

# Incidence and prevalence of Lyme-Disease in Germany

"Lyme-Borreliosis is the most frequent TBD in Germany. Its incidence is estimated between **50-thousand and 100-thousand** each year."

Wilske, Fingerle et al. on CD: "Lyme-Borreliose", version 1.0, Hoffmann-La-Roche AG; Nov. 2001

Incidence of Lyme-Disease in Germany:  
**50.000 - 60.000 / year**  
and  
150 -200 of TBE

BgVV, Press-release 15 / 2001, 27. April 2001

Incidence of Lyme-Borreliosis in the region  
**"Oder-Spree" : 89,3 / 100 000**  
and in  
**„Amt Scharmützelsee“ 237/ 100 000**  
inhabitants

Talaska, Brandenburgisches Ärzteblatt 11 / 2002; 338-340

**"0.5% of the population are getting ill every year!"**

"In the area examined by us, namely Kraichgau in Nord-Baden, about 17% of the population are seropositive. Most of these seropositive patients also have characteristic complaints. By prospective investigation of the cohort over nearly ten years we show that the annual rate of new illnesses (incidence) amounts to about 0.5% of the population."

[http://www.dieterhassler.de/diagnostik\\_und\\_therapie.htm](http://www.dieterhassler.de/diagnostik_und_therapie.htm)

**"Every 5th inhabitant** of Baden-Württemberg is infected with *Borrelia burgdorferi*. There are 40.000 new cases of infection every year according to bio-toxicologist Thomas Hartung at a workshop in the University of Konstanz. The danger of illness is particularly high in the large region of Konstanz, because an average of 35 percent - in individual areas up to 57 percent - of ticks are infected with *Borrelia* which they could pass on to humans."<sup>1</sup>

Comparison: in the area of Berlin-Brandenburg up to 60% of ticks are infected with *Bb* (Burmester)<sup>2</sup>

1. Stuttgarter Zeitung v. 22. Februar 2001; S.7
2. G.-R. Burmester (Charite), lecture in "Institut für Laboratoriumsmedizin", Berlin, 13. März 2002

## PREVALENCE AND INCIDENCE OF LYME-BORRELIOSIS IN SOUTH AND EAST BAVARIA

"We observed an incidence of Lyme-Disease of 1.5 % per year and 0.6 % of symptomless new infections per year."

Poster: B. Reimer<sup>1</sup>, A. Marschang<sup>1</sup>, V. Fingerle<sup>2</sup>, B. Wilske<sup>2</sup>, F. v. Sonnenburg<sup>1</sup>.

<sup>1</sup> Abteilung für Infektions- und Tropenmedizin, <sup>2</sup> Max-von-Pettenkofer Institut für Mikrobiologie, Universität München, 1999

## Vectors / transmission of *Borrelia burgdorferi*

**Mites ?**<sup>1</sup> "Work, which we completed this autumn, showed that mites feeding on *Borrelia burgdorferi* infected mice take up *Borrelia*. This puts mite-problems in a new light and requires epidemiological investigations ..."

**Mosquitoes ?**<sup>2</sup> *Aedes vexans* for *B. afzelii*

1. <http://www.meb.uni-bonn.de/parasitologie/wissensch.htm>
2. Faulde et al.: "Vorkommen und Verhütung Vektor-assoziiierter Erkrankungen des Menschen in Deutschland unter Berücksichtigung zoonotischer Aspekte" Bundesgesundheitsblatt (2), 4, 2001, 921-93
2. Ann Agric Environ Med 2002;9(1):55-7 Detection of *Borrelia burgdorferi* sensu lato in mosquitoes (Culicidae) in recreational areas of the city of Szczecin. Kosik-Bogacka D, Bukowska K, Kuzna-Grygiel W.
2. Infection 1999;27(4-5):275-7 Isolation of *Borrelia afzelii* from overwintering *Culex pipiens* biotype molestus mosquitoes; Halouzka J, Wilske B, Stunzner D, Sanogo YO, Hubalek Z.
2. Halouza; Med Vet Entomol 1998 Jan; 12(1): 103-5: Isolation of *B. afzelii* from the mosquito *Aedes vexans* in Czech Republic
2. contra mosquitoes; Matuschka, Parasitol Res (2002) 88: 283-284

