Information session for the National Reference Center for Borrelia burgdorferi (UCL St Luc and UZ Leuven)

16-11-2023

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UZ LEUVEN
National Reference Center
Borrelia burgdorferi

Recap important information

- **Accreditation** requested: please enter your name (and RIZIV/INAMI if applicable) in the chat box
- **Interactive sessions**: you can speak up by unmuting your microphone to ask questions or raise comments in the chat box

- No recording of the session but slides will be shared
  https://www.uzleuven.be/nl/laboratoriumgeneeskunde/nationale-referentiecentra-en-referentielaboratoria
  https://www.sciensano.be/nl/nrc-nrl/nationaal-referentiecentrum-nrc-voor-borrelia-burgdorferi-lymeziekte

National Reference Center
Borrelia burgdorferi

NRC Lyme Borreliosis

Université Catholique de Louvain

UZ Leuven (associated)

UZ LEUVEN
National Reference Center
Borrelia burgdorferi

Available tests

Cliniques universitaires SAINT-LUC
UCLouvain BRUXELLES

UCL St Luc and UZ Leuven

- Diagnosis by serology: Borrelia (serum) and neuroborreliosis (CSF)
- Confirmation by serology: Borrelia (serum) and neuroborreliosis (CSF)

UCL St Luc only

- Molecular diagnostics and identification of Borrelia (species)
- Culture and direct examination (studies)

UZ Leuven only

- CXCL13 biomarker (CSF) in the context of acute neuroborreliosis

National Reference Center
Borrelia burgdorferi

NRC for Borrelia burgdorferi (Lyme's disease)

Nationaal Referentiecentrum (NRC) voor Borrelia burgdorferi (Lymeziekte)

Belangrijk bericht

De tests moeten aangevraagd worden met het volledig ingevuld NRC-formulier met aanwijzing van de klinische en anamnestische redenen. **Indien geen enkele serologische of andere bevestiging gevraagd wordt, zullen alle tests uitgevoerd door het NRC als onderaanneming beschouwd worden en zal betaling volgens de normale RIZIV-procedure gevraagd worden.**

Meer informatie?

Voor meer informatie betreffende de werking van het NRC Borrelia burgdorferi (Lymeziekte) kan u de volgende website contacteren: https://uclouvain.be/fr/instituts-recherche/irec/mblg

Nuttige links:

Onderaan deze pagina kan u de **NRC-rapporten** terugvinden.

De bijhorende **epidemiologische surveillance-rapporten** kan u consulteren via:

Gezondheidsonderwerp Ziekte van Lyme , **Gezondheidsonderwerp Tekenoverdraagbare ziekten** , **Gezondheidsonderwerp Vectoroverdraagbare ziekten** .

Pathogen:

Borrelia burgdorferi (Ziekte van Lyme)

Verantwoordelijke laboratoria

Coördinator

- Université Catholique de Louvain

Geassocieerd

- UZ Leuven/KU Leuven

Erkend door

- National Institute for Health and Disability Insurance (INAMI-RIZIV)

Aanvraagformulieren

- Aanvraagformulier Borrelia

National Reference Center
Borrelia burgdorferi

Scientific reports (including NRC data)

sciensano

Nuttige links:

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Gezondheidsonderwerp Vectoroverdraagbare ziekten .

Epidemiologische surveillance van Lyme borreliose - 2019-2021

Ziekten en gezondheid in kaart brengen

Sci. report, recommendat°, guidance doc., directive, monograph

Nederlands

SCIENSANO

Downloaden 668.93 KB

Public Access

Published

Auteurs

inne Lernout; Cuypers, Lize; Sherihane Bensemmane; Dessilly, Géraldine; Scohy, Annaïs; Lagrou, Katrien; Depypere, Melissa; Kabamba-Mukadi, Benoît

UZ LEUVEN

National Reference Center
Borrelia burgdorferi

Application form

Cliniques universitaires SAINT-LUC UCLouvain BRUXELLES

***IDENTIFICATION DU LABORATOIRE QUI ENVOIE L'ÉCHANTILLON**

Nom du laboratoire : ……

Biologiste responsable : ……

Tél. : …… Fax : ……

Adresse email : ……

Médecin prescripteur :

……

……

N° INAMI : ……

RENSEIGNEMENTS CONCERNANT LE PATIENT

*Nom/prénom : ……

*Sexe : ❑ H ❑ F

*Date de naissance (ou âge) : ……

*Adresse : ……

*Code postal/Localité : ……

Nationalité : ……

N° national : ……

RENSEIGNEMENTS CONCERNANT L'ÉCHANTILLON

*Numéro d'identification : ……

*Nature : ……

*Date de prélèvement : ……

Tests effectués par le laboratoire demandeur ? ❑ Oui ❑ Non

Nom test 1: ……

Résultat : ……

Nom test 2 :……

Résultat : ……

IgG totales ou protéines LCR……g/L Sérum…….g/L

* Renseignements indispensables
** Requis pour le financement dans le cadre des CNR*** Pour les tests de sérologie, si demande en 1ère ligne, les tests seront remboursés par la nomenclature de l'INAMI
****Matrices en cours de validation
EFFECTIF A PARTIR DU 28/10/2021
RESPONSABLE DU DOCUMENT : A. BUCHELOT

CADRE RÉSERVÉ AU CENTRE DE RÉFÉRENCE

***INFORMATIONS CLINIQUES**

Date de début des symptômes :……

Symptômes :

❑ Erythema migrans

❑ Arthrite

❑ Neuroborréliose :……cellules/uL ; ……..% Lymphocytes

❑ Autres.

Spécifiez : ……

……

***INFORMATIONS ÉPIDÉMIOLOGIQUES**

❑ Morsure de tique. Date : ……

❑ Province probable de contamination : ……

❑ Traitement avant prélèvement diagnostique.

❑ Borréliose préalable. Année : ……

❑ Autres données épidémiologiques : ……

……

ANALYSES DEMANDÉES

❑ Sérologie diagnostique***

- ❑ Borrelia IgG (SANG)
- ❑ Borrelia IgM (SANG)
- ❑ Borrelia IgG (LCR)

❑ Sérologie de confirmation (Immunoblot)***

- ❑ Borrelia Confirmation IgG (SANG)
- ❑ Borrelia Confirmation IgM (SANG)
- ❑ Borrelia Confirmation IgG (LCR)
- ❑ Borrelia Confirmation IgM (LCR)

❑ PCR - Diagnostic et typage moléculaire (LCR, liquide articulaire, peau**** ou biopsie articulaire**** non fixée)
Dosage CXCL13 dans LCR (**à envoyer directement à UZLeuven**)

Analyse réalisée par un sous-traitant :

16SADN2E ❑ PCR 16s – Fièvre récurrente (*B. recurrentis, B. miyamotoi, B. hispanica, …sur sang total EDTA*)

***GEGEVENS OVER HET LABORATORIUM DAT HET STAAL OPSTUURT**

Naam laboratorium: ……

Klinische Bioloog: ……

Tel: …… Fax: ……

Emailadres: ……

Aanvragende arts :

……

RIZIV n° : ……

PATIENTGEGEVENS (OF STICKER)

