included 166 women without any history of previous ectopic pregnancy and who experienced an uneventful pregnancy. Blood samples, fallopian tubes or placental biopsies were collected for each EP and C patient. Local ethical committees of both hospitals (clinical part in Vietnam & 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 ectopic pregnancies, or between patients with and without *Waddlia* positive serology by the Pearson $\chi 2$ test (or the Fisher 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 ectopic pregnancies and miscarriages. All statistical analyses were performed using STATA 13.0 (Stata Corporation, College Station, USA).

Sociodemographical data are presented in Table 1. All sera were tested for antibodies against *Chlamydia trachomatis* (Table 1), as previously described [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 others [7, 12, 13]. *C. trachomatis* seroprevalence was higher for women who experienced an ectopic pregnancy (24.9%) than for women with an uneventful pregnancy (6.6%, $p<0.001$).

For *Waddlia* and other *Chlamydia*-related bacteria Micro-immunofluorescence (MIF) were performed as previously described [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) titer ≥1:64 were tested for IgG and IgM reactivity using corresponding anti-human Fluorescein-labelled Ig (FluolineG or FluolineM, BioMerieux, Marcy l`Etoile, France) and serial two-fold 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 microimmunofluorescence and ectopic pregnancy ($p=0.04$). However, there was no statistical association with EP when anti-*Waddlia* IgG, or anti-*Waddlia* IgM, were considered. *Waddlia* ELISA was performed as previously described [14] and confirmed the

association between *Waddlia* seropositivity and ectopic pregnancies (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 with ectopic pregnancies (Table 1). When all variables from Table 1 were considered (stepwise logistic regression analysis), the only three independent factors associated with ectopic pregnancy were a positive *C. trachomatis* serology (adjusted Odds Ratio [aOD] 5.41; 95% Confidence Intervals [95%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 ectopic pregnancy [8, 9]. Patients' characteristics according to their *C. trachomatis* serological status are shown in Supplementary Table 1.

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). Association between *Waddlia* seropositivity and miscarriage remains 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 lifelong 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-centimeter piece of fallopian tube (EP) or placental (C) tissue using Wizard SV genomic DNA purification kit (Promega Corporation, USA), and a pan-C*hlamydiales* PCR was performed as previously described [17]. This Pan-*Chlamydiales* PCR is

able to detect up to 5 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 ectopic pregnancy. However, neither the fallopian tubes nor placenta of women with positive *Chlamydia* or *Waddlia* serologies demonstrated presence of respective bacteria, as also shown by others [12]. Moreover, IgG but not IgM antibodies were detected during ectopic pregnancies. Thus, these results suggest that the persistence of the bacteria is not necessary to induce tubal damage, and reinforces the role of an immuno-pathological process due to a previous chlamydial infection [18, 19]. However, the physiopathology mechanism by which tubal scaring 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 Vietnamese 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 ectopic pregnancies ($p=0.04$), 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 ectopic pregnancy (i.e. other infectious agent) and miscarriage (i.e. chromosomal anomalies).

In conclusion, this study confirmed the serologic association of *C. trachomatis* with ectopic pregnancy [8] and of *Waddlia* with miscarriage [4, 6]. Moreover, we showed an association between anti-*Waddlia* antibodies and ectopic pregnancy 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 ectopic pregnancy. Further investigations are needed to understand the high prevalence of *Waddlia* in this Asian population and to precise its role in ectopic pregnancy.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. **Corsaro D, Greub G.** Pathogenic potential of novel Chlamydiae and diagnostic approaches to infections due to these obligate intracellular bacteria. *Clinical microbiology reviews* 2006; **19**: 283-297.
2. **Baud D, Greub G**. Intracellular bacteria and adverse pregnancy outcomes. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2011; **17**: 1312-1322.
3. **Darville T, Hiltke TJ**. Pathogenesis of genital tract disease due to Chlamydia trachomatis. *The Journal of Infectious Diseases* 2010; **201 Suppl 2**: S114-S1125.
4. **Baud D, *et al*.** Waddlia chondrophila, a potential agent of human fetal death. *Emerging infectious diseases* 2007; **13**: 1239-1243.
5. **Baud D, *et al***. Waddlia chondrophila: from bovine abortion to human miscarriage. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2011; **52**: 1469-1471.
6. **Baud D, *et al***. Role of Waddlia chondrophila placental infection in miscarriage. *Emerging infectious diseases* 2014; **20**: 460-464.
7. **Baud D, *et al***. Role of Chlamydia trachomatis in miscarriage. *Emerging infectious diseases* 2011; **17**: 1630-1635.
8. **Rana P, *et al***. Ectopic pregnancy: a review. *Archived of Gynecology and Obstetrics* 2013; **288**: 747-757.
9. **Farquhar CM**. Ectopic pregnancy. *Lancet* 2005; **366**: 583-591.

