

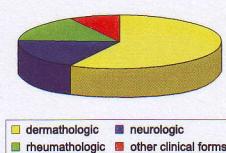
Molecular detection and identification of Lyme disease spirochetes in humans and animals

Hulínská D., Votýpka J., Langrová K., Dřevová H., Plch J., Bojar M., Valešová M.

Objective:

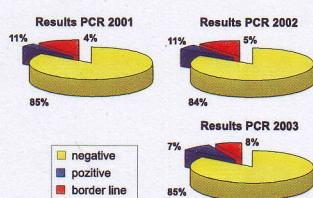
To unveil the prevalence of different *Borrelia* strains in patients and animals and to find relationship between strains and different clinical symptoms, dermatologic, neurologic, rheumatologic and others as shown in graph.

Basic clinical forms 2001-2003



Methods:

Direct proof of agents by immunosorbent (ISEM) method using transmission (TEM) or scanning (SEM) electron microscopy, PCR and real-time PCR with sequence analysis of products



Results:

Borrelia garinii and *B.afzelii* spirochetes were detected by ISEM in the tissue biopsy of the epidermis (Figs.1a,b) dermis (Figs.2a,b) skeletal muscle and heart (Fig.3) liver (Figs.4a,b) synovium (Fig.5a), body fluid (Fig.6a) and brain biopsy (Figs.6b and 7).

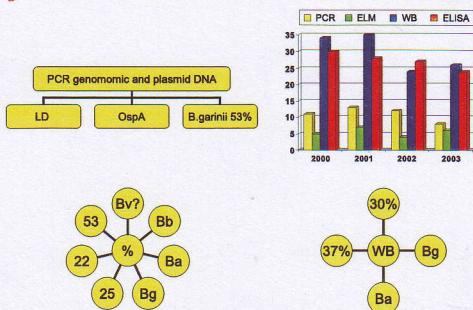
We were successful in culturing that agent in 16 cases (4 blood, 6 skin, 6 CSF isolates). Real-time PCR targeted, 16S DNA (Fig.8), OspA (Fig.9), recA (Fig.10) genes were utilized for detection and quantification (Fig.11) of *Borrelia* spp. in 18% infected patients (out of 300) that had involvement of the nervous system, joints and heart caused by *B.garinii* (54%), *B.burgdorferi* (20%), *B.afzelii* (22%). The remaining 4% comprise mixed infection.

Among wild animals, roe deer (Fig.12), red deer, fallow deer and mouflons 22% (out of 75) tested positive with *B.garinii* and *B.burgdorferi*. Mice were positive to *B.afzelii* in 12%.

Conclusion:

Borrelia garinii predominantly involved nervous system and joints as revealed by real-time PCR. Sequence analysis detected serotypes 3, 5 and 6, while serotypes 1, 2 and 4 were rare.

Comparison of the results from direct and undirect methods



Prevalence of *Borrelia burgdorferi* and *borrelia garinii* in 2001 and 2003 in the three wotched regions

