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Research Paper

High seroprevalence of *Simkania negevensis* in Jordan

Hesham M. Al-Younes[1], Mika Paldanius[2]

[1]Department of Biological Sciences, Faculty of Science, University of Jordan, Amman, Jordan.
[2]Northern Finland Laboratory Centre, Nordlab, Oulu, Finland

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Abstract

The bacterium *Simkania negevensis* is a germ associated with respiratory diseases. This study aims at estimating the prevalence of *Simkania* in the Jordanian population. Serum samples from 664 Jordanian males and females, aged 2 to 86 years were collected. IgG and IgM *Simkania*-specific antibodies were detected using an indirect immunofluorescence test. Seropositivity titers for IgG and IgM were defined as 1:8 and 1:10, respectively. The overall prevalence of IgG antibody in all examined Jordanian nationals was 58.4%. IgG seropositivity was low in children under the age of 10 years (34.2%), and increased rapidly with age and ranged between 49.4% and 72%. *Simkania*-specific IgM was detected in 24.8% of subjects. IgM prevalence in children under 10 years was lowest (10.5%) and increased in older ages and remained above 20%. Overall detection rates of both IgG and IgM were significantly higher in females than males (60.7% *vs.* 54.5% for IgG and 26.7% *vs.* 21.7% for IgM). These data indicate that *Simkania* infection is highly prevalent in Jordan. The high level of seropositivity is most likely maintained by re-infections or chronic infections. Our data may serve as a basis to elucidate the pathogenesis of *Simkania* in Jordan.

Key words: Jordan, immunofluorescence, prevalence, *Simkania*.

Introduction

Simkania negevensis, formerly called microorganism 'Z' or '*Simkania* Z', is a *Chlamydia*-related bacterium that belongs to the novel family Simkaniaceae within the order Chlamydiales. It was first described in 1993 as a cell culture contaminant of unknown origin (Kahane *et al.*, 1993). This microorganism shares some biological properties with members of the family Chlamydiaceae; for instance, it is an obligate intracellular microorganism and has a typically two-stage developmental cycle, consisting of infectious elementary bodies and replicative reticulate bodies (Kahane *et al.*, 1993). *S. negevensis* appears to be transmitted via domestic water (Kahane *et al.*, 2007).

Seroepidemiological studies from different parts of the world demonstrated remarkable differences in seropositivity rates that range from as low as 4.3% to approximately 80% (Friedman *et al.*, 1999, 2003, 2006; Johnsen *et al.*, 2005; Yamaguchi *et al.*, 2005; Korppi *et al.*, 2006; Donati *et al.*, 2013). In addition, infection with *Simkania* has been associated with respiratory diseases, such as pneumonia, exacerbation of chronic obstructive pulmonary disease and bronchiolitis (Lieberman *et al.*, 1997, 2002; Kahane *et al.*, 1998; Greenberg *et al.*, 2003; Friedman *et al.*, 2003, 2006; Kumar *et al.*, 2005; Fasoli *et al.*, 2008; Heiskanen-Kosma *et al.*, 2008; Nascimento-Carvalho *et al.*, 2009). However, Niemi and coworkers (2001) were not able to detect an association of *Simkania* with respiratory diseases. Importantly, DNA of *Simkania* has been amplified from an aortic aneurysm (Ossewaarde and Meijer, 1999). In a very recent report, this bacterium has been linked to gastrointestinal infections (Donati *et al.*, 2013).

Simkania organisms can be detected in clinical specimens by cultivation in cell culture, PCR and serology (Friedman *et al.*, 2003, 2006). The microimmunofluorescence test (MIF) was found to be specific for the pathogen (Yamaguchi *et al.*, 2005; Fasoli *et al.*, 2008) and, therefore, is frequently used for the diagnosis of *Simkania* infections (Korppi *et al.*, 2006; Fasoli *et al.*, 2008; Heiskanen-Kosma *et al.*, 2008; Nascimento-Carvalho *et al.*, 2009; Donati *et al.*, 2013).

Send correspondence to H.M. Al-Younes. Department of Biological Sciences, Faculty of Science, University of Jordan, 11942 Amman, Jordan. E-mail: alyounes@ju.edu.jo.

The prevalence of *Simkania* infection in subjects and the correlation of this pathogen with respiratory infections and other illnesses have not yet been investigated in Jordan. The present study aimed, using MIF, to determine the prevalence of *Simkania* infection among Jordanian volunteers from both sexes and of different ages.