10. **Baud D, *et al***. Performance of an automated multiplex immunofluorescence assay for detection of Chlamydia trachomatis immunoglobulin G. *Diagnostic microbiology and infectious disease* 2014; **78**: 217-219.

11. **Baud D, Regan L, Greub G**. Comparison of five commercial serological tests for the detection of anti-Chlamydia trachomatis antibodies. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2010; **29**: 669-675.

12. **Shaw JL, Horne AW**. The paracrinology of tubal ectopic pregnancy. *Molecular and Cellular Endocrinology* 2012; **358**: 216-222.

13. **Yongjun T, *et al***. The prevalence of sexually transmitted and other lower reproductive tract infections among rural women in Sichuan Province, China. *Southeast Asian Journal of Tropical Medicine and Public Health* 2009; **40**: 1038-1047.

14. **Lienard J, *et al***. Undressing of Waddlia chondrophila to enrich its outer membrane proteins to develop a new species-specific ELISA. *New Microbes and New Infections* 2014; **2**: 13-24.

15. **Goy G, *et al***. Development of a real-time PCR for the specific detection of Waddlia chondrophila in clinical samples. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology* 2009; **28**: 1483-1486.

16. **Lienard J, *et al***. Development of a new chlamydiales-specific real-time PCR and its dapplication to respiratory clinical samples. *Journal of Clinical Microbiology* 2011; **49**: 2637-2642.

17. **Croxatto A, *et al***. Presence of Chlamydiales DNA in ticks and fleas suggests that ticks are carriers of Chlamydiae. *Ticks and tick-borne diseases* 2014; **5**: 359-365.

18. **Witkin SS, Ledger WJ**. Antibodies to Chlamydia trachomatis in sera of women with recurrent spontaneous abortions. *American Journal of Obstetrics and Gynecolgy* 1992; **167**: 135-139.

19. **Baud D, Regan L, Greub G**. Emerging role of Chlamydia and Chlamydia-like organisms in adverse pregnancy outcomes. *Current opinion in infectious diseases* 2008; **21**: 70-76.

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

* MOMP-R, CT pELISA (R-Biopharm, Darmstadt, Germany)

** Similar p-value when doubtful were excluded

OD: optical density

MIF: micro-immunofluorescence

Characteristics	**Control** (n=166)		**Ectopic pregnancy** (n=177)		**p value**
Age in years (years ± SD)	28 ± 5.2		30.3 ± 6.4		0.0003
≥ 40	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
Lifelong sexual partners (≥2)	2	(1.2%)	12	(6.8%)	0.012
Chlamydia trachomatis* ELISA					
Negative	153	(92.2%)	126	(71.2%)	<0.0001
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* IgG MIF**	4	(2.4%)	5	(2.8%)	1.0

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 in years (years ± SD)	28.7 ± 5.7	30.4 ± 6.3	0.02
≥ 40	10 (4.1%)	13 (13%)	0.007
Nulliparity	137 (56.4%)	46 (46%)	0.08
Previous miscariage	34 (14%)	24 (24%)	0.005
Comorbidity	18 (7.4%)	7 (7%)	1
Pets at home	166 (68.31%)	70 (70%)	0.759
Lifelong 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* IgG MIF**	5 (2.1%)	4 (4%)	0.292

Supplementary Table 1: Patient`s characteristics according to their *C. trachomatis* serological status. *C. trachomatis* doubtful results were excluded from the analysis.