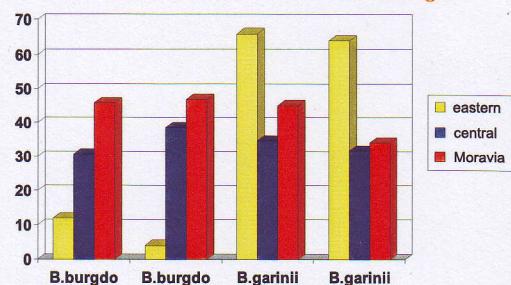


Figure 1

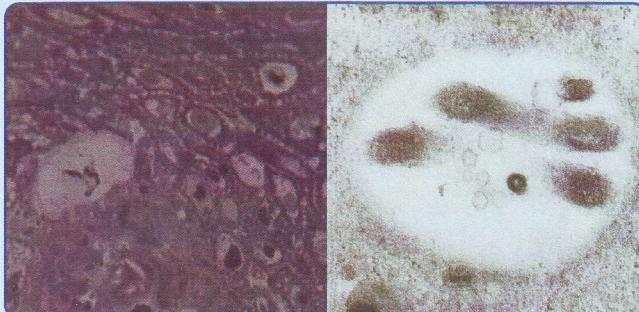


Figure 2

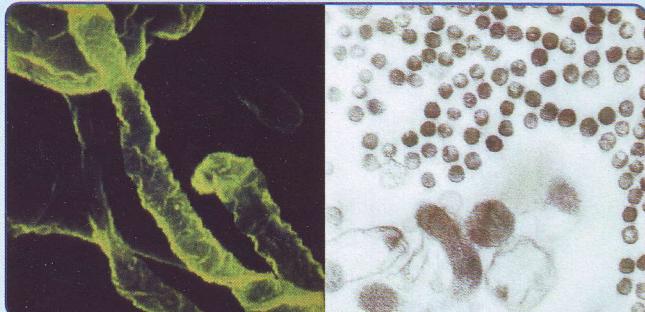


Figure 3

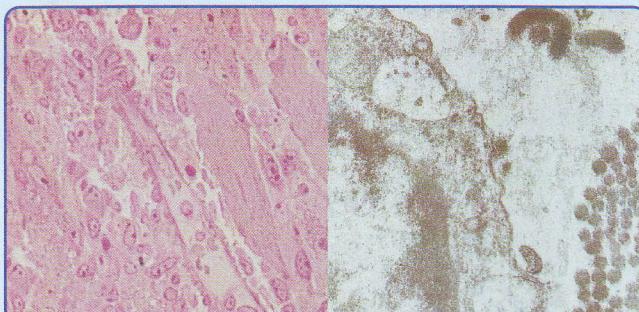


Figure 4a

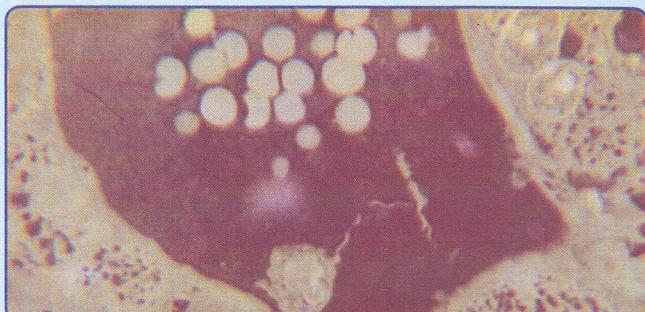


Figure 4b

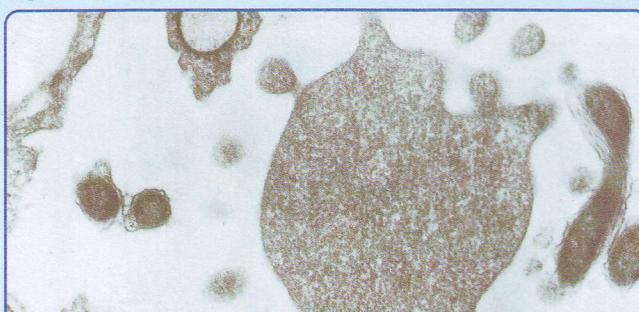


Figure 5a



Figure 5b

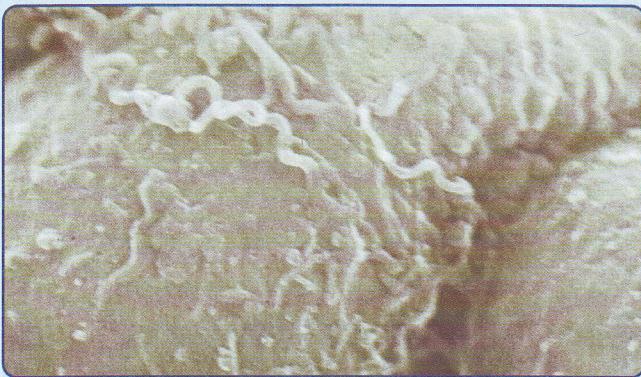