*Naam/voornaam: ……

*Geslacht: ❑ M ❑ V

*Geboortedatum (of leeftijd): ……

*Adres: ……

*Postcode of woonplaats: ……

Nationaliteit: ……

Rijksregister n° ……

GEGEVENS OVER HET STAAL

*Identificatienummer: ……

* Staaltype: ……

*Afnamedatum: ……

TESTEN IN HET AANVRAGEND LABORATORIUM UITGEVOERD? ❑Ja ❑ Neen

Naam test 1: ……

Resultaat: ……

Naam test 2: ……

Resultaat: ……

*** Verplicht in te vullen**
**** Vereist voor financiering in het kader NRC**
*** Als er geen test is uitgevoerd, zullen de tests worden terugbetaald door RIZIV nomenclatuur behalve PCR en CXCL13. PCR en CXCL13 worden niet aangerekend.
****Matrix wordt gevalideerd**

VOORBEHOUDEN VOOR HET REFERENTIECENTRUM

***KLINISCHE GEGEVENS**

Datum van aanvang symptomen : ……

Symptomen:

❑ Erythema migrans

❑ Artritis

❑ Neuroborreliose:……cellen/ul ; ……% Lymfocyten

❑ Andere, specificeer: ……

……

……

***EPIDEMIOLOGISCHE GEGEVENS**

❑ Tekenbeet. Datum : ……

❑ Vermoedelijke provincie van besmetting : ……

❑ Behandeling reeds gestart voor staalname.

❑ Vroegere Lyme borreliose. Jaar : ……

❑ Andere epidemiologische gegevens :

……

……

AANGEVRAAGDE TESTEN***

❑ Serologie – diagnose

- *2315* ❑ Borrelia antistoffen (BLOED)
- *2434* ❑ Borrelia antistoffen (CSV)

❑ Serologie – bevestiging (Immunoblot)

- *2318* ❑ Borrelia Confirmatie IgG (BLOED)
- *2319* ❑ Borrelia Confirmatie IgM (BLOED)
- *2437* ❑ Borrelia Confirmatie IgG (CSV)
- *2438* ❑ Borrelia Confirmatie IgM (CSV)

❑ Dosering CXCL13

- *2439* ❑ CXCL13 biomerker (CSV)

PCR – Moleculaire diagnostiek en typering
(CSV, gewrichtsvocht, niet gefixeerde huid**** of gewrichtsbiopt****)
(Rechtstreeks naar UCLouvain verzenden)
PCR 16s – Febris recurrens (*B. recurrentis, B. miyamotoi, B. hispanica, … op vol bloed*)
(Rechtstreeks naar UCLouvain verzenden)

INTRODUCTION

UCLouvain

First, a little history…

In 1975… in “Old Lyme”, a small town in Connecticut / USA.

“Epidemic” of asymmetric arthritis of the large joints in 39 children and 12 adults.
13 patients had noticed an annular skin lesion of increasing diameter over the preceding weeks.

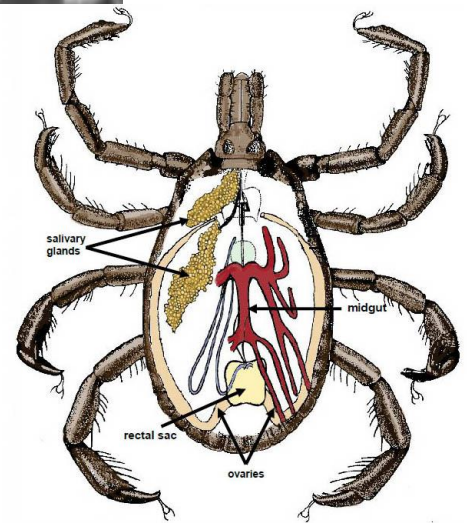
The maximum frequency was observed in summer and early autumn.

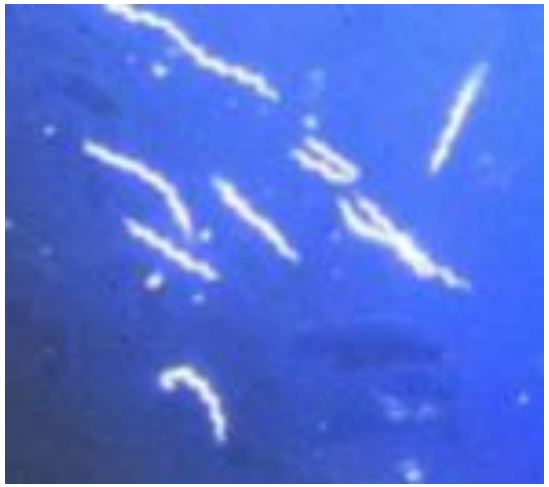
Lyme disease was born.
(But the Swedes had described chronic erythema migrans as early as 1909)

Source:

The beginning of the end of the story...

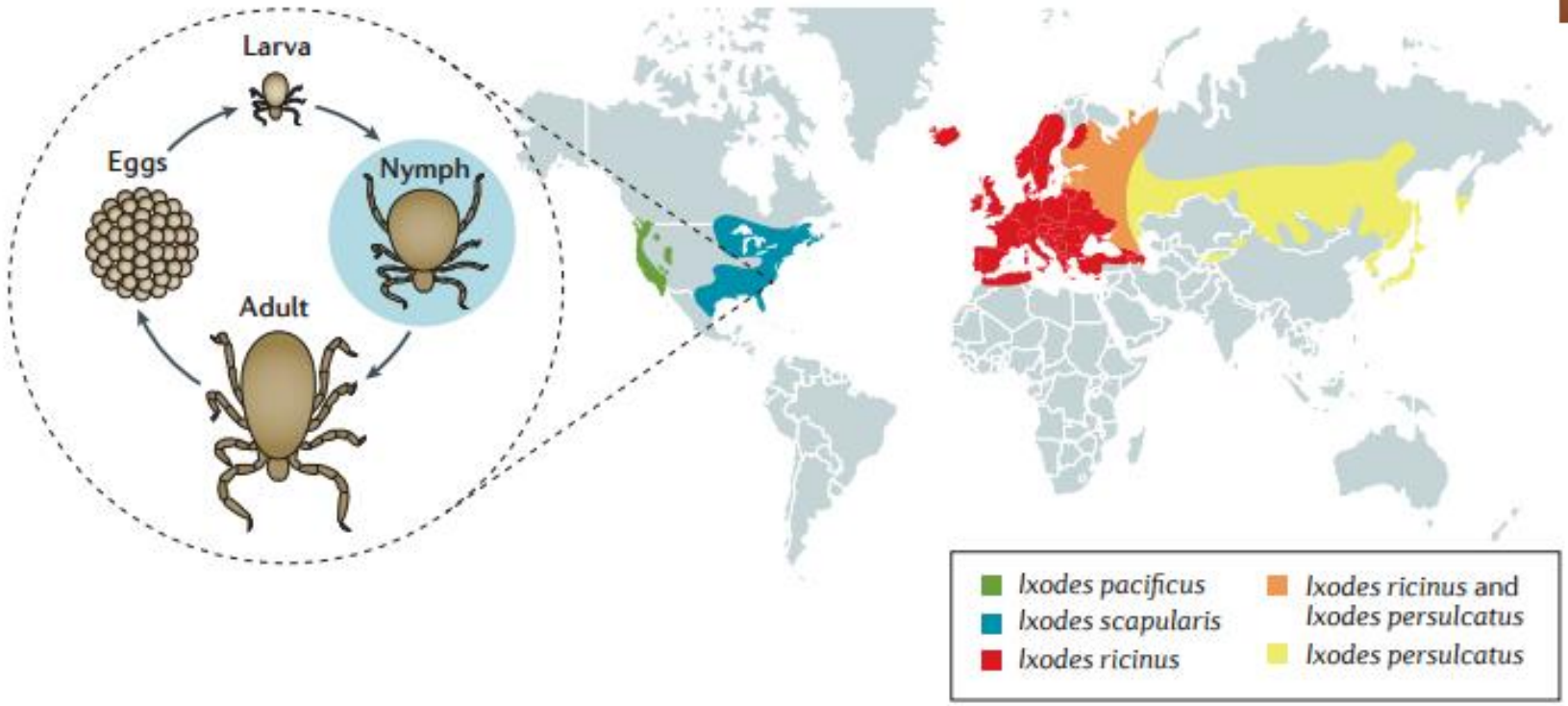
1982 : Mr. Burgdorfer discovered the presence of spirochetes in the intestine of an *Ixodes*