### Ticks

Larvae (transovarial infection)

Nymphs  
Adult ticks

1. Burgdorfer, Transovarial and transstadial passage of *Borrelia burgdorferi* in the western black-legged-tick, *Ixodes pacificus*; Am.J.Trop.Med.Hyg.37 (1987), 188-192
2. H.Horst: "Einheimische Zeckenborreliose bei Mensch und Tier", 1997, S.44

- Highly infectious ticks may carry more than 100 spirochetes<sup>1</sup>
- In spring-time *Bb* often is transmitted via ticks by cystic forms<sup>2</sup>

1. L. Gern, Schweiz; oral presentation, IPS-VI; Berlin 2001
2. Alekseev; Acarina 9 (2): 299-307: "The alteration between spirochete and cystic forms of the tick-borne borreliosis agent: it's relationship .."

### Transmission of *Bb* during pregnancy and by blood-transfusion

- *Bb* survives longer than *treponema-pallidum* in a refrigerator: 25 days at 4°C<sup>1</sup>
- histological proof of foetal organs: mostly no inflammation, negative serology<sup>2</sup>

1. Pantanowitz, Transfusion Medicine, 2002, 12, 85-106 (overview)
2. H.Horst: "Einheimische Zeckenborreliose bei Mensch und Tier", 1997, S. 122-127

### It's not always a tick: "biting flies" may be vectors of *Borrelia burgdorferi* as well as other insects

1. MMW 131 (1989) Nr. 18, S. 93
2. Luger; N Engl J Med, 1990 Jun 14; 332 (24): 1752
3. G.R. Burmester; 13. März 2002; Vortrag im Institut für Laboratoriumsmedizin; Berlin
4. Magnarelli, J Inf Dis Vol 54 No.2 Aug 1986 p.355 ff; The etiologic agent of LD in Deer Flies, Horse Flies and Mosquitoes
5. Magnarelli, J Clin Microbiol; Aug 1988; p 1482-1486 Ticks and Biting Insects with the Etiologic Agent of Lyme Disease, *Borrelia burgdorferi*

# Pathomechanisms

## of *Borrelia burgdorferi* sensu lato and their implications for diagnostics, clinical appearance and treatment of Lyme-Disease

### ***Borrelia burgdorferi* grows slowly**

Bb needs ca. 12-20 (8-35) hours for one generation-time

- cf *E. coli* needs ca. 20 min for one generation-time
- Bb sometimes needs 10 weeks for culturing

1. Preac-Mursic et al, Infection 1996 Jan-Feb, 24 (1) 9-16; Kill kinetics of Bb and bacterial findings in relation to the treatment of LB
2. Hassler, [http://www.dieterhassler.de/diagnostik\\_und\\_therapie.htm](http://www.dieterhassler.de/diagnostik_und_therapie.htm)

### **The slow growing of Bb means for the infected human being:**

- He / she can become ill a long time after infection (latency) <sup>1</sup>
- treatment has to take a long time to reach as many generations as in treatment of fast-growing-bacteria ( 60 -100 x?)
- Consider using therapy-principles of other slow-growing-bacteria; e.g. : *M. leprae*, *M. tuberculosis*, *T. pallidum*
  - treatment of TBC: combi for at least 6 months;
  - similar to leprosy: ca. 2 years combi-therapy (*E. Freeksen*, Borstel, Malta); before at least 10 years of Dapson <sup>2</sup>

1. Holger Blenk, Vorsitzender des Bundesverbandes der Ärzte für Mikrobiologie und Infektionsepidemiologie; Saarland online – 16 years latencywysiwyg://19/http://www.sol.de/news/boulevard/fitness/139682.php3 :
2. Hans Schadewaldt, Über die Rückkehr der Seuchen; VGS Köln 1994, S. 68; Robugen

### ***Borrelia burgdorferi* sequester in tissue which is poorly vascularised**

- connective tissue (present in all organs) and which is
- poorly infiltrated by defence cells - the immune system

Hauptl, Burmester et al.: Persistence of Bb in ligamentous tissue from a patient with chronic LB; Arthritis Rheum 1993 Nov; 36(11): 1621-6

"Considering an early germ-dissemination into CNS .. it seems being necessary to reach high antibiotic-levels in target-tissues like joint-synovia or CNS.. even in treatment of erythema migrans or *Borrelia-lymphozytom*."

