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## PREVALENCE OF RICKETTSIOSES IN POLAND IN 2006-2012

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### ABSTRACT

**INTRODUCTION.** Tick-borne diseases, such as rickettsioses, anaplasmosis, Lyme boreliosis and bartonellosis are often difficult to correctly diagnose. All these diseases are present in Poland.

**OBJECTIVES.** The aim of the study was to estimate a prevalence of *Rickettsia* spp. infections in humans in Poland in 2006 to 2012 based on the results made in the Laboratory of Rickettsiae, Chlamydiae and Spirochetes, NIPH-NIH in Warsaw.

**MATERIAL AND METHODS.** The levels of *Rickettsia* spp. and *Anaplasma phagocytophilum* IgM and IgG antibodies were determined by indirect immunofluorescent assay (IFA). From 2006 to 2012, serum samples derived from 180 humans suspected for rickettsioses, including 84 patients suspected for the infections with typhus and spotted fever group (SFG) rickettsiae, and 96 patients suspected for anaplasmosis.

**RESULTS.** Specific serum antibodies to the SFG rickettsiae have been detected in 5 persons (2.7%). Granulocytic anaplasmosis has been recognized in 9 patients (4.9%). While the reporting and registration of rickettsioses are obligatory in Poland less than 50% of detected cases are reported.

**CONCLUSIONS.** Presented data indicate that in Poland rickettsioses are often unrecognized resulting in their underestimation. If research for rickettsiosis are made immediately after infection, antibodies will not be detected.

**Key words:** *Rickettsia* spp., *Anaplasma phagocytophilum*, prevalence

### INTRODUCTION

Rickettsioses are diseases caused by obligate intracellular bacteria belonging to the order *Rickettsiales* within the  $\alpha$ -Proteobacteria, genus *Rickettsia* and *Anaplasma*. Rickettsioses are usually separated into two groups: typhus group (TG) and spotted fever group (SFG) (1, 2). The pathogenic rickettsiae multiply in endothelial cells and cause a vasculitis, which is responsible for the clinical and laboratory abnormalities that occur in rickettsioses (3). Human (granulocytic ehrlichiosis) anaplasmosis is acute infectious disease caused by *Anaplasma phagocytophilum* (formerly human granulocytic ehrlichia) (4). These bacteria belong (4). Each *Rickettsia* sp. has one or several arthropod's vectors, and its geographical distribution, seasonal activity, host-seeking behavior or tendency of these arthropods to bite humans that underlies the epidemiology of the disease (5).

Tick-borne rickettsioses are an important problem for public health in the world and belong to emerging/re-emerging diseases. Recently, in the field of rickettsi-

ology, an explosion of new isolates of pathogens have received species designation and new disease names, all of which have been relatively neglected by primary care and infectious disease physicians (6,7). A broad group of other tick-associated rickettsial, ehrlichial and anaplasma agents of unknown pathogenicity exist (eg, *R. amblyommii*) that may cause confusion in interpreting serologic surveys or a single elevated antibody titer. Rickettsial, ehrlichial and anaplasma diseases are clinically difficult to distinguish and therefore misdiagnosis and failure to treat have unfortunate and sometimes tragic outcomes (8).

The precise serological identification of the etiological agent of rickettsioses in humans may be difficult because of the strong cross-reactivity in serological studies. In some cases, cross-absorption studies and western-blot may help, but these techniques work only in cases in which the suspected agent is known and isolated. In all cases of new and emerging rickettsioses, the serological study may indicate only the group etiology (TG/SFG). One of the first studies performed with *R. conorii* antigen showed the strikingly high incidence

of rickettsial antibodies in human sera. The research was made on people from different parts of the world, mostly there were people from African countries. A recent worldwide report from 2010 has demonstrated a 5.6% incidence of rickettsial infections in a group of travelers who developed acute febrile infections after returning from endemic areas. These infections are the one of the most frequently identified etiology for systemic febrile illness among travelers, following malaria (9). Moreover, pathogenicity of *R. slovaca*, *R. helvetica* and *R. raoulti* bacteria, known until recently as saprophytic species, has been recognized in central and northern Europe. They are the etiologic agents of the TIBOLA/DEBONEL disease recognized in this area increasingly (10).

Rickettsioses and granulocytic anaplasmosis are tick-borne diseases present in Poland (11). The aim of the study was to estimate a prevalence of *Rickettsia* spp. infections in humans in Poland in 2006-2012 based on the results made in the Laboratory of Rickettsiae, Chlamydiae and Spirochetes, NIPH-NIH in Warsaw.