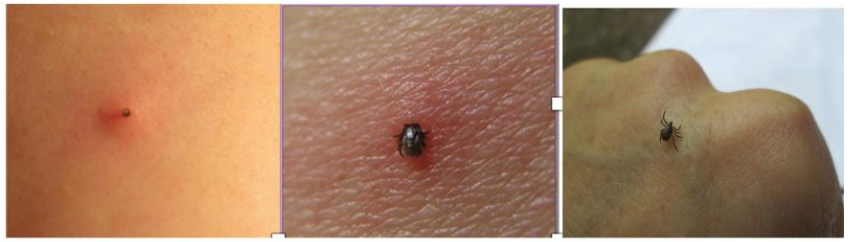




UCLouvain

Vector: arthropods of the *Ixodidae* family





a: larva b: nymph c: adult female tick

PMID: 27976670
PMID: 31097370

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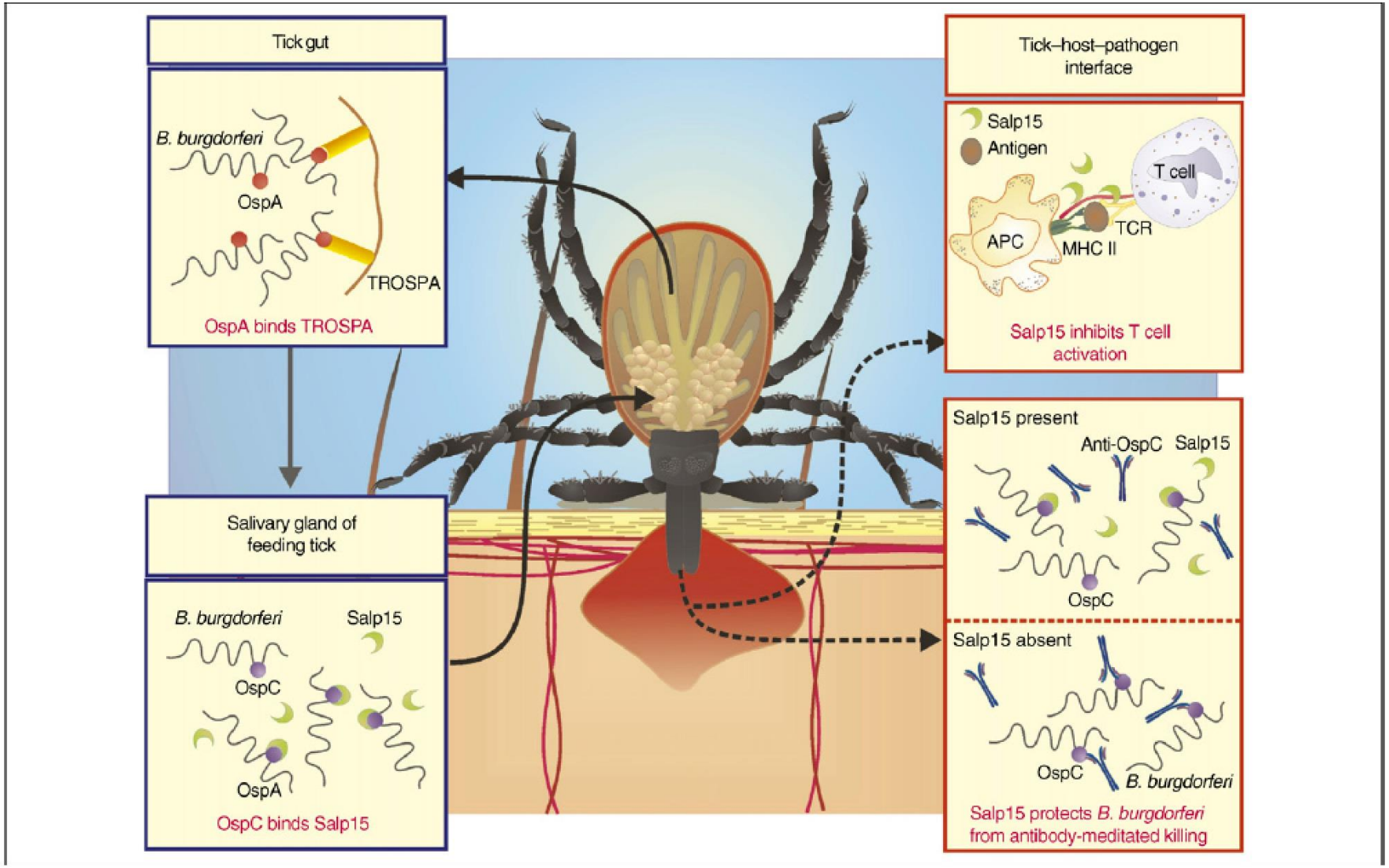