Materials and Methods

Study participants and serum collection

Blood samples were drawn from 664 Jordanian individuals who attended outpatient clinics at the hospital of the University of Jordan, Amman, Jordan, during December, 2008 and May, 2009 for various reasons. Serum samples were separated and stored at -20°C until tested. The study population comprised individuals aged 2 to 86 years (mean age 40.1 years) with 244 males and 420 females (mean age 42.3 and 39 years, respectively). Subjects were divided into eight age groups: 2-9 years (n = 38), 10-19 years (n = 62), 20-29 years (n = 143), 30-39 years (n = 100), 40-49 years (n = 93), 50-59 years (n = 83), 60-69 years (n = 54) and ≥ 70 years (n = 91). Written informed consent was obtained from adult individuals or from parents of children, who participated in the study. The study was approved by the appropriate Committees in the institution and in the Deanship of Academic Research, The University of Jordan.

Simkania antigen preparation

Elementary bodies of *S. negevensis* (American Type Culture Collection VA, USA; no. VR-1471) were used as antigen in the serologic test. Bacterial growth, purification of elementary bodies and preparation of antigen were performed as previously described (Korppi *et al.*, 2006; Heiskanen-Kosma *et al.*, 2008).

Serologic test

Detection of IgG and IgM antibodies specific to *S. negevensis* in collected sera was performed using in-house MIF test. Positive and negative control sera were also applied in each run. Diluted sera added onto slides dotted with the antigen were incubated in a humid chamber at 37°C for either 1 h or 3 h to detect IgG or IgM, respectively. After being washed, antigen spots were overlaid with fluorescein-labeled goat anti-human IgG or IgM antibody (Bio-Rad, CA, USA) and incubated as before. Slides were then washed, mounted and examined under an epifluorescence microscope (Nikon, Japan) at 400X. The cut-off values for seropositivity were 1:8 for IgG and 1:10 for IgM (Yamaguchi *et al.*, 2005; Heiskanen-Kosma *et al.*, 2008).

Statistical analysis

The statistical analysis of the data obtained for gender and age groups was determined using chi-square test. A probability value (p) of < 0.05 was considered statistically significant.

Results

IgG and IgM seropositivities for *Simkania* infection in Jordan were evaluated using sera collected from a total of 664 Jordanian nationals (420 females and 244 males). The mean age of female and male participants was 39 and 42.3 years, respectively. Tables 1 and 2 summarize the presence of *Simkania* IgG and IgM antibodies, respectively, in males and females, grouped into eight age groups. Moreover, Tables 1 and 2 show the percentages of IgG and IgM prevalence within the age groups after combining the genders.

When both genders were combined, the IgG and IgM detection rates were the lowest in participants under the age of 10 years and remained relatively high in older age groups. Peak IgG and IgM seropositivities were detected in

Table 1 - Seroprevalence of anti-*S. negevensis* IgG antibodies in Jordanian population in relation to age and gender.

Age group (years)	Males			Females			Both genders		
	number examined	No. IgG[a] positive	% IgG positivity	number examined	No. IgG[a] positive	% IgG positivity	number examined	No. IgG[a] positive	% IgG positivity
2-9*	28	8	28.6	10	5	50	38	13	34.2
10-19	28	18	64.3	34	23	67.6	62	41	66.1
20-29*	33	16	48.5	110	60	54.5	143	76	53.1
30-39*	22	14	63.6	78	58	74.4	100	72	72
40-49	25	16	64	68	37	54.4	93	53	57
50-59	32	13	40.6	51	28	54.9	83	41	49.4
60-69	23	16	69.6	31	20	64.5	54	36	66.7
≥ 70*	53	32	60.4	38	24	63.2	91	56	61.5
Total*	244	133	54.5	420	255	60.7	664	388	58.4

[a]Cut-off value for IgG seropositivity was 1:8.
*Statistically significant difference between males and females ($p < 0.05$).

Table 2 - Seroprevalence of IgM antibodies specific to *S. negevensis* in Jordanian subjects.

Age group (years)	Males			Females			Both genders		
	number examined	No. IgM[a] positive	% IgM positivity	number examined	No. IgM[a] positive	% IgM positivity	number examined	No. IgM[a] positive	% IgM positivity
2-9*	28	3	10.7	10	1	10	38	4	10.5
10-19	28	4	14.3	34	12	35.3	62	16	25.8
20-29*	33	9	27.3	110	28	25.5	143	37	25.9
30-39*	22	7	31.8	78	27	34.6	100	34	34
40-49	25	5	20	68	18	26.5	93	23	24.7
50-59	32	7	21.9	51	11	21.6	83	18	21.7
60-69	23	5	21.7	31	8	25.8	54	13	24.7
≥ 70*	53	13	24.5	38	7	18.4	91	20	22
Total*	244	53	21.7	420	112	26.7	664	165	24.8

[a]Cut-off value for IgM seropositivity was 1:10.
*Statistically significant difference between males and females ($p < 0.05$).

individuals aged between 30 to 39 years (Tables 1 and 2). The overall seropositivities of IgG and IgM antibodies in all subjects tested were 58.4% and 24.8%, respectively. The detection rates of both antibodies were significantly higher in females compared with males ($p < 0.05$).