## MATERIAL AND METHODS

From 2006 to 2012 serum samples derived from 180 humans suspected for rickettsioses including 84 and 96 patients suspected for the infections *Rickettsia* spp. and anaplasmosis respectively, were tested in Laboratory of Rickettsiae, Chlamydiae and Spirochetes of National Institute of Public Health – National Institute of Hygiene (NIPH-NIH). Infections due to fever of unknown etiology, skin lesions, visits to endemic area and infestation with ticks and not specified reasons given by the physicians.

The levels of *Rickettsia* spp. and *Anaplasma phagocytophilum* IgM and IgG antibodies were determined by indirect immunofluorescence assay (IFA). Specific IgM and IgG antibodies to *Rickettsia typhi*, and spotted fever group rickettsiae such as *R. conneri*, *R. rickettsii*, *R. slovaca* were tested with indirect immunofluorescence assay using Rickettsia IFA IgM and Rickettsia IFA IgG and Anaplasma phagocytophilum IFA IgM and Anaplasma phagocytophilum IFA IgG commercial kits of Focus Diagnostics, USA.

All positive serum samples in the titer  $\geq 128$  were tested with *Rickettsia* Screen IFA IgG Antibodies Kit (Fuller Laboratories, Fullerton, California, USA) to differentiate immune response to the following rickettsial species: *R. conneri*, *R. helvetica*, *R. felis*, *R. slovaca*, *R. sibirica*, and *R. massilae*. The titers of: IgM and IgG antibodies *R. typhi* and *R. rickettsii*  $\geq 64$ , IgM *A. phagocytophilum*  $\geq 20$ , and IgG *A. phagocytophilum*  $\geq 64$  were considered positive.

## RESULTS

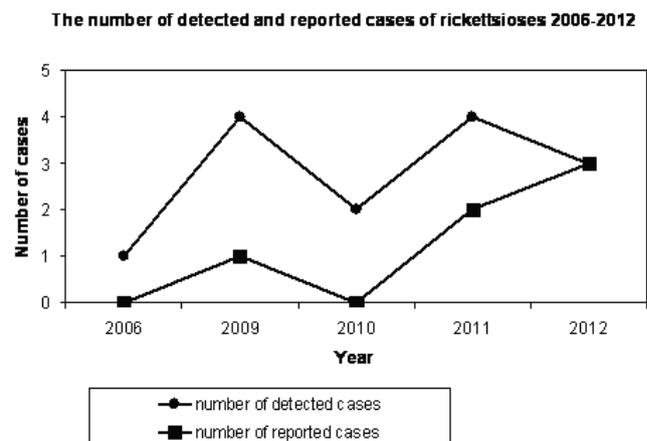
In serum samples derived from humans suspected for rickettsioses, specific serum antibodies to the SFG rickettsiae have been detected in 5 persons (2.7%). These infections have been recognized in Mazowieckie (3 cases) and Dolnośląskie (2 cases) districts. Two cases have been imported from South Africa. Detected SFG rickettsiae have been classified as: *R. conneri*, *R. slovaca*, *R. raoulti*, *R. africae*.

Granulocytic anaplasmosis has been recognized in 9 patients (4.9%). The disease has been distributed in various parts of Poland. In southern Poland it has been found in Podkarpackie (2 cases) and Małopolskie (1 case) districts, in central Poland in Mazowieckie district (4 cases) and in north-western Poland, in Zachodniopomorskie district (1 case). Significant levels of serum antibodies to the Typhus group rickettsiae were not found. Low levels (32 titer) of IgG antibodies to *R. typhi* have been found in sera of 3 patients, suggesting previous contact with this rickettsial species.

## DISCUSSION

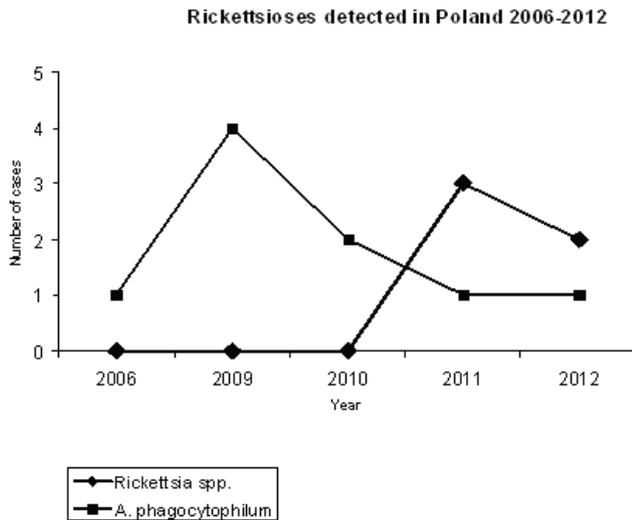
According to the Polish regulations (Dziennik Ustaw 2008, No. 234, poz.1570 and Dziennik Ustaw 2012, poz. 892), the reporting and registration of rickettsioses are obligatory. In 2006-2012, six cases of various rickettsioses have been reported. At the same time, in the following years 14 cases of rickettsioses including SFG rickettsioses and granulocytic anaplasmosis have been detected in Laboratory of Rickettsiae, Chlamydiae and Spirochetes (fig. 1 and fig.2). It's only one lab in Poland, where these tests are carried out.