Hovious et coll. 2007

UCLouvain

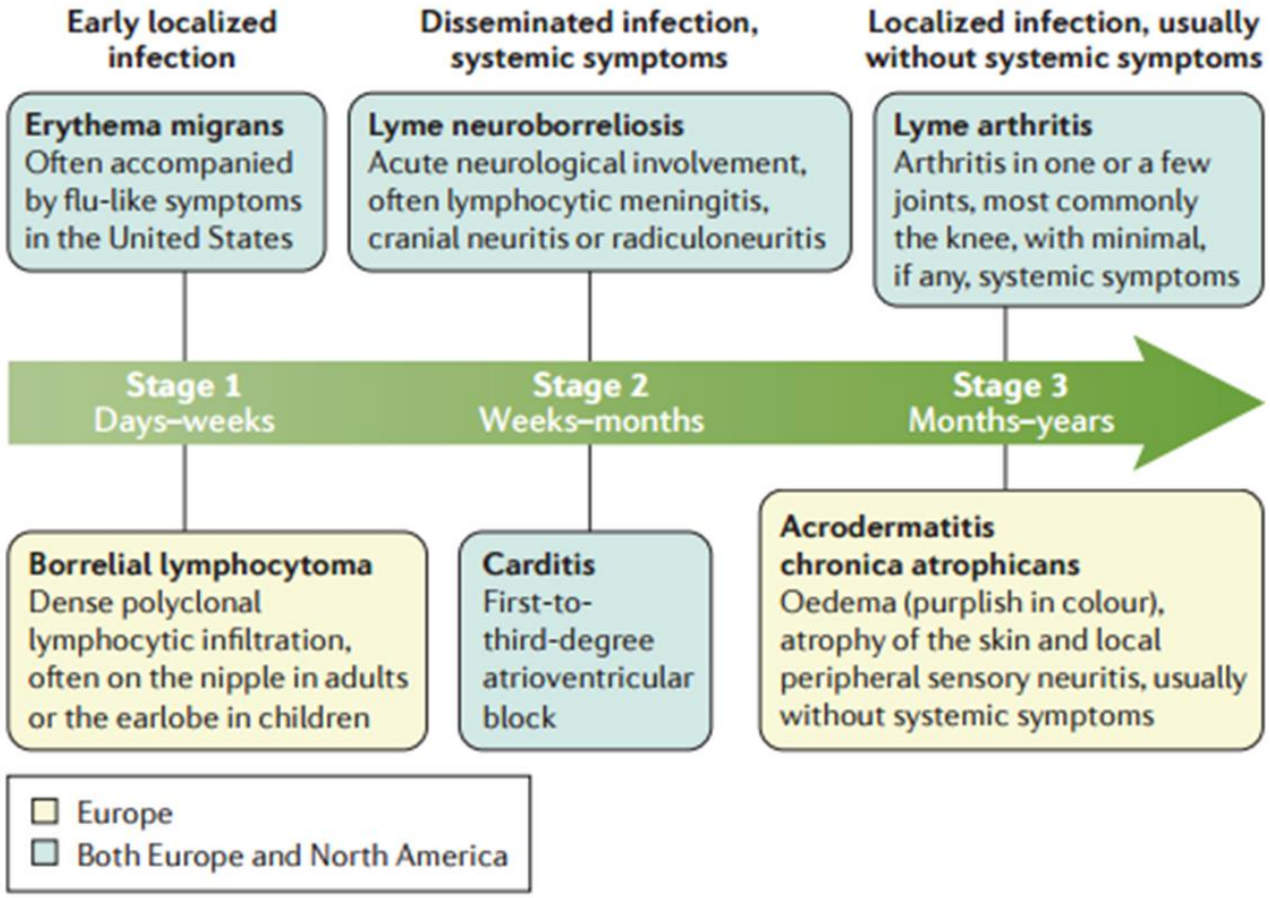
Lyme borreliosis in Belgium: transmission

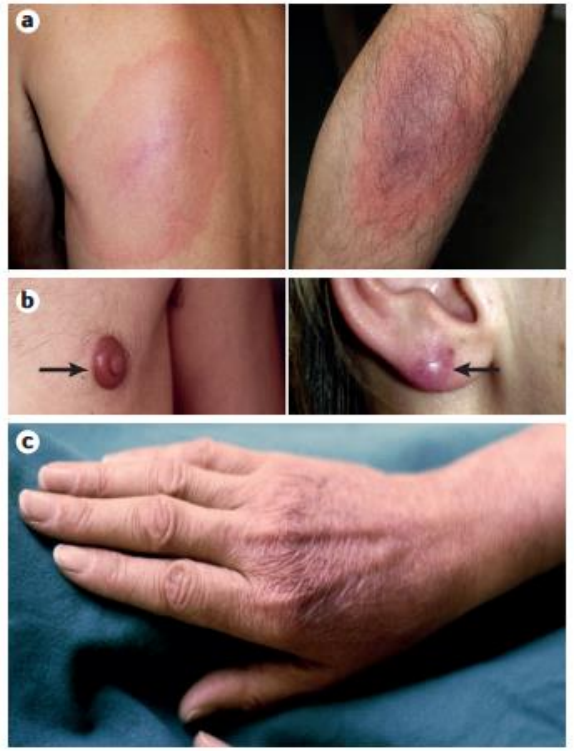
- Different factors influence the risk of transmission
    - Duration of blood meal on host (12-24 hours)
    - Density of ticks in the environment
    - Prevalence of Borrelia infection in ticks (10%)
    - Climatic conditions, type of vegetation
    - Host behavior

- Final risk after bite <3%

UCLouvain

Stages of infection – clinical manifestations





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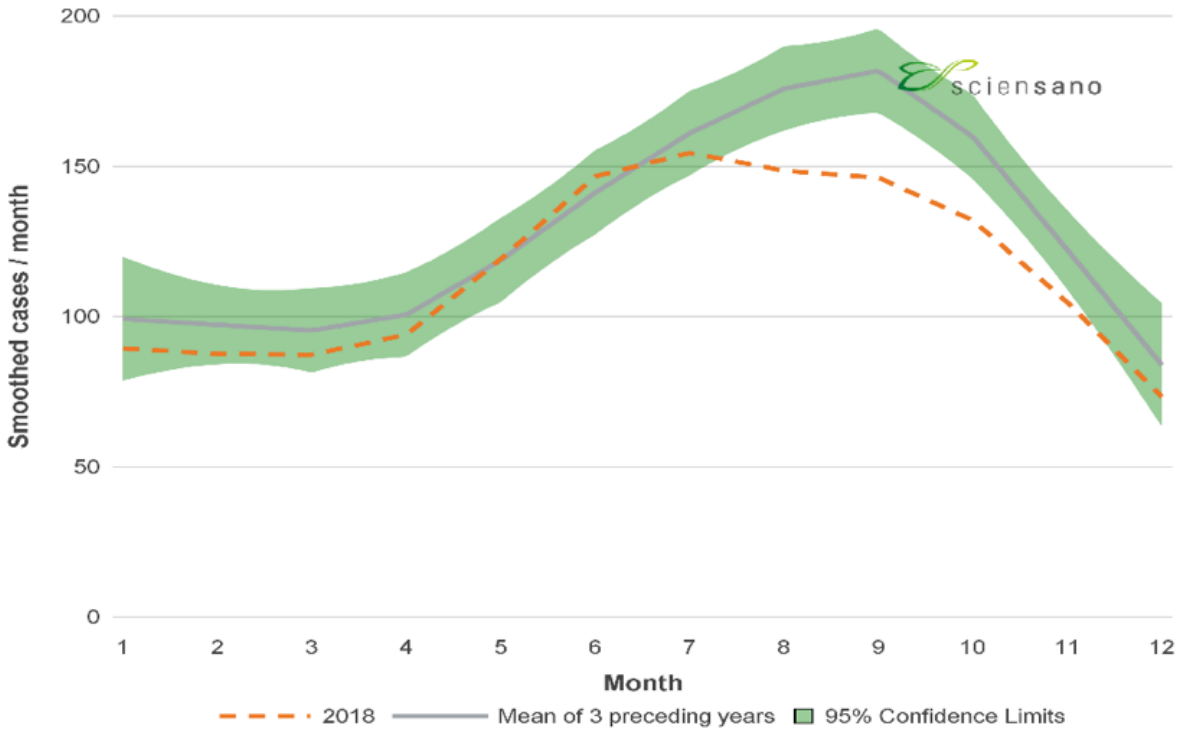
EPIDEMIOLOGY

UCLouvain

Lyme borreliosis: seasonal variability

- The majority of cases are recorded in summer and early autumn (from June to October)



Monthly number of positive serological results for *B. burgdorferi s.l.* reported in 2018 and average over the previous 3 years (2015-2017), Belgium
(Source: network of sentinel laboratories)

