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Seroprevalence of different *Chlamydia*-like organisms in an asymptomatic population

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BACKGROUND

Accumulating evidence suggests that new obligate intracellular *Chlamydia*-like organisms may play a role as human pathogens [1–3]. *Waddlia chondrophila*, an abortigenic agent in bovines, might play a role in human miscarriage [2], while *Parachlamydia acanthamoebae* and *Protochlamydia naegleriophila* represent new aetiologic agents of pneumonia [1,3]. However, the modes of transmission of these *Chlamydia*-related organisms and the populations at risk for infection with these strict intracellular bacteria are incompletely defined. Here, we tested sera taken from asymptomatic men in Switzerland for antibodies directed against these *Chlamydia*-like organisms to determine seroprevalence and to identify risks factors for seropositivity, i.e. exposure to these bacteria. Sera were also tested for a fourth *Chlamydia*-related organism, *Criblamydia sequanensis* [4], which was isolated from the water of the Seine river and whose pathogenic role has not yet been studied.

METHODS

Young Swiss men were enrolled in this cross-sectional study during their medical examination for compulsory military service [5]. Demographic data, animal exposure and sexual risks factors were recorded using a questionnaire [5]. Sera were first screened by immunofluorescence for antibody reactivity against *Waddlia chondrophila*, *C. sequanensis*, *P. acanthamoebae* and *P. naegleriophila* using as secondary antibody FluolineH (BioMerieux, Marcy l'Etoile, France) as reported [2]. Sera that exhibited a total Ig titre ≥1:64 were then tested for IgG and IgM reactivity using corresponding anti-human Ig fluorescein (FluolineG or FluolineM, BioMerieux). Each immunofluorescence was read blindly by two independent observers and congruent results were considered positive (98.1% and 97.5% concordance for *Waddlia* and *Criblamydia*, respectively). IgG and IgM positivity cut-offs were ≥1:64 and ≥1:32, respectively [2]. Positive immunofluorescences were confirmed by Western-blot, as described earlier [2]. Prevalence, confidence interval (95%CI) and odds ratio (OR) were calculated using STATA (College Station, TX, USA).

RESULTS

Among 517 young Swiss men enrolled [5], 482 gave their consent for blood sampling. Demographic characteristics and potential risk factors for seropositivity for *Chlamydia*-like organisms are shown in Table 1.

Among 66 volunteers (13.7%, 95% CI 10.6–16.8) screened positive for *Waddlia*, 40 (8.3%, 95% CI 5.9–11.3) had IgG antibodies against this agent. Only three men (0.6%, 95% CI 0–1.3) exhibited IgM antibodies against *Waddlia*. No specific risks factors for *Waddlia* infection were identified.

A total of 101 subjects (21%, 95% CI 17.7–25.5) were screened positive for *Criblamydia*. The prevalences of IgG and IgM antibody reactivity against *Criblamydia* were 8.3% (40/482, 95% CI 5.9–11.3) and 1.7% (8/482, 95% CI 0.5–2.8), respectively. Presence of dogs at home was associated with *Criblamydia* total Ig seropositivity, suggesting that this *Chlamydia*-like organism may be zoonotically transmitted. However, this association was not observed when considering patients with IgG or IgM positivity. Conversely, *Criblamydia* IgG seropositivity was associated with asthma (OR 2.9, 95% CI 1.2–7.1). This association between *Criblamydia* and asthma was stronger (OR 4.1, 95% CI 1.1–15.6) when an IgG cutoff of ≥1/256 was chosen.

No volunteers exhibited significant IgG or IgM reactivity against *Protochlamydia* or *Parachlamydia*,

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Table 1. Demogrphic characteristics, potential risk factors of chlamydial infection and underlying diseases according to *Waddlia* and *Criblamydia* serostatus.

