

What, when and why in the laboratory diagnostics of Lyme-borreliosis of dogs

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Laboratory diagnosis of Lyme-borreliosis in dogs

Global trend?

Google:

Results **1 - 10** of about **641,000** for lyme disease dog. (**0.26** seconds)

Results **1 - 10** of about **890** for Lyme kutya. (**0.15** seconds)

Overview

- Lyme-disease: history and prevalence
- Vectors
- Etiology and pathophysiology
- Syndromes and symptoms
- Differentials
- Specific testing
- Consequences

Lyme-disease: history and prevalence

- 1975 Lyme, Connecticut, USA –Alan Steere
- Museum specimens from 1895 (USA, UK) PCR-positive!
- Proven prevalence:
Northern hemisphere

Prevalence in ticks

- USA 30-70% Európa: 10-35%
- Up to 4 genogroup in one tick

Seropositivity in dogs:

Endemic regions: 70-90%. 5% of that is clinically ill !!

Vectors

- Ixodes, Dermacentor
- In Hungary:
- **Ixodes ricinus** (Földvári G. PhD-thesis) Dermacentor reticulatus?



Borreliosis-etiology

- **Borrelia burgdorferi sensu lato group**

Borrelia burgdorferi sensu stricto (USA, Europe)

Borrelia lusitanae (USA, Europe)

Borrelia afzelii (Európa, Asia)

Borrelia garinii (Európa, Asia)

Borrelia valaisiana (Európa)



Pathomechanisms facts

- Infection with the saliva of the vector
- No horizontal and vertical spreading
- Perzistent infection
- Migration in connective tissue
- Bacteraemia?

Syndroms, symptoms

Symptoms to appear 2-5 months fter experimental infection

Fever , anorexia, lymphadenopathia, intermitting and wandering lameness

Arthritis- 1st in the joint closest to tick bite

Natural infection

As above

Glomerulonephritis

Erythema chronicum migrans?

Neuroborreliosis?

Myocarditis?

Uveitis, chorioretinitis?

Lyme-nephritis

- Golden/Labrador retriever (USA)
- Bernese mountain dog? (Europe)
- Acute or chronic renal failure

Anorexia, PU/PD +/-, weight loss, dehydration

Proteinuria, (glucose-, hematuria)

azotaemia, non reg. anaemia, hypercholesterinaemia,
hypoalbuminaemia, hyperfosfataemia, hyperkalaemia stb.

Lameness, fever, anorexia

Painfull, swollen joint

Tick borne pathogens:

Borreliosis, Anaplasmosis,
Ehrlichiosis, RMSF, Bartonellosis,

Mycoplasmosis

Immun-medated poliartthritis

Systemic lupus erythematosus

Rheumatoid arthritits

Septic arthritis

Degenerative joint disease

Trauma

Lyme+, proteinuria:

urinalysis

urine protein/crea ratio

Blood pressure

Pedigré-analysis

Kidney biopsy

Periarticular swelling, pain

Panostitis

Osteomyelitis

Hyperthrophic osteodystrophy

Hyperthrophic osteopathia

Polymiosytis

Neoplasma

Paralytic, weak limbs

Metabolic disorder

Cardiopulmonary disease

Neurológc syndroma

Vascular disease

Further testing as indicated

- CBC, chemistries, CRP, UA
- Serologies
- ANA, RF, Coomb's-test
- Joint tap-cytology, microbiology
- RTG
- Tumor-search(RTG, UH, lymph node, bone marrow cyology)

„Specific”-tests

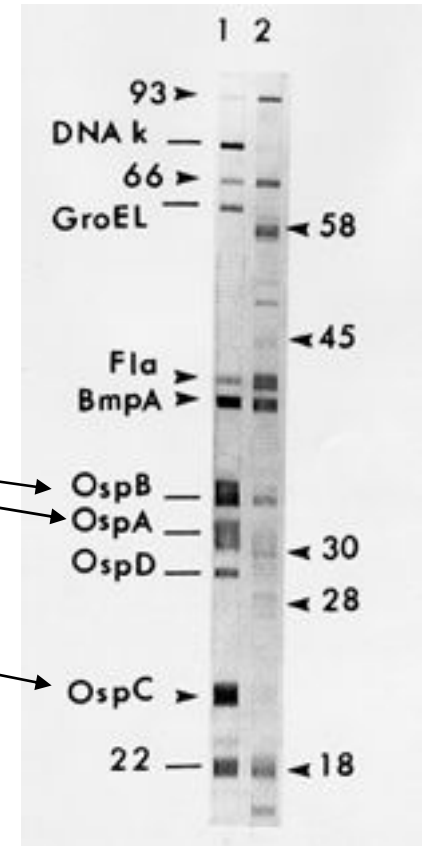
Conventional serology

- **Borrelia ELISA/IFT**
 - Whole bacterin antibodies
 - Numerous cross reacting species (mostly Spirochaetes-CAVE-oral flora!, Leptospirák)
 - Difficulties in comparison of test done by different labs (test kits)
(different Ag-preparates)
- Paired titers? (persisting bacteria-persisting antibodies)
- IgG/IgM?
 - IgM 3-6 weeks after infection. után+
 - IgG- 4-6 weeks after+
- + titer before symptoms appear in experimental cases

Serology-more specific tests

Western immunoblot

- Separates the antibody spectrum
- No titer
- Vaccine induced titer is usually distinguishable
- Early infection?
- Paired titers?