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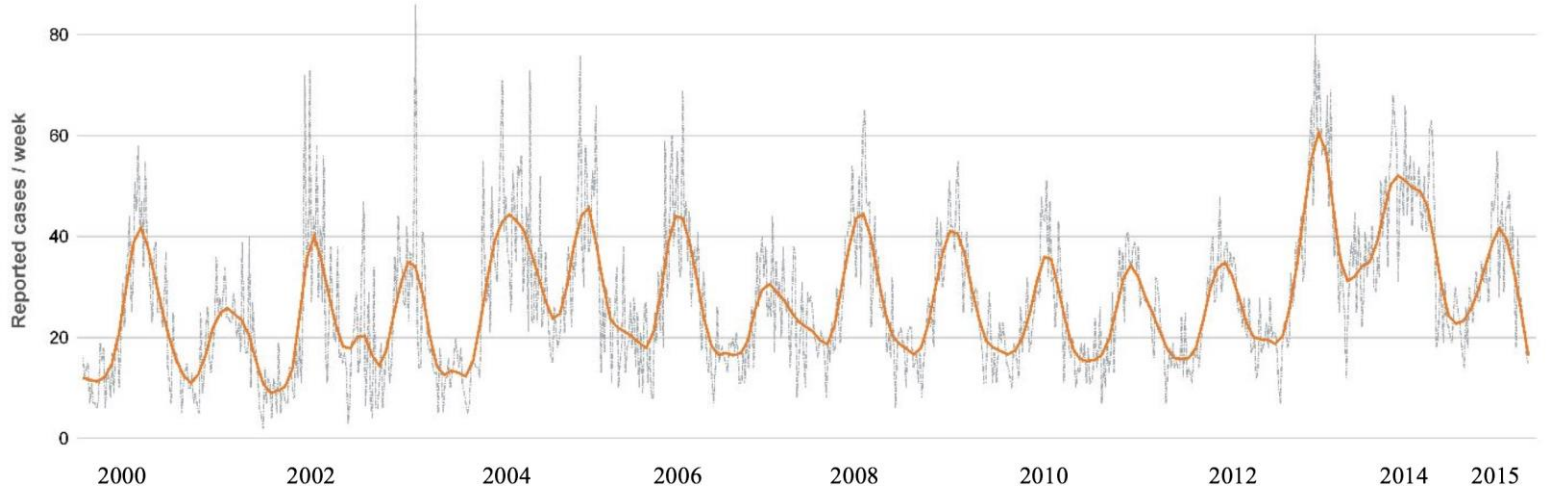
Lyme borreliosis in Belgium: epidemiology

- The number of positive serological tests is mainly reported
  - Network of sentinel laboratories covering 67% of tests
  - National Reference Center for Borreliosis
- The serology positivity rate remains stable (2 to 3%, 2007-2015).

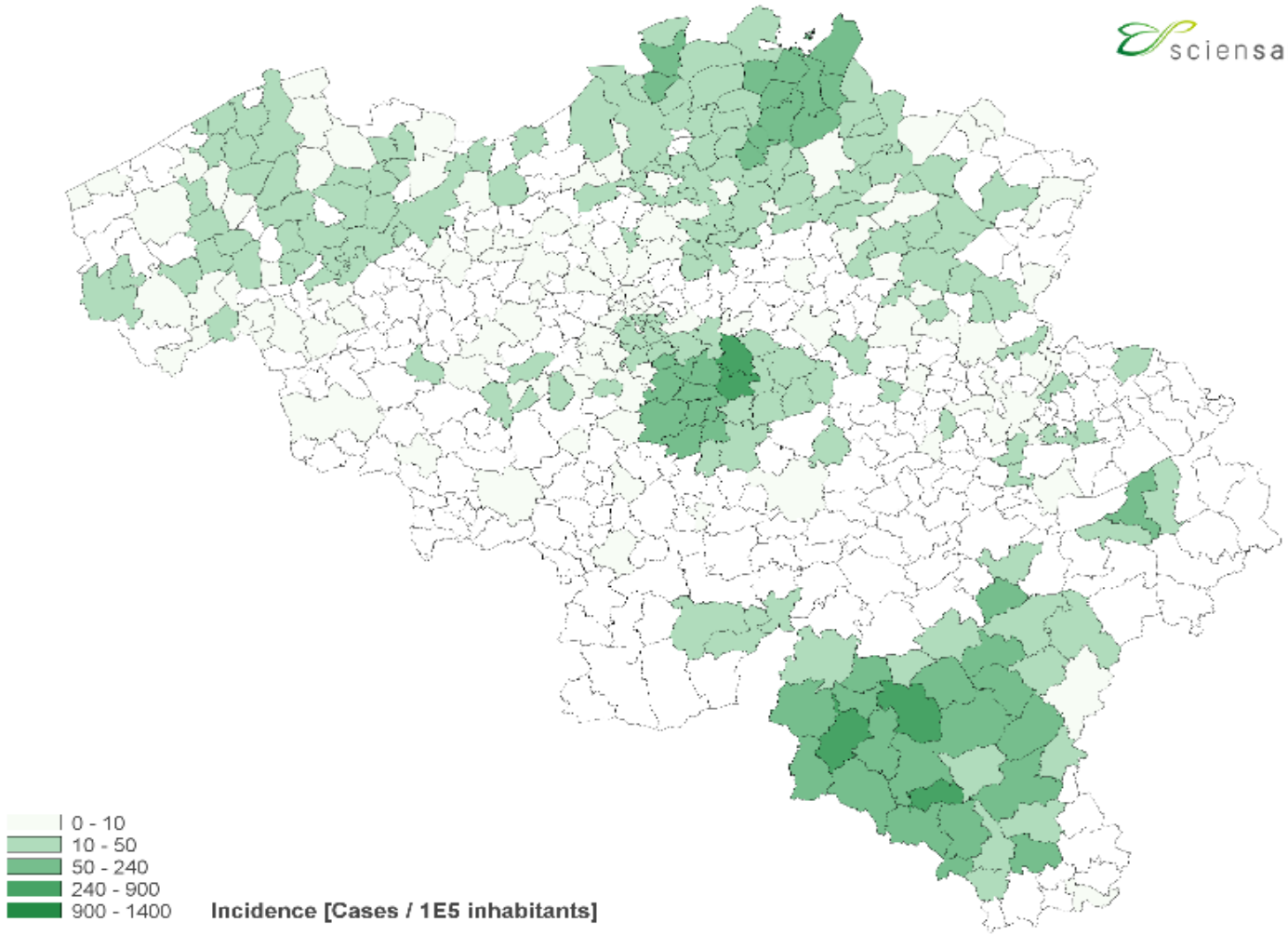
Figure 1: Nombre hebdomadaire de tests sérologiques positifs pour Borrelia effectués par les laboratoires vigies, Belgique, 2000-2015, WIV-ISP



http://organesdeconcertation.sante.belgique.be/fr/documents/recommandations-borreliose-de-lyme-2017

UCLouvain

Lyme borreliosis in Belgium: transmission and epidemiology



Reported incidence of positive serological results for B. burgdorferi s.l. by municipality, Belgium, 2018
(Source: sentinel laboratory network)