U. Neubert, Borreliosen – Therapie 1998, Fortschritte der praktischen Dermatologie und Venerologie; ISBN 3-540-64352-4

"..In principle the disease symptoms result from the high affinity of the *Borreliae* to collagen fibre. Thus connective tissue (collagen) is particularly prone to chronic inflammatory processes. The result is vessel inflammation (vasculitis processes with perivascular infiltrates of lymphocytes and plasma cells) (literature: Meier, de Koning, Duray).

Capillary occlusions lead to disturbances of the tissue-supply, e.g. the vessels by which nerves are supplied (Epineurium). This again leads to (ischaemia -) pain and increased vulnerability. So probably the periarticular decalcifying process is a consequence of the poor local supply in the bone. *Borreliae* can probably partly evade the the immune system by sequestering in collagen where they are inaccessible to antibiotics ."

[http://www.dieterhassler.de/diagnostik\\_und\\_therapie.htm](http://www.dieterhassler.de/diagnostik_und_therapie.htm)

Decorin-binding-proteins (Dbp A, DbpB) are thought to be adhesion-molecules of Bb to collagen-associated extracellular matrix (decorin)

1. Exner M., Successful vaccination for LD...; Expert Opin. Biol. Ther. (2001) 1(5): 783-793
2. Guo et al.: Mol.Microbiol (1998) 30:711-723 and Infect.Immun. (1995) 63:3467-3472

### ***Borrelia burgdorferi* is able to invade human cells and persist there:**

e.g. blood-cells (macrophages), fibroblasts, endothelial, and synovial cells  
Perhaps Bb can even survive in CNS-cells?

1. Malawista: J Immunol 1993 Feb; 150(3) 909-15; Persistenz in Maus-Makrophagen
2. Ma Y, A Sturrock, JJ Weis: Intracellular localization of *Borrelia burgdorferi* within human endothelial cells. Infection and Immunity 59, 1991 671-678
3. Hauptl, Burmester et al.: Persistence of Bb in ligamentous tissue from a patient with chronic LB; Arthritis Rheum 1993 Nov; 36(11): 1621-6
4. Arthritis Rheum 2001 Jan; 44(1):151-62; Insights from a novel three-dimensional in vitro model of Lyme arthritis: standardized analysis of cellular and molecular interactions between *Borrelia burgdorferi* and synovial explants and fibroblasts. Franz JK, Fritze O, Rittig M, Keysser G, Priem S, Zacher J, Burmester GR, Krause A.

### **Cell wall permeable antibiotics are required to treat intracellular Bb**

- Tetracycline, Doxycycline, Minocycline
- Macrolides: Roxithromycin, Azithromycin, Clarithromycin, - **no Erythromycin!**

1. Hunfeld et al: Standardised in vitro susceptibility testing of Bb against well-known and newly developed antimicrobial agents - possible implications for new therapeutic approaches to LD; Int.Med.Microbiol.291; Suppl.33, 125-137 (2002)
2. Terekhova, Antimicrobial Agents and Chemotherapy, Nov 2002, p.3637-3640, Vol.46, No.11; Erythromycin Resistance in Bb

### ***Borrelia burgdorferi* can change its appearance**

- by "starvation" (antibiotics, CSF) Bb can change its appearance:
  - cyst, bleb, mesosom, granulum
- a "cyst" / L-form / spheroblast can later convert to living spirochetes again

1. Brorson; Infection 1997 Jul-Aug 25(4) 240-6, Transformation of cystic forms of *Borrelia burgdorferi* to normal, mobile spirochetes.
2. Kersten; Antimicrobial Agents and Chemotherapie; May 1995; p.1127-1133: Effects of Penicillin, Ceftriaxon and Doxycycline on Morphology of Bb
3. Gruntra, Cinco: APMIS 2001 May; 109(5): 383-8; Conversion of *B. garinii* cystic forms to motile spirochetes in vivo
4. Brorson, O., & Brorson S, Infection, 1998;26(3):144-50 (R) In vitro conversion of *Borrelia burgdorferi* to cystic forms in spinal fluid, and transformation to mobile spirochetes by incubation in BSK-H medium.