Figure 5c



Figure 6a

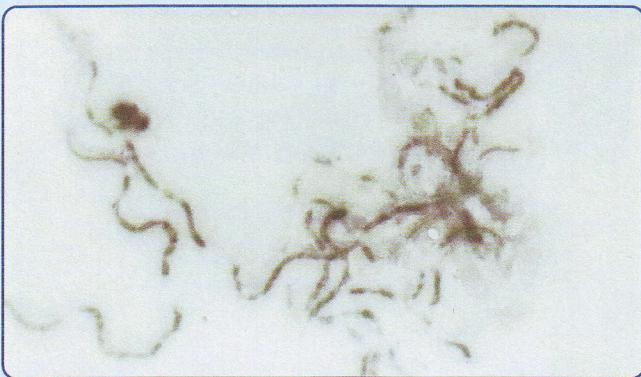


Figure 6b



Figure 7

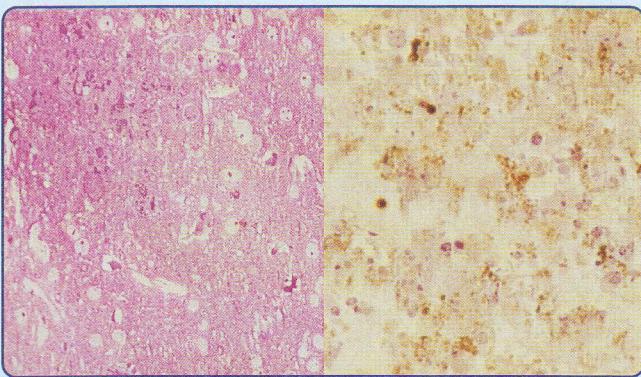


Figure 8

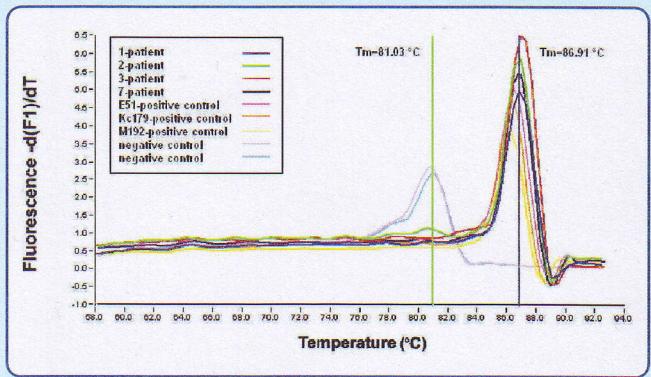


Figure 9

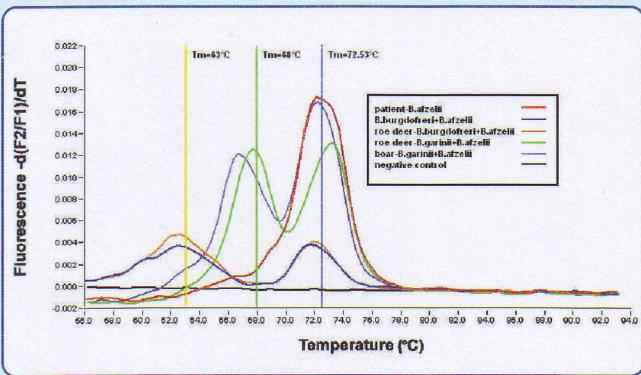


Figure 10

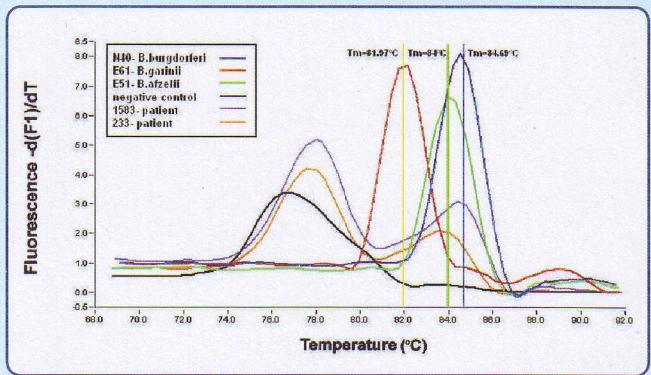


Figure 11

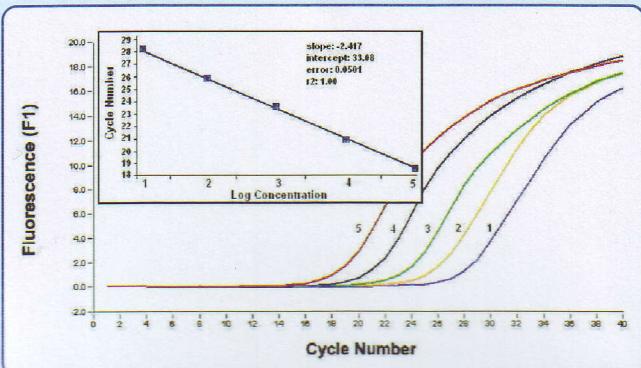


Figure 12