	Total		*Waddlia* positive					*Criblamydia* positive				
Characteristics	***n***	**(%)**	***n***	**(%)**	**p-value***	**Odds ratio**	**95% CI**	***n***	**(%)**	**p-value***	**Odds ratio**	**95% CI**
Age (year ± SD)	20.6 ± 1.4		20.9 ± 1.7					20.8 ± 1.6				
≤20	264	54.8	20	50	0.619	ref		22	46.8	0.281	ref	
>20	218	45.2	20	50		1.23	0.64–2.35	25	53.2		1.42	0.78–2.61
Nationality at birth												
Switzerland	407	84.4	35	87.5	0.941	ref		42	89.4	0.408	ref	
Europe	39	8.1	3	7.5		0.89	0.26–3.02	4	8.5		0.99	0.34–2.93
Other	36	7.5	2	5		0.63	0.14–2.71	1	2.1		0.25	0.03–1.86
Place of residence												
>10 000 inhabitants	128	73.4	14	35	0.261	ref		8	17	0.163	ref	
<10 000 inhabitants	354	26.6	26	65		1.55	0.78–3.07	39	83		0.54	0.24–1.19
Main occupation												
Studies	218	45.2	20	50	0.949	ref		24	51.1	0.249	ref	
Work	251	52.1	19	47.5		1.1	0.57–2.12	20	42.6		0.96	0.51–1.78
Declined to respond	13	2.7	1	2.5		0.96	0.12–7.79	3	6.4		2.84	0.73–11.02
Monthly income												
<l000 Frs.	283	62.3	22	55	0.152	ref		24	51.1	0.048	ref	
1000–2000 Frs.	64	14.1	2	5		0.38	0.09–1.67	3	6.4		0.53	0.15–1.82
>2000 Frs.	107	23.6	12	30		1.5	0.71–3.15	14	29.8		1.62	0.81–3.27
Declined to respond	28	5.8	4	10		1.98	0.63–6.21	6	12.8		2.94	1.09–7.96
Contact with animal	250	51.9	22	55	0.742	1.15	0.60–2.20	26	55.3	0.648	1.17	0.64–2.14
Farm animals	13	2.7	1	2.5	1	0.92	0.12–7.25	1	2.1	1	0.77	0.10–6.03
Dog	108	22.4	10	25	0.693	1.17	0.55–2.48	13	27.7	0.361	1.37	0.69–2.70
Cat	166	34.4	14	35	1	1.03	0.52–2.03	19	40.4	0.419	1.33	0.72–2.46
Smoke/drugs												
Cigarettes	225	46.7	12	30		0.46	0.23–0.93	20	42.6	0.645	0.83	0.45–1.53
Cannabis	125	25.6	10	25	1	0.95	0.45–2.00	10	21.3	0.489	0.75	0.36–1.56
Drugs	19	3.9	2	10.5	0.666	1.32	0.29–5.91	2	10.5	0.707	1.09	0.24–4.88
Alcohol > 2×/week	326	67.6	24	60	0.293	0.7	0.36–1.35	28	59.6	0.251	0.68	0.37–1.26
Any reported disease	48	9.9	5	12.5	0.579	1.33	0.49–3.56	9	19.2	0.038	2.4	1.08–5.34
Asthma	32	6.6	4	10	0.326	1.64	0.55–4.94	7	14.9	0.027	2.87	1.17–7.05
Any regular treatment	52	10.8	9	17.3	0.028	2.69	1.20–6.03	10	19.2	0.024	2.53	1.17–5.45
Food												
Vegetarian	6	1.2	1	2.5	0.407	2.24	0.26–19.7	1	2.1	0.462	1.87	0.21–16.35
Milk allergy	11	2.3	0	0				0	0	0.611	-	-
Maternal feeding at birth	440	91.3	36	90	0.768	0.85	0.29–2.51	44	93.6	0.786	1.44	0.43–4.87
Drink												
Water from city	377	78.2	31	77.5	0.844	ref		34	72.3	0.351	ref	
Water in bottle	105	21.8	9	22.5		1.05	0.48–2.27	13	27.7		1.43	0.72–2.81
Sport practice ≥ 1×/week	340	70.5	31	77.5	0.368	1.48	0.69–3.20	36	76.6	0.402	1.41	0.70–2.86
Number of life sexual partners												
0	65	13.5	7	17.5	0.373	ref		7	14.9	0.643	ref	
1	109	22.6	7	17.5		0.57	0.19–1.70	8	17		0.66	0.23–1.90
≥2	297	61.6	24	60		0.73	0.30–1.77	32	68.1		1	0.42–2.38
Declined to respond	11	2.3	2	5		1.84	0.33–10.3	0	0		-	-
Sexual orientation												
Heterosexual	363	75.3	29	72.5	0.282	ref		36	76.6	0.943	ref	
Homo/bisexual	9	1.9	2	5		3.29	0.65–16.6	1	2.1		1.14	0.14–9.34
Declined to respond	110	22.8	9	22.5		1.02	0.47–2.24	10	21.3		0.91	0.44–1.90
Condom use												
Always	156	32.4	12	30	0.189	ref		19	40.4	0.422	ref	
Sometimes	224	46.5	16	40		0.93	0.42–2.01	22	46.8		0.78	0.41–1.51
Never	70	14.5	6	15		1.13	0.40–3.13	5	10.6		0.55	0.20–1.55
Declined to respond	32	6.6	6	15		2.77	0.95–8.04	1	2.1		0.24	0.03–1.80

