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IgG and IgA response to *Simkania negevensis* in sera of patients with respiratory and gastrointestinal symptoms

Manuela Donati[1], Natascia Fiani[1], Antonietta Di Francesco[2], Maria Di Paolo[1], Manuela Vici[3], Roberto Cevenini[1]

[1]Section of Microbiology DESOS, Policlinico S. Orsola, University of Bologna, Bologna, Italy;
[2]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia (Bologna), Italy;
[3]Department of Experimental Pathology, University of Bologna, Bologna, Italy

SUMMARY

The presence of IgG and IgA antibodies to *Simkania negevensis* in adult Italian patients with respiratory or gastrointestinal symptoms was investigated by the microimmunofluorescence test. In patients with respiratory infections, IgG (50%) and IgA (13%) seropositivity was consistent with previous data. In patients with gastrointestinal disorders, IgG (68%) and IgA (18%) seroprevalence was significantly higher than in healthy controls. These results, in association with the previously described detection of *S. negevensis* in water sources, could suggest an oral route of infection other than droplets or close contact, and a possible association of *S. negevensis* with gastrointestinal infections.

KEY WORDS: Simkania negevensis, Antibody response, Microimmunofluorescence test.

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Simkania negevensis is an obligate intracellular Gram-negative bacterium belonging to the family of *Simkaniaceae* in the order *Chlamydiales* (Everett *et al*., 1999), discovered as a contaminant in a variety of cell cultures (Kahane *et al*., 1993). *S. negevensis* is also able to replicate in various environmental free-living amoebae such as *Acanthamoeba polyphaga* and persists for long periods of time in amoebal cysts (Kahane *et al*., 2001).

Previous studies detected *S. negevensis* in water sources and showed that it is relatively resistant to chlorination procedures used for routine treatment of drinking water supplies (Kahane *et al*., 2004). Additional studies compared *S. negevensis* partial 16S rDNA sequences from clinical samples of children with pneumonia and domestic water samples from their homes, indicating a "common strain" of Simkania (Kahane *et al*., 2007a).

Epidemiologic studies reported widespread human exposure to this bacterium (Friedman *et al*., 2003) both in healthy subjects and in association with respiratory diseases in infants and adults (Lieberman *et al*., 1997; Kahane *et al*., 1998; Lieberman *et al*., 2002; Greenberg *et al*., 2003; Kumar *et al*., 2005; Fasoli *et al*., 2008). Few studies have been reported on the humoral and cell-mediated response in *S. negevensis* infections, unlike chlamydial infections (Meoni *et al*., 2009).

The seropositivity to *S. negevensis* in healthy population groups suggested the organism is a simple colonizer. However, *in vitro* studies have shown that *S. negevensis* can infect human cell cultures of various tissue origins, such as respiratory epithelial cells, gastrointestinal tract, genital tract and endothelial cells (Kahane *et al*., 2007b).

The aim of this study was to retrospectively evaluate the IgG and IgA response to *S. negevensis* in

Corresponding author
Manuela Donati
Section of Microbiology DESOS
Policlinico S. Orsola
University of Bologna
Via Massarenti, 9 - 40138 Bologna, Italy
E-mail: manuela.donati@unibo.it

sample sera in Italy from patients with respiratory and gastrointestinal symptoms in comparison to healthy controls. Serum antibodies were detected by the microimmunofluorescence (MIF) test. The possible cross-reactivity between *S. negevensis* and *Chlamydia pneumoniae* serology was evaluated.

A total of 326 sera, collected over a 24 month period in 2008-2010 for diagnostic investigations from patients admitted to S. Orsola Hospital (Bologna, Italy) were tested. In particular, 102 samples were from patients (aged 52.4±19.3 years) hospitalized with symptoms of lower respiratory tract infections and 224 from patients (aged 53.2±20.3 years) with gastrointestinal symptoms (diarrhoea, vomiting), fever and elevated inflammatory blood parameters (VES and neutrophils). The sera from the patients with enteric disorders were negative for antibody to enterovirus and *Salmonella* spp. As controls, 104 sera from blood donors (aged 45.6±12.4 years) were used.

S. negevensis Z (American Type Culture Collection VR-1471) and *C. pneumoniae* IOL-207 reference strains were grown in LLC-MK2 cells in six large well plates (Donati *et al*., 2003) and elementary bodies (EBs) were purified by use of sucrose gradients (Fukushi & Hirai, 1988).

The MIF test was performed according to the method of Wang and Grayston (1986). Sera were screened at 1:16 and 1:8 dilution in phosphate buffered saline (PBS) supplemented with 2% foetal calf serum, for IgG and IgA detection, respectively. FITC-conjugated goat antibody anti-human IgG diluted 1:40 or anti-IgA diluted 1:30 in PBS (Dako, Copenaghen, Denmark), were used. Positive sera were tested by serial dilutions and the reciprocal of the highest serum dilution considered positive represented the antibody titre of the sample. χ^2 test ($p<0.05$) was used in the statistical analysis of the data.

