**EV0446**

**ePoster Viewing**

**Clinical ID: Lyme borreliosis, toxoplasmosis**

# Is erythema migrans always caused by *Borrelia burgdorferi*?

A. Moniuszko[1], J. Dunaj[2], M. Andersson[3], S. Pancewicz[2], P. Czupryna[2], K. Guziejko[2], M. Kondrusik[2], S. Grygorczuk[2], R. Swierzbinska[2], J. Zajkowska[2]

[1]*Medical University of Bialystok, Bialystok, Poland*

[2]*Medical University in Bialystok, Bialystok, Poland*

[3]*Molecular Ecology and Evolution Lab MEEL- Biology Department, Lund University Sweden, Lund, Sweden*

## Objectives

It is well established that erythema migrans (EM), as the symptom of early phase of Lyme disease, is caused by *Borrelia burgdoferi*. However, molecular diagnostic does not always let us to detect genetic material of the spirocheates. Therefore we aimed to search for *Anaplasma phagocytphilum*, as an etiological factors of EM skin lesions.

## Methods

Skin and blood samples of 20 patients with EM were examined for DNA of *B. burgdorferi* and *A. phagocytophilum*.

Molecular detection of *Borrelia* species was performed by using the *B. burgdorferi* PCR kit (GeneProof, Czech Republic) - amplification of the a specific DNA sequence of a 276 bp fragment of the flagellin encoding gene by nested one -tube PCR. Amplification was performed according to the following reaction program: UDG decontamination at 37°C for 2 minutes, initial denaturation at 96°C for 10 minutes, first amplification for 30 cycles (denaturation at 96°C for 20 s, annealing at 68°C for 20 s, extension at 72°C for 40 s), second amplification for 45 cycles (denaturation at 96°C for 20 s, annealing at 54°C for 20 s, extension at 72°C for 30 s) and final extension at 72°C for 2 minutes.

*A. phagocytophilum* DNA concentration was measured with a Nanodrop 2000 spectrophotometer (Thermo scientific, Wilmington DE, USA) and diluted to a concentration of 10ng/µl DNA. PCR was performed with primers ehr521 and ehr747 according to Pancholi et al. targeting a part of the 16s rRNA gene, with the following conditions; 94ºC for 2 min followed by 35 cycles consisting of 94ºC for 30 s, 55ºC for 30 s and 74ºC for 30 s, followed by at 72 ºC for 10 minutes. These primers have been shown to amplify various Anaplasmataceae species.

**Results:** Blast searches showed 100% similarity with published *A. phagocytophilum* sequences in 6 out of 20 examined skin samples and in 4 out of 20 blood samples. In none of these blood and skin samples *B. burgdorferi* DNA was detected. In the skin samples of 10 patients, but not in any blood sample, *B. burgdorferi* DNA was detected. In the remaining 4 samples neither *B. burgdorferi* nor *A. phagocytophilum* DNA was found.

**Conclusions:** Despite popular opinion that EM is caused only by *B. burgdorferi* it seems that many skin lesions after a tick bite may be misdiagnosed. Our study shows, that *A. phagocytophilum* may be a frequent etiologic factor of EM and should always be considered in differential diagnostic any skin lesion after a tick bite. Interpretation of these data can bring important contribution to establish the role of *A. phagocytophilum* in EM and forming the principle of precaution with laboratory diagnosis eg. patients treated with amoxicillin.