Characteristics	*C.trachomatis* IgG negative (n= 279)	*C.trachomatis* IgG positive (n=55)	p value
Age in years (years ± SD)	29.2 ± 5.8	29.4 ± 6.5	0.768
≥ 40	16 (5.7%)	6 (10.9%)	0.228
Nulliparity	126 (45.2%)	29 (52.7%)	0.375
Previous miscariage	40 (14.3%)	15 (27.3%)	0.027
Comorbidity	21 (7.9%)	3 (5.5%)	0.778
Pets at home	82 (29.4%)	22 (40%)	0.151
Lifelong sexual partners (≥2)	12 (4.3%)	2 (3.6%)	1
***Waddlia IgG* MIF**	77 (27.6%)	18 (32.7%)	0.513
***Parachlamydia* IgG MIF**	24 (8.6%)	5 (9.1%)	1.0
***Estrella* IgG MIF**	36 (12.9%)	15 (27.3%)	0.012
***Criblamydia* IgG MIF**	6 (2.2%)	3 (5.5%)	0.171

ANNEX

METHOD

Subject

A total of 343 patients were recruited at Tu Du Hospital, Hô Chi Minh (Vietnam) in 2007. The "Ectopic pregnancy" group (EP) included 177 women with an ectopic pregnancy which was treated by laparoscopy. The "Control" group (C) included 166 women who experienced a normal pregnancy, without any history of previous ectopic pregnancy, preterm labor or miscarriage. One blood sample, respectively affected fallopian tube or placental biopsy were collected for each EP and C patient. Local ethical committees of both hospitals have approved the study protocol and all patients included in the study gave their written consent.

All blood samples have been centrifuged as soon as possible and only sera have been kept and stored frozen (at - 20 C). Both ectopic pregnancy product and placenta have also been stored frozen (at -20 C). Each sample has been anonymized with a code, according to the patient's group. (EP1, EP2, EP3,… for ectopic pregnancies or C1, C2, C3, … for controls)

For each patient, a case report form has been filled to investigate for potential risk factors (date of birth, number of pregnancies, number of children, animals at home, number of previous sexual partner,...).

All the samples collected at Tu Du Hospital (Vietnam) have been sent by express frozen courier to the CHUV (Lausanne, Switzerland) where the experimental part of this research has been performed. Local ethical committees of both hospitals have approved the study protocol and all patients included in the study gave their written consent.

W. chondrophila and C. trachomatis micro-immunofluorescence assay

Immunofluorescence test were performed by using *W. chondrophila* strain ATCC VR-1470 as antigen[1]. All immunofluorescence were read blindly by two independent observers and only congruent results were considered positive. Sera that exhibited total immunoglobulin (Ig) titer ≥ 1:64 were tested for IgG and IgM reactivity using corresponding anti-human Ig flourescein (FluolineG or FluolineM, BioMerieux, Marcy l`Etoile, France) and serial two-fold dilutions of serum. IgG and IgM positivity cut-offs were ≥ 1:64 and ≥ 1:32, respectively, as proposed for other chlamydia-like organisms. The sera collected for a previous study from two women identified to be positive, respectively negative, for chlamydia were used as positive and negative controls[2]. All sera were also tested for IgG antibodies against *Chlamydia trachomatis* with the MOMP-R, CT pELISA (R-biopharm, Darmstadt, Germany). These ELISA use a recombinant peptid of the major outer membrane protein (MOMP) of C.trachomatis and showed a good sensitivity/specificity ratio in previous studies[3].

DNA extraction and PCR

DNA extraction was performed for Women with *Waddlia chondrophila* IgG title > 1:64. Practically, a two centimeter piece of ectopic or placental tissue was dissected and DNA extraction was performed using Wizard SV genomic DNA purification kit (Promega Corporation, USA).

A PAN-CHLAM was performed on the DNA extract from ectopic or placental tissues for the women with an IgG title > 1:64 in order to correlate a positive serology against Waddlia with the presence of this bacterium in the tissues[4]. This Pan-*Chlamydiales* PCR is able to detect up to 5 DNA copies per reaction and demonstrated similar performance compared to specific *Chlamydiales* PCRs.

Waddlia chondrophila ELISA

ELISA was performed for *Waddlia chondrophila* based on a recent study protocol[5].

Statistical analysis

Serological status and epidemiological data were compared between patients with and without ectopic pregnancies, or between patients with and without *Waddlia* positive serology by the Pearson $\chi 2$ test (or the Fisher 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 ectopic pregnancies and miscarriages. All statistical analyses were performed using STATA 13.0 (Stata Corporation, College Station, USA).