Immunblot: paired titers?

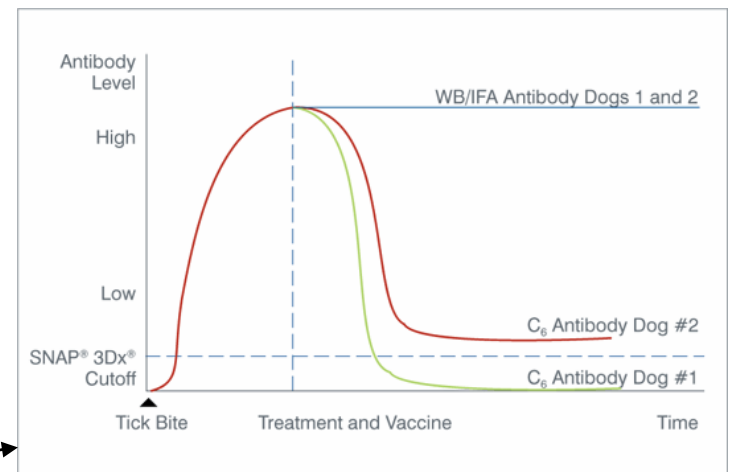
Owner	Györi, Barbala	Order ID	
Animal	Dog Maugli	Laboratory No.	VM436926/28.07.2006
Test	Result	Sign	
18 kDa (specific)	positive	++	
19 kDa (specific)	positive	++	
21 kDa (highly specific)	negative		
22 kDa (specific)	positive	++	
25 kDa (OspC specific)	positive	++	
28/29 kDa (OspD highly spec.)	positive	++	
30 kDa (highly specific)	positive	++	
31 kDa (OspA highly spec.)	positive	++	
34 kDa (OspB highs spec.)	positive	++	
39 kDa (highly specific)	negative		
41 kDa (cross reactiv)	positive	++	
45 kDa (specific)	negative		
58 kDa (specific)	negative		
100/83 kDa (highly spec.)	negative		
VLSE (highly specific)	negative		
IgG Immunoblot	positive	++	

Owner	Györi, Borbala	Order ID	90039943
Animal	Dog Maugli	Laboratory No.	VM505452/01.09.2006
Test	Result	Sign	
18 kDa (specific)	positive	++	
19 kDa (specific)	positive	++	
21 kDa (highly specific)	negative		
22 kDa (specific)	positive	++	
25 kDa (OspC specific)	positive	++	
28/29 kDa (OspD highly spec.)	weak positive	++	
30 kDa (highly specific)	positive	++	
31 kDa (OspA highly spec.)	positive	++	
34 kDa (OspB highs spec.)	positive	++	
39 kDa (highly specific)	weak positive	++	
41 kDa (cross reactiv)	positive	++	
45 kDa (specific)	negative		
58 kDa (specific)	negative		
100/83 kDa (highly spec.)	weak positive	++	
VLSE (highly specific)	negative		
IgG Immunoblot	positive	++	

Serology-more specific tests

C6-ELISA

- Immunodominant conservative region IR6/VlsE protein C6-synthetic peptid Ag
- Specificity 99,6%
- Sensitivity 94,4%
- Not influenced by vaccination
- Correlates with bacterial load
- Paired titer
- 3Dx, 4Dx (Ehrlichia, D. immitis, Anaplasma), Quantitv C6-ELISA



More specific tests: PCR

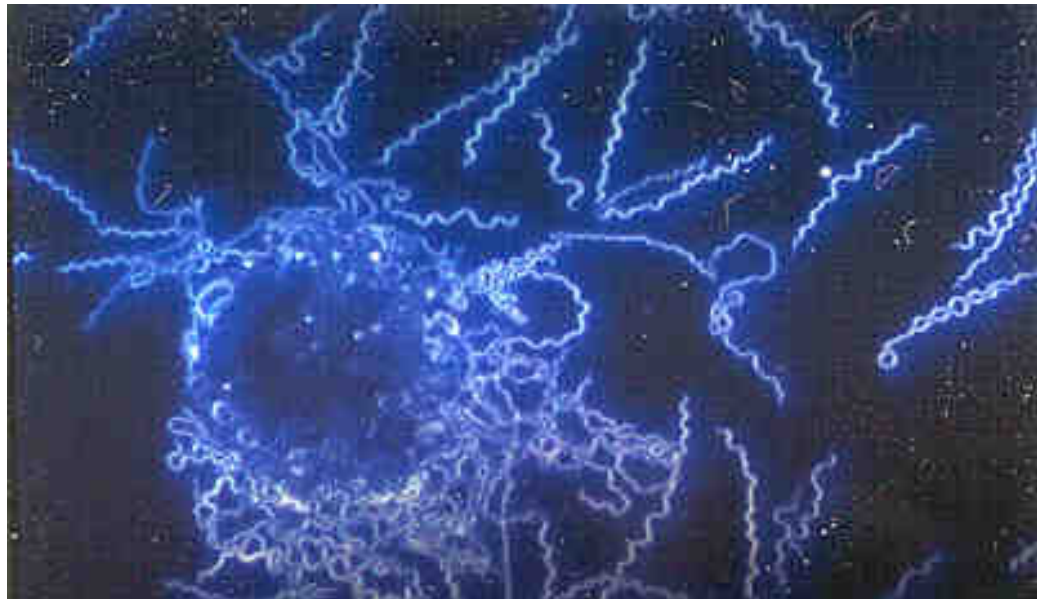
- Highly specific, sensitive but:
- „only” DNS-detection (vs. Living organism)
- Genotype can be determined (eg sequencing)
- Optimal sample:
- NB: patomechanisim: bacteraemia is rare or very short
- Skinbiopsy, synovia, liquor
- tick