Discussion

This study confirmed for the first time the presence of *S. negevensis* in Jordanian population. Serum samples from Jordanian natives from both sexes, aged 2 to 86 years, were utilized to estimate the seroprevalence of *S. negevensis* antibodies using MIF method. The IgG prevalence of *Simkania* infection was 34.2% in children aged between 2 and 9 years old and doubled in older children and teenagers aged 10-19 years. In older individuals, the seropositivity remained relatively high and fluctuated between approximately 50% and 72%. Other population-based studies performed in our geographic area demonstrated results almost comparable to our findings (Friedman *et al.*, 1999, 2003), suggesting that, in the Middle East, *S. negevensis* infections are very common in children and adult population.

Intriguingly, the overall prevalence of *Simkania* IgG and IgM was significantly higher in females than in males, suggesting a possible relationship between gender and the prevalence of bacterial infection. This finding is fully inconsistent with those reported for the prevalence of *Simkania*-related *C. pneumoniae*, which was higher in males than in females in many countries including Jordan (Al-Younes, 2014). The higher prevalence rate among females observed here might be due to the relatively low numbers of males investigated compared with female numbers. Therefore, the gender-associated differences in the seropositivity of *S. negevensis* remain inconclusive and deserve to be validated by prospective studies.

The prevalence of *Simkania* in apparently healthy individuals and possible association of this bacterium with various clinical manifestations, such as respiratory and gastrointestinal diseases, have been investigated in a limited number of countries in North America, Western Europe, the Middle East and East Asia (Yamaguchi *et al.*, 2005; Korppi *et al.*, 2006; Donati *et al.*, 2013). Most epidemiological studies on healthy teenagers and adults detected relatively high seropositivity rates, ranging from 35-68% in Western Europe, 39-68% in North America and 55-80% in the Middle East (Lieberman *et al.*, 1997; Friedman *et al.*, 1999, 2003, 2006; Johnsen *et al.*, 2005; Kumar *et al.*, 2005). In Japan, by contrast, broader seroprevalence analysis, including that in healthy children, youths and older persons, estimated an overall rate of only 4.3% (Yamaguchi *et al.*, 2005). In this study, the exposure rate to *Simkania* was analyzed in Jordanian natives from both sexes and of different ages. The overall prevalence of *Simkania* IgG antibodies was high in the Jordanian population (~58%) and comparable to those estimates recorded previously in the Middle East (Friedman *et al.*, 1999, 2003). Differences in global seropositivity rates may be partially due to variations in geographic regions and ethnic groups (Ossewaarde and Meijer, 1999). In our geographic area (the Middle East), infection with *Simkania* seems to be relatively common as observed here and by others (Friedman *et al.*, 2003).

In the first studies on the prevalence of *S. negevensis*, antibodies to this bacterium were measured by an enzyme-linked immunosorbent assay (ELISA), developed to detect IgA and IgG antibodies (Lieberman *et al.*, 1997; Kahane *et al.*, 1998; Friedman *et al.*, 2003). Serologic studies using ELISA test in countries in North America, West Europe and the Middle East indicated antibody rates of IgG in healthy population that ranged from 39 to 80%, suggesting apparently high seroprevalences of *Simkania* infection. By contrast, a recently developed immunofluorescence-based method (MIF) detected relatively very low overall IgG seropositivity (4.3%) against *Simkania* in healthy Japanese children and adults (Yamaguchi *et al.*, 2005). These contra-

dictory epidemiological findings obtained using ELISA and MIF raised questions about the clinical significance of antibody reactivity against *Simkania*, especially when using ELISA (Corsaro *et al.*, 2006). Utilizing MIF, it was subsequently shown that there was a 20 to 30% seropositivity in Italian children (Fasoli *et al.*, 2008) - a rate higher than that observed by Yamaguchi *et al*. in Japan (Yamaguchi *et al.*, 2005), but, interestingly, consistent with the high prevalence observed in this study in children aged 2-9 years. Our MIF-based results and those reported by Fasoli and coworkers (2008) are in accordance with those obtained by ELISA in North America, West Europe and Middle East, indicating the feasibility of both tests for diagnosing cases of *Simkania* infections.

This study shows for the first time the prevalence of *S. negevensis* infection in Jordanian children and adults among both sexes. The first exposure to *Simkania* begins at a very early age, with most primary infections occurring within the first two decades of life. The stable high detection rates of *Simkania* IgG antibodies in subjects older than 20 years may be the result of past infection or frequent reinfection. Our data show that the seroprevalence in the Jordanian population is one of the highest worldwide, when compared to estimates in other populations. Further studies are recommended to link this *Chlamydia*-like pathogen with pulmonary and non-pulmonary diseases in Jordan.

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Declaration of Interest

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