UCLouvain

Lyme borreliosis in Belgium: transmission and epidemiology

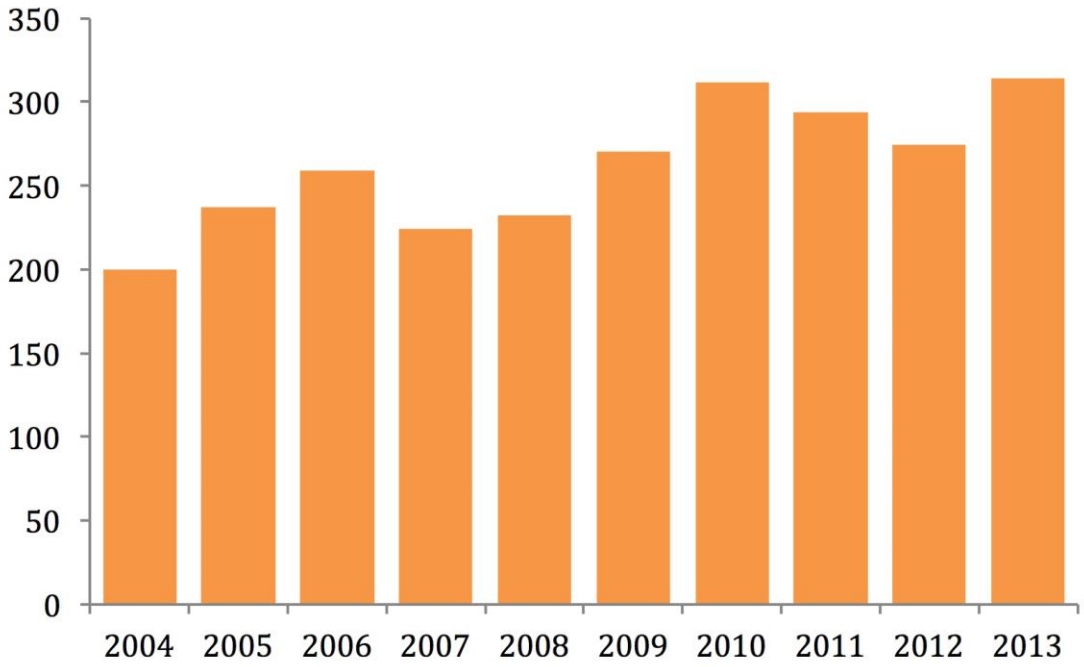
- Epidemiological surveillance based on clinical data
  - Number of patients consulting for EM (one-off studies),
  - Number of hospitalizations for Lyme borreliosis.
- In 2003-2004 and 2008-2009, study of the network of medical monitors
  - Tick bite 18.6 per 10,000 people
  - Erythema migrans 8.3 and 9.0 per 10,000 people
  - 7,360 to 9,270 cases of ME in 2003 and 8,080 to 10,003 cases in 2009, difference not statistically significant.
- In 2015-2016, the number of consultations
  - Erythema migrans 10.6 (95% CI 9.5-11.9) per 10,000 people
  - (WIV-ISP, D. Vancauteren, personal communication)

UCLouvain

Lyme borreliosis in Belgium: transmission and epidemiology

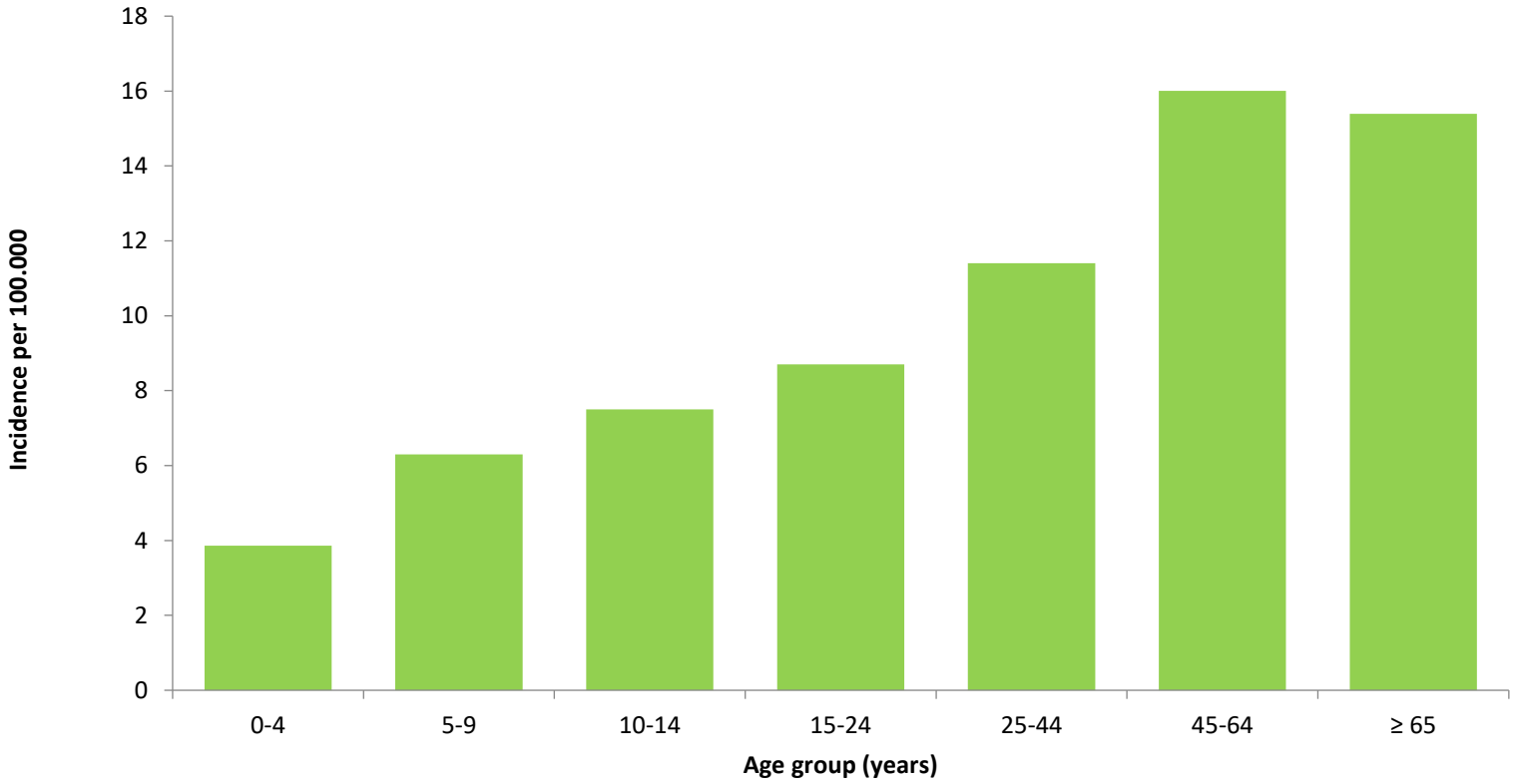
- From 1999 to 2010, an average of 300 people hospitalized per year
- Three sources indicate stability over the past 10 years.

Figure 4: Nombre d'hospitalisations pour maladie de Lyme (ICD9) de 2004-2013, RHM, Belgique, WIV-ISP



UCLouvain

Lyme borreliosis in Belgium: transmission and epidemiology



Reported incidence of positive serological results for *B. burgdorferi s.l.* by age group, Belgium, 2018 (Source: sentinel laboratory network)

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Lyme borreliosis in Belgium: transmission and epidemiology

Stability in the number of cases linked to Lyme disease over the last 10 years (even if apparent peak in 2013) but other approaches are necessary in terms of epidemiological surveillance.