Fig. 1. The number of detected and reported cases of rickettsioses.



Any cases of rickettsioses were not detected in 2007 and 2008

Fig. 2. Rickettsioses detected in Poland in 2006-2012.



Any cases of rickettsioses were not detected in 2007 and 2008

These data show that less than 50% of recognized rickettsial disease cases are registered only. On the other hand, among 180 patients suspected for rickettsioses, laboratory testing has confirmed clinical recognition in 14 cases (7.7%). It means that symptoms suggesting rickettsioses finally have been due to these group of diseases (tab. I). The other possibility low number of reported cases is that antibodies were not detected since in the first week of symptomatic disease antibodies are still undetectable.

Table I. Symptoms in patients suspected of rickettsial infection recognized by clinicians.

Disease suspected	Symptoms
<i>Anaplasmosis</i>	Fever, hepatitis, contact with tick
	Symptoms of Lyme borreliosis
<i>Typhus group rickettsioses</i>	Not said
<i>Spotted fever rickettsioses</i>	Fever, rash, contact with tick
	Fever rash, leucopenia
<i>Spotted fever rickettsioses</i>	Fever, rash
	Fever of 38°C, eschar, muscle pain, weakness, ticks attached, return from South Africa
	Fever, rash, tick's bite in South Africa
	Fever, skin rash, leukopenia, thrombocytopenia
	Enlarged painful lymph nodes, maculopapular rash, eschar behind ear concha

Incubation period of rickettsioses lasts from 8 to 18 days. After that time the symptoms of the diseases appear and specific rickettsial antibodies can be detected in the second week of the disease. On the other hand a broad group of other tick-associated rickettsial agents of unknown pathogenicity exist (eg, *R amblyommii*) and may cause confusion in interpreting serologic surveys or a single elevated antibody titer (8).

## CONCLUSIONS

The mentioned evidences suggest that rickettsioses infections in Poland are commonly unrecognized and in consequence unreported and unregistered. Lack of any information about the patients' symptoms and their duration, patients' age and region where the infection has been contracted, it makes impossible to evaluate laboratory tests results and suggest clinicians for further examination. If research for rickettsiosis are made immediately after infection, antibodies will not be detect.

## REFERENCES

- Roux V, Rault D. Phylogenetic analysis of the genus *Rickettsia* by 16S rDNA sequencing. *Res Microbiol* 1995;146:385-396.
- Dumler JS, Barbet AF, Bekker CP, et al. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *Int J Syst Evol Microbiol* 2001;51:2145-2165.
- Chen SM, Dumler JS, Bakken JS, et al. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol* 1994;32:589-595.
- Rault D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. *Clin Microbiol Rev* 1997;10:694-719.
- Parola P, Rault D. Ticks and tickborne bacterial diseases in humans: an emerging infectious threa. *Clin Infect Dis* 2001;32:897-928.
- Halliday J, Daborn C, Auty H, et al. Bringing together emerging and endemic zoonoses surveillance: shared challenges and a common solution. *Philos Trans R Soc Lond B Biol Sci.* 2012 ;367(1604):2872-2880.
- Carithers HA.: Cat-scratch disease. An overview based on a study of 1,200 patients. *Am J Dis Child.* 1985;139(11):1124-33.
- Walker DH, Paddock CD, Dumler JS. Emerging and re-emerging tick-transmitted rickettsial and ehrlichial infections. *Med Clin North Am.* 2008 ;92(6):1345-1361.
- Mediannikov O, Diatta G, Fenollar F, et al. Tick-borne rickettsioses, neglected emerging diseases in rural Senegal. *PLoS Negl Trop Dis* 2010;14;4(9).
- Parola P, Rovey C, Rolain JM, et al. *Rickettsia slovaca* and *R. raoultii* in tick-borne Rickettsioses. *Emerg Infect Dis.* 2009;15(7):1105-8.
- Tylewska-Wierzbanowska S, Chmielewski T: Tick-borne zoonoses occurring in Poland. *Post Mikrobiol.* 2010;49:191-197.

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