although three men (0.6%, 95% CI 0.1–1.8) were positive for *Parachlamydia* during the screening phase that detected total antibody reactivity against *Parachlamydia*. Positivity was confirmed by Western-blots (data not shown). The three patients positive for *Parachlamydia* were negative for all other *Chlamydia*-like organisms tested. Interestingly, they lived in the same rural area (within a less than 20 km radius) and two of them were occupationally exposed to farm animals (2/12 vs. 1/467, p 0.002).

Interestingly, 53.2% of IgG positive *Criblamydia* serologies were positive for *Waddlia*. Conversely, 62.5% of IgG positive *Waddlia* serologies were also positive for *Criblamydia*. None of the positive serologies observed here were associated with *Chlamydia trachomatis* infection [5]. To identify cross-reacting immunogenic proteins, we performed Western blot analyses on sera positive only for *Criblamydia* and on sera positive for both *Waddlia* and *Criblamydia*, using *C. sequanensis* as antigen. Immunogenic proteins of 77, 69, 62, 57, 54

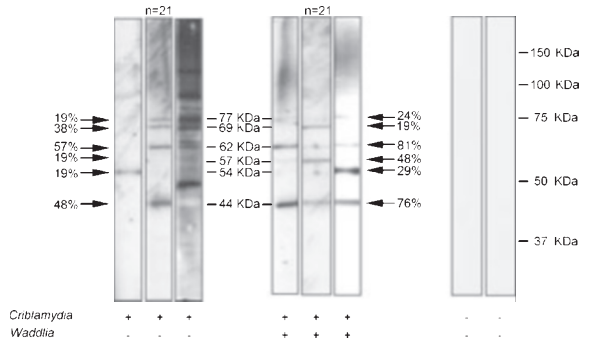

Fig. 1. Representative western blot pattern of IgG positive human sera tested using *Criblamydia sequanensis* as antigen. Percentage of bands were calculated on 2 x 21 positive sera.

and 44 KDa were identified in both groups (Fig. 1). Rate of positivity was higher in patients with a concomitant positive *Waddlia* serology for proteins exhibiting 62, 57 and 44 kDa, suggesting that these proteins cross-reacted.

CONCLUSIONS

This work demonstrates that exposure of humans to *W. chondrophila* and *C. sequanensis* is quite common, while exposure to *P. acanthamoebae* and *P. naegleriophila* may be limited. The *Waddlia* IgG seroprevalence of 8.3% we observed here was similar to that of 7.1% observed in 169 healthy pregnant women in the UK [2].

To our knowledge, we describe here the first hint suggesting a possible association of *C. sequanensis* with the presence of asthma. Human infection might occur through exposure to contaminated water. Indeed, *C. sequanensis* has been isolated from water [4] and free-living amoebae, which are widespread in water networks, may serve as hosts for *Criblamydia* [4]. *Criblamydia* and *Parachlamydia* might also be zoonotically transmitted, because we observed an association with dog exposure and farm animals, respectively. However, these associations were only present when considering screening results.

The absence of anti-*Parachlamydia* IgG reactivity observed in all 482 healthy subjects investigated when considering an IgG titre ≥1/64 is consistent with a report by Marrie *et al*. who did not identify any seropositive subjects among 511 healthy volunteers [1].

This work also shows some serological cross-reactivity between *Criblamydia* and *Waddlia* and confirms the absence of cross-reactivity between *Parachlamydia* and the other bacteria we investigated here [2,3].

In conclusion, further investigations are needed to confirm whether *C. sequanensis* may be involved in the pathogenesis of asthma and whether *C. sequanensis* and *P. acanthamoebae* should be, like *W. chondrophila* [2], considered as zoonotic pathogens.

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