### **"Cysts" are resistant to the usual antibiotics**

- Metronidazole can be used against cysts
- CNS tissue is highly permeable to it
- Metronidazole can cause cancer or harm an embryo / foetus
- Possible to use other treatment options against cysts: Hydroxychloroquin (anti-malaria-drug); ranitidine bismuth citrate

1. Brorson; An in vitro study of the susceptibility of mobile and cystic forms of Bb to hydroxychloroquine; Int Microbiol 2002 Mar;5 (1) :25-31
2. Brorson; Brorson O, Brorson SH, APMIS 1999 Jun; 107 (6): 566-76, An in vitro study of the susceptibility of mobile and cystic forms of *Borrelia burgdorferi* to Metronidazole
3. Brorson; Int.Microbiol 2001 Dec; 4(4):209-15; Susceptibility of motile and cystic forms of Bb to ranitidine bismuth citrate

### The human body may confuse *Borrelia burgdorferi* with its own tissue

- LYMERix, the US-Bb-vaccine on Osp-A-basis, has been recalled because of side-effects (Feb. 2002)
- Some of side-effects may be caused by "molecular mimicry" of Osp-A with hLFA-1<sup>2</sup>
  - arthritic complaints
    - o possibly impaired leucocyte-funktion
- Part of the side-effect can be induced (in this way) without vaccination
- Cross-reactivity of 41kD with myelin of peripheral nerves<sup>1</sup>

1. Aberer, Ann Neurol 26, 1989, 732-737: Molecular Mimicry and Lyme Borreliosis: A Shared Antigenic Determinant Between *Borrelia burgdorferi* and Human Tissue
2. Steere AC, Gross D, Meyer AL, Huber BT; J Autoimmun 2001 May;16(3):263-8; Autoimmune mechanisms in antibiotic treatment-resistant Lyme arthritis

### Quorum sensing - Bacterial interactions: What's that ?

Why are micro-organisms simultaneously active?  
How do they communicate?  
Do we find this phenomenon at Bb ?  
LuxS

Possible implications for LD:

- "up-regulation" of erp / OspE (CRASPs)
- "flares" - symptoms can be active at different points of the body

Stevenson B, Babb K.; Infect Immun 2002 Aug;70(8):4099-105; LuxS-mediated quorum sensing in *Borrelia burgdorferi*, the Lyme disease spirochete.

### Parts of Bb cell wall protect Bb from the host defence system

by manipulation of complement Systems (CRASPs)  
Factor H binding  
OspE

1. The Journal of Immunology, 2002, 169: 3847-3853. Complement Inhibitor Factor H Binding to Lyme Disease Spirochetes Is Mediated by Inducible Expression of Multiple Plasmid-Encoded Outer Surface Protein E Paralogs, Alitalo et al.
2. Brade, Kraiczy: Immunevasion of Bb: Insufficient killing of the pathogens by complement and antibody; Int.J.Med.Microbiol.291; Suppl.33; 141-146 (2002)

## Co-Infections with

### Babesia

- ca. 5% seropositive, "healthy" adults<sup>1</sup>

### Ehrlichia

### Bartonella

- cat-scratch-disease
- cases of death at Swedish "elite-cross-runners", eg myocarditis<sup>2</sup>