UCLouvain

Lyme borreliosis in Belgium: tick bite report (tiquesnet.be) January - December 2022

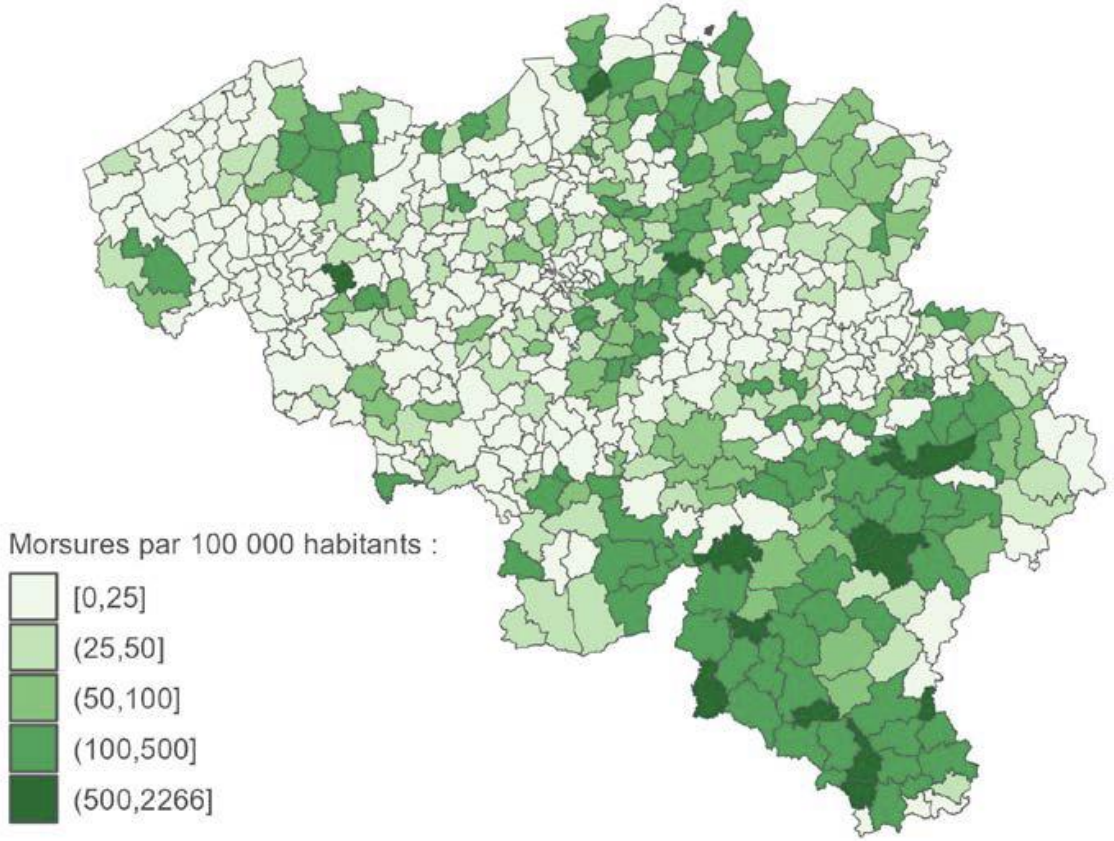


Figure 2 presents the geographical distribution of the number of tick bites per 100,000 inhabitants, established on the basis of the municipality where the bite took place.

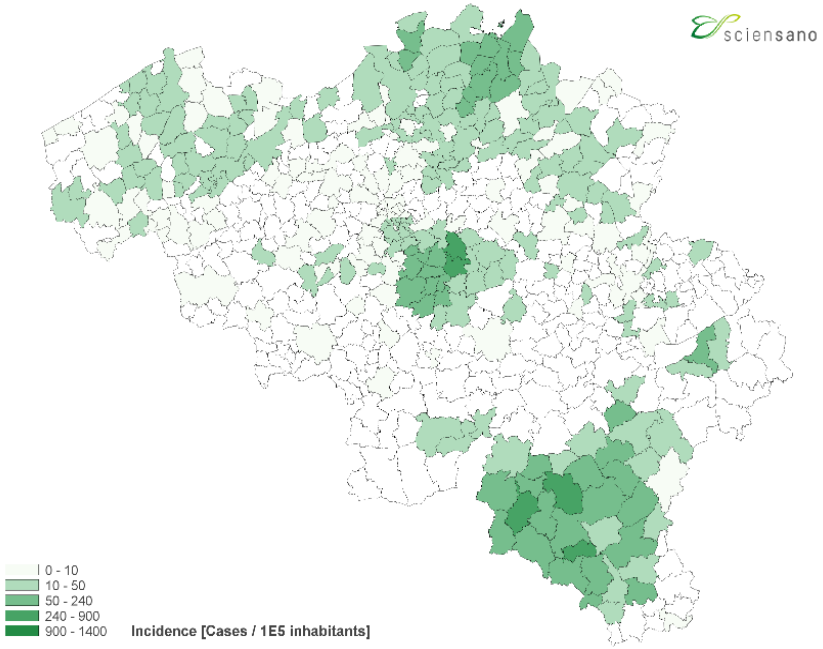
https://tiquesnet.wiv-isp.be/results

UCLouvain

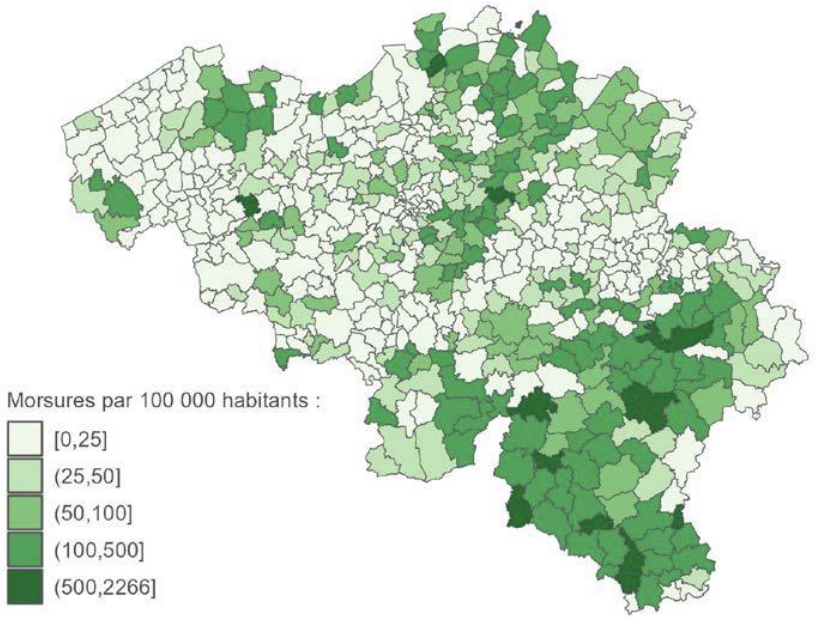
Lyme borreliosis in Belgium:

Positive tests (2018) and tick bite locations (2020) per 100,000 inhabitants

Reported incidence of positive serological results for B. burgdorferi s.l. by municipality, Belgium, 2018 (Source: surveillance laboratory network)