Borrelia PCR -blood

- *Borrelia burgdorferi* sensu lato
 - (nucleic acid by PCR) negative (negativ) hjb
- No detection of nucleic acid of *Borrelia burgdorferi*, *B.afzelii* or *B.garinii* by PCR.
- When interpreting a negative result it must be taken into account that bacteraemia occurs only for a short period of time during the course of infection. Depending on the clinical symptoms the material needed for detection of *Borrelia* nucleic acid is joint aspirate, cerebrospinal fluid or a skin biopsy.

Borreliosis: culture

- Modified Barbour-Stoenner
Kelley–medium, very long
incubation
- **Insensitive**
- + eredmény 100%-ban
specifikus



A Lyme-disease diagnostic algorithm

Indikáció: tick bite
symptom adequate
rule out infection prior to vaccination

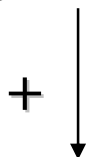
IF treat with antibiotics

NOT TRATED with anitbiooitics

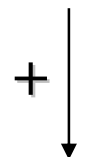
„Traditional testing in 2 steps

„C6-series”

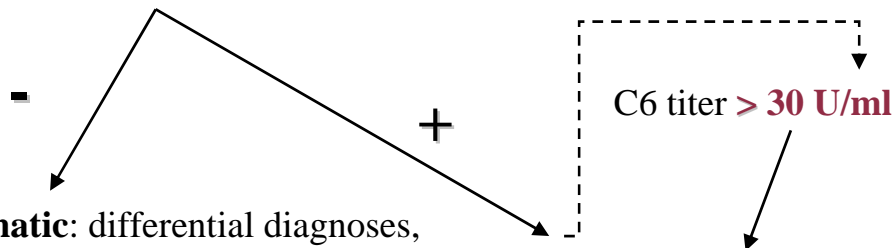
1. „screening” ELISA



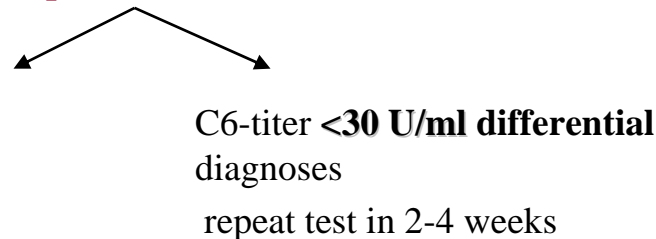
1. **qualitatív C6-ELISA**



2. **Confirmation by Western immunoblot**



2. **quantitatív C6-ELISA**



If symptomatic: differential diagnoses, repeat testing in 2-4 weeks

If asymptomatic: not infected (or repeat test in 2-4 weeks)

TREAT

Checking therapy in 3-6 months

A minimum of **50%-decline in quant. Titer** indicates an effective treatment

Borreliosis: treatment

Most joint disease are intermitting and self limiting

Doxycyclin: chondroprotective in noninfectious arthritisben s well!

Treat if:

Characteristic symptoms!

ELISA+Immunblot positive / C6-ELISA positive

- Doxycyclin
- Amoxycillin/ampicillin
- III.-generáció cephalosporins (Ceftriaxone)
- Makrolids
- Cave: a bacteria cannot be eliminated may have recidiva on steroid treatment

Take home

1. Search for differentials
2. ELISA/C6-ELISA if negative –not Lyme
3. If positive: immunblot/C6-ELISA quant.
4. If vaccinated or status questionable: Immunblot/C6-ELISA
5. If localised symptoms: Biopsy- PCR

References

- Littman MP, Goldstein RE, Labato MA, Lappin MR, Moore GE. ACVIM small animal consensus statement on Lyme disease in dogs: diagnosis, treatment, and prevention. *Vet Intern Med.* 2006 Mar-Apr;20(2):422-34.
 - Liang FT, Jacobson RH, Straubinger RK, Grooters A, Philipp MT. Characterization of a *Borrelia burgdorferi* VisE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. *J Clin Microbiol.* 2000;38(11):4160–66.
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