IgG and IgA response to *S. negevensis* detected by MIF test are shown in Table 1. The prevalence of IgG antibodies to *S. negevensis* was 50% (51/102) in sera of patients with lower respiratory tract infections, 68% (152/224) in sera of patients with gastrointestinal disorders and 35% (36/104) in sera of healthy controls. IgG positive sera showed titres ranging from 16 to 128.

The IgG seropositivity rate to *S. negevensis* showed a statistically significant difference in patients with respiratory infections ($p=0.03$, odds ratio 1.9, 95% confidence interval [CI]:1.08-3.31) and in patients with gastrointestinal problems ($p<0.001$, odds ratio 4, 95% CI: 2.26%-7.02) when compared with healthy controls. In patients with respiratory symptoms, IgG antibodies were increasingly prevalent with increasing age, starting from 18% in patients aged 30-40 years and increasing to 67% in patients aged over 65 years. The patients with gastrointestinal symptoms and the healthy controls did not show any age-related change in prevalence.

TABLE 1 - *IgG and IgA antibodies to* S. negevensis *in adult patients by MIF technique*

Sera	*No. of MIF positive sera/ No. of sera tested (%)*	
	IgG	*IgA*
Patients with respiratory infections	51/102 (50)	13/102 (13)
Patients with gastrointestinal signs	152/224 (68)	40/224 (18)
Blood donors	36/104 (35)	2/104 (2)

S. negevensis IgA were found in 13% (13/102) of the sera of patients with signs of respiratory infection, 18% (40/224) of the sera of patients with gastrointestinal disorders and 2% (2/104) of the healthy controls. IgA positive sera showed titres ranging from 8 to 32.

IgA positive sera to *S. negevensis* did not present IgA to *C. pneumoniae*. In relation to IgG positive sera to *S. negevensis*, 26 sera from patients with respiratory symptoms, 92 sera from patients with gastrointestinal signs and 21 sera from healthy controls reacted to *C. pneumoniae* at low titre. To evaluate cross-reacting antigens, a limited number of 20 sera reacting to *S. negevensis* and *C. pneumoniae* were absorbed with *C. pneumoniae* according to Yamaguschi *et al*. (2005) and then tested again by MIF. All these sera no longer reacted with *C. pneumoniae* and showed IgG titres to *S. negevensis* reduced within one dilution. The adsorption results showed that there was no substantial cross-reactivity between *S. negevensis* and *C. pneumoniae* antibodies, according to Yamaguschi *et al*. (2005) and Fasoli *et al*. (2008). Reports of exposure to *S. negevensis* are available

from various parts of the world, both in healthy subjects and in association with respiratory diseases in infants and adults. In Italy, the *S. negevensis* seroprevalence was first investigated in North Italian children with community-acquired pneumonia (CAP), showing that 20-30% of the children had measurable antibodies to *S. negevensis* (Fasoli *et al*., 2008). To our knowledge, no investigation has been performed on *S. negevensis* exposure in Italian adults to date.

The present study investigated the IgG and IgA response to *S. negevensis* in two groups of adult Italian patients with respiratory and gastrointestinal problems by MIF test. The seropositivity IgG (50%) and IgA (13%) rates found in the subjects with respiratory signs were consistent with those previously published for adults (Friedman *et al*., 2006). According to other reports (Kumar *et al*., 2005; Yamaguschi *et al*., 2005; Friedman *et al.*, 2006), the seropositivity rates increased with age.

There are no comparative studies on seroprevalence to *S. negevensis* in patients with gastrointestinal disorders, although Lieberman *et al.* (1997) reported gastrointestinal symptoms in some CAP patients with serological evidence of acute *S. negevensis* infection. In subjects with gastrointestinal symptoms, we detected a significantly higher IgG (68%) and IgA (18%) seroprevalence than in the healthy controls. Organism-specific IgA may be an indication of current or recent infection (Friedman *et al*., 2006). The IgG and IgA response in subjects with gastrointestinal disorders, in association with the already described detection of *S. negevensis* in water sources, could support a possible oral route of infection other than droplets or close contact. Previous *in vitro* studies have shown that *S. negevensis* can infect the human gastrointestinal tract and is able to assume a persistent form of infection, which may lead to a prolonged inflammatory response (Kahane *et al*., 2007b). Furthermore, in an *in vitro* simulation model Kahane *et al*. (2008) suggested a role of monocyte/macrophages as vehicles of dissemination of *S. negevensis* to other body compartments.

More extensive molecular studies and attempts to isolate the microorganism are needed to elucidate our observations on a possible association of *S. negevensis* with gastrointestinal infections and its pathogenic role.

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