RESULTS

1. *Socio-demographic data and pregnancy outcomes:*

   Women experienced ectopic pregnancy (n=177) were compared with women experienced an uneventful pregnancy (n=166) according to their epidemiological information (Table 1). Risks factors for an ectopic pregnancy were maternal age ($p = 0.031$), parity ($p < 0.001$) and number of sexual partner ($p=0.012$). They were no statistical difference between our two groups in term of medical comorbidity, gynecological complains during pregnancy, work activity or animal possession.

   When all variables from Table 1 were considered (stepwise logistic regression analysis), the only three independent factors associated with ectopic pregnancy were a positive *C. trachomatis* serology (adjusted Odds Ratio [aOD] 5.41; 95% Confidence Intervals [95%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)

2. *Chlamydial serologies*:

   Waddlia chondrophila :

   There was a borderline significant association between total anti-*Waddlia* antibodies There was a significant association between total anti-*Waddlia* antibodies detected by microimmunofluorescence and ectopic pregnancy ($p=0.04$). However, there was no statistical association with EP when anti-*Waddlia* IgG, or anti-*Waddlia* IgM, were considered. *Waddlia* ELISA was performed as previously described [14] and confirmed the association between *Waddlia* seropositivity and ectopic pregnancies ($p=0.046$). and ectopic pregnancy ($p=0.04$; Table 2). However, there was no statistical difference when anti-*Waddlia* IgG or IgM were considered. A total of 36 and 5 women exhibited high anti-*Waddlia* IgG titers $\geq$ 1/256 and anti-*Waddlia* IgM $\geq$

1/32, respectively. *Waddlia* ELISA and confirm the borderline association between *Waddlia* seropositivity and ectopic pregnancies (p=0.046).

Women positive 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). They were no statistical difference between both groups in terms of medical comorbidity, gynecological complains during pregnancy, work activity, lifelong sexual partner or presence of pets at home.

Chlamydia trachomatis and other *Chlamydia* related bacteria :

C.trachomatis seroprevalence (Table 1) was higher for women who experienced an ectopic pregnancy (24.9%) than for women with an uneventful pregnancy (6.6%, $p < 0.001$). Serological evidence of human exposure to other *Chlamydia*-like organisms, such as *Parachlamydia acanthamoebae* (p=0.125), *Estrella lausannensis* (p=0.081), *Criblamydia sequanensis* (p=0.187) were not associated with ectopic pregnancies.

There was no cross-reaction between *Waddia* and *C.trachomatis* serologies, since 77 patients (23.1%) were positive only for *Waddlia* IgG and 37 (11.1%) positives only for *C.trachomatis* IgG. Only 18 patient (5.4%) were positive for both bacteria (p= 0.512).

3. *Tissues identification of bacteria :*

None of the 50 Fallopian tube and 43 placenta with a positive *Waddlia* and/or *C.trachomatis* serology demonstrated presence of *Waddlia* or *Chlamydia* DNA. All 20 control patients with a negative serology (10 EP and 10 C) were also negative by PCR.

BIBLIOGRAPHY

1. **Greub G, et al.** Serological hint suggesting that Parachlamydiaceae are agents of pneumonia in polytraumatized intensive care patients. *Annals of the New York Academy of Sciences*, 2003, 990:311-319.

2. **Baud D, et al**. Waddlia Chondrophila, a Potential Agent of Human Fetal Death. *Emerging Infectious Diseases* 2007, **13**; 1239-1243.

3. **Baud, D, Regan L, et Greub G**. Comparison of Five Commercial Serological Tests for the Detection of Anti-Chlamydia Trachomatis Antibodies. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology* 2010, **6**; 669-675.

4. **Lienard, J et al**. Development of a New Chlamydiales-Specific Real-Time PCR and Its Application to Respiratory Clinical Samples. *Journal of Clinical Microbiology* 2011, **49**; 2637-2642.

5. **Lienard, J et al**. Undressing of Waddlia chondrophila to enrich its outer membrane proteins to develop a new species-specific ELISA ». *New Microbes and New Infections*, 2014; **2**: 13-24