### Rickettsia

- spotted fever<sup>3</sup>

### FSME / TBE (Virus)

### Other *Borrelia*-species

- relapsing fever<sup>4</sup>

1. Hunfeld KP et al. Wien Klin Wochenschr, 1998; 110: 901-8
2. McGill S et al. Scand J Infect Dis, 2001; 3: 423-8
3. Appl Environ Microbiol 2002 Sep;68(9):4559-66 *Rickettsia monacensis* sp. nov., a Spotted Fever Group Rickettsia, from Ticks (*Ixodes ricinus*) Collected in a European City Park. Simser JA, Palmer AT, Fingerle V, Wilske B, Kurti TJ, Munderloh UG.
4. Richter D, Schlee DB, Matuschka F-R. Relapsing fever-like spirochetes infecting European vector tick of Lyme disease agent; Emerg Infect Dis 2003 June; Vol. 9, No. 6; <http://www.cdc.gov/ncidod/EID/vol9no6/02-0459.htm>

### *Borrelia burgdorferi*-debris can make you ill

Lysis of Bb, e.g by taking antibiotics, may release cell wall pieces

- acute: Herxheimer-reaction  
It's estimated that parts of Bb cell wall of gram negative bacteria are responsible for a severe course of illness (Lyme-encephalopathy)
- ca. 3% dry-weight of Bb are LPS
  - Perhaps colestyramine inhibits the entero-hepatic (cephalic) circulation of toxins
    - ? production of ectotoxins, Bbtox-1, (like *Botulinus*.-toxin?)

1. pro NT: Zajkowska, Juchnowitz; Przegł Epidemiol 2002; 56 Suppl 1:37-50 (Abstract)
2. Beck; Inf. Dis 152 (1985), 108-117, Chemical and biological characterization..
3. contra LPS: Takayama; 1987, Absence of LPS in Bb; Infect.Immun, 55, 2311-13

### Heterogeneity, antigenshift and antigendrift may lead to

- immunevasion
- difficulties in serological testing
- symptom-flares like relapsing-fever

1. Onishi et al.; Antigenic and genetic heterogeneity of Bb populations transmitted by ticks; Proc.Natl.Acad.Sci. USA 2001;January 16; 98 (2): 670-675
2. Fang et al.: An Immune Evasion Mechanism for Spirochetal Persistence in Lyme Borreliosis; JEM Vol 195; No.4; Febr. 18, 2002 415-422
3. Hefty et al.: Changes in Temporal and Spatial Patterns of Outer Surface Lipoprotein Expression Generate Population Heterogeneity and Antigenic Diversity in the Lyme-DiseaseSpirochete, *Borrelia burgdorferi*: Infection and Immunity July 2002; p. 3468-3478
4. Wilske et al.:Impact of Strain Heterogeneity on Lyme Disease Serology in Europe:Comparison of Enzyme-Linked Immunosorbent Assays Using Different Species of *Borrelia burgdorferi* sensu lato; JCM Febr. 1998, 427-436

### *Borrelia burgdorferi* may lead to immune-deficiency

Bb suppresses inflammation (TNF- $\alpha$ ,  $\gamma$ -interferon, G-CSF)  
-possibly no fever  
-lack of : ESR, CRP, leucocytes-funktion  
Trial: supplication of G-CSF (Hartung, Konstanz)

1. Diterich,Hartung; Modulation of Cytokine Release in Ex Vivo-Stimulated Blood from Borreliosis Patients, Infection and Immunity, Febr. 2001, p.687-694
2. Current study of Universität Konstanz about antibiotics plus Neutropen for the treatment of LD
3. Immunology Volume 107 Issue 1 Page 46 - September 2002 ; Cytokines in Lyme borreliosis: lack of early tumour necrosis factor-alpha and transforming growth factor-beta1 responses are associated with chronic neuroborreliosis, Mona Widhe

**"When diagnosed early, borreliosis can be treated successfully with antibiotics. If the infection is not diagnosed, diagnosed too late or treated with an inadequate or overly short course of antibiotics, a chronic course of the disease may develop affecting the nervous system, joints and heart which is difficult or even impossible to treat."**

1. BgVV, IPS VI, Berlin 2001, press release 15/2001, 27. April 2001;
2. D.T. Dennis, CDC; oral presentation, IPS VI, Berlin 2001 - "...sometimes it's severe or disabling, in particularly, when the diagnosis is missed early..."