Geographical distribution of tick bites per 100,000 inhabitants, by municipality in Belgium, January-December 2022 (Source: https://tiquesnet.sciensano.be/results)



https://tiquesnet.wiv-isp.be/results

UCLouvain

Lyme borreliosis in Belgium: Tick bite report (tiquesnet.be) 2022

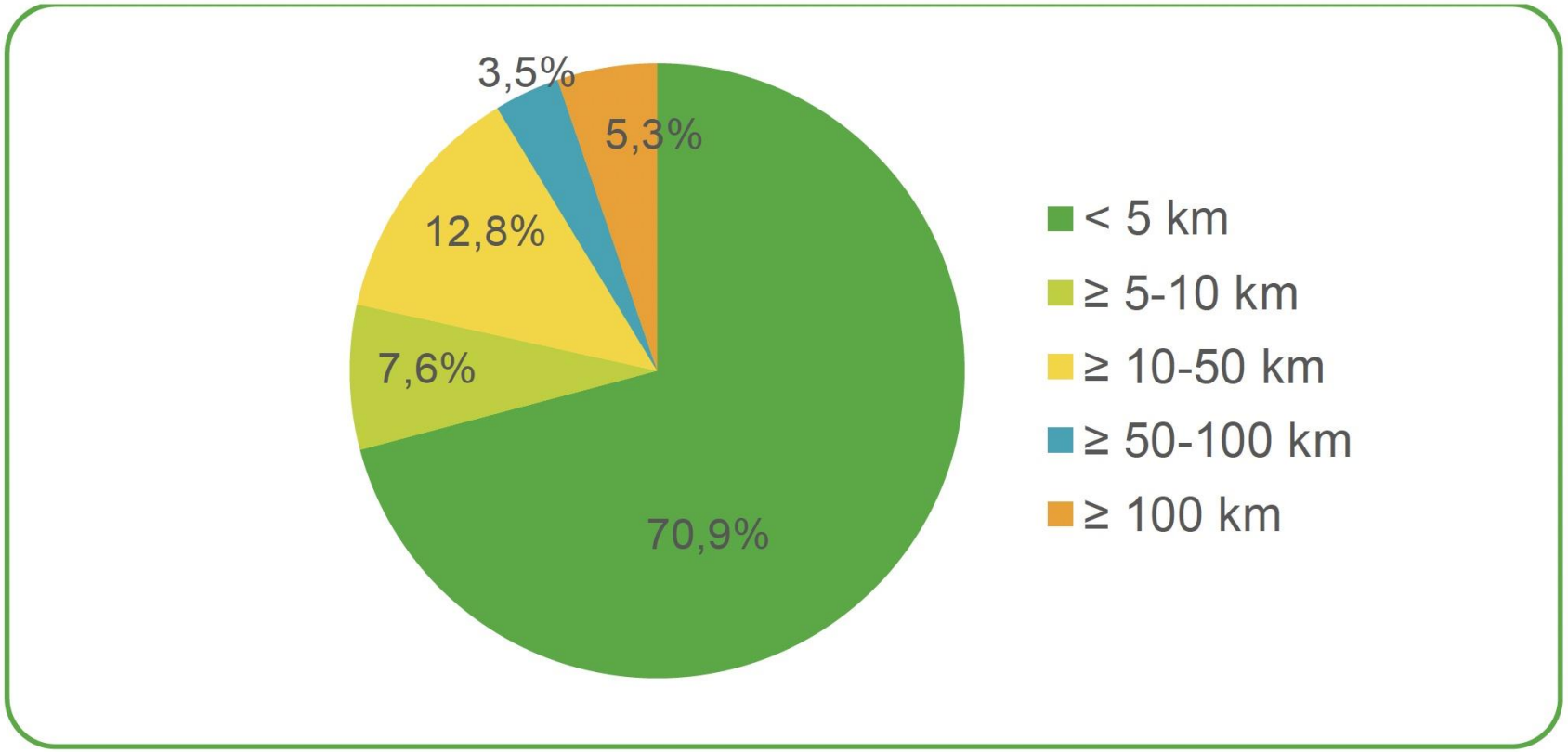


Figure 3: Distance between the location of the bite and the place of residence in 2022

https://tiquesnet.wiv-isp.be/results

UCLouvain

Lyme borreliosis in Belgium: Tick bite report (tiquesnet.be) 2022

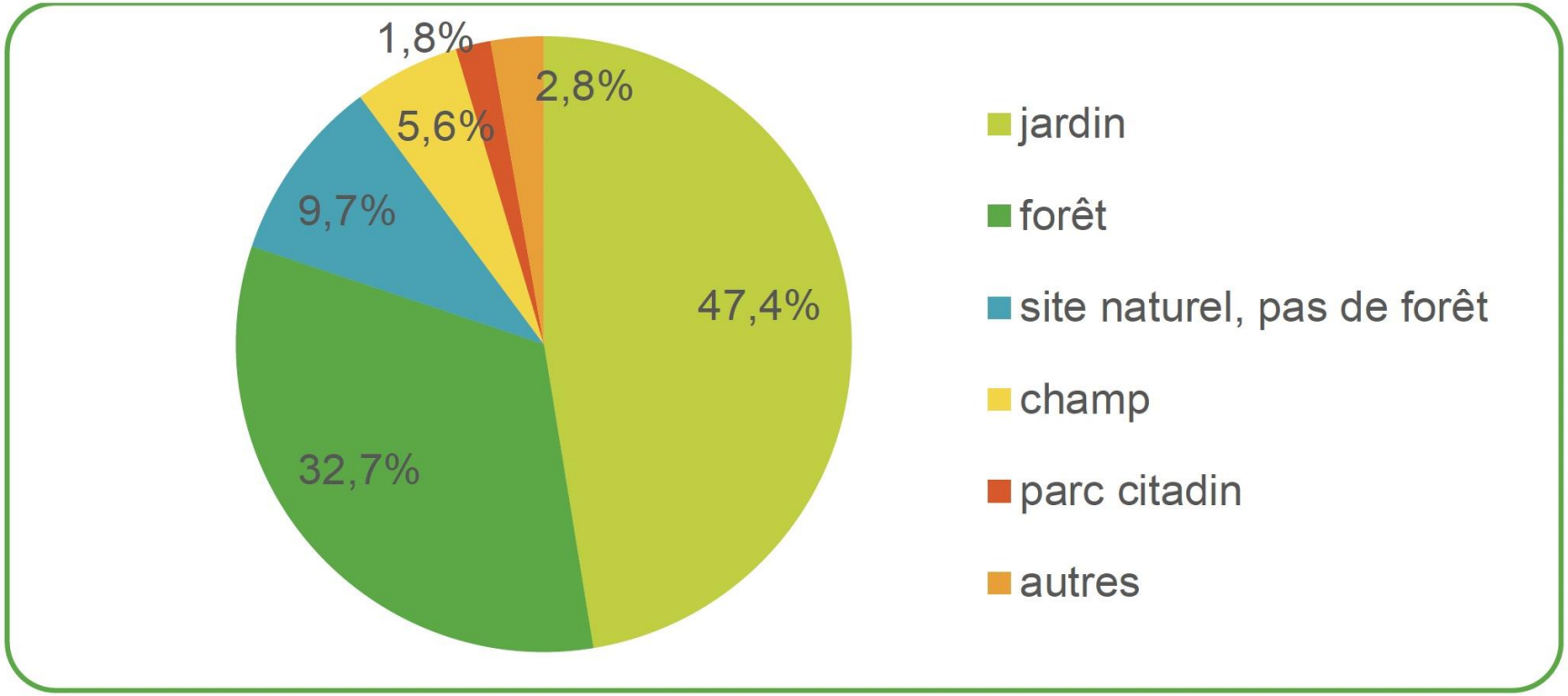


Figure 4: Proportion of tick bite notifications by environment type

https://tiquesnet.wiv-isp.be/results

UCLouvain

Lyme borreliosis in Belgium: Tick bite report (tiquesnet.be) 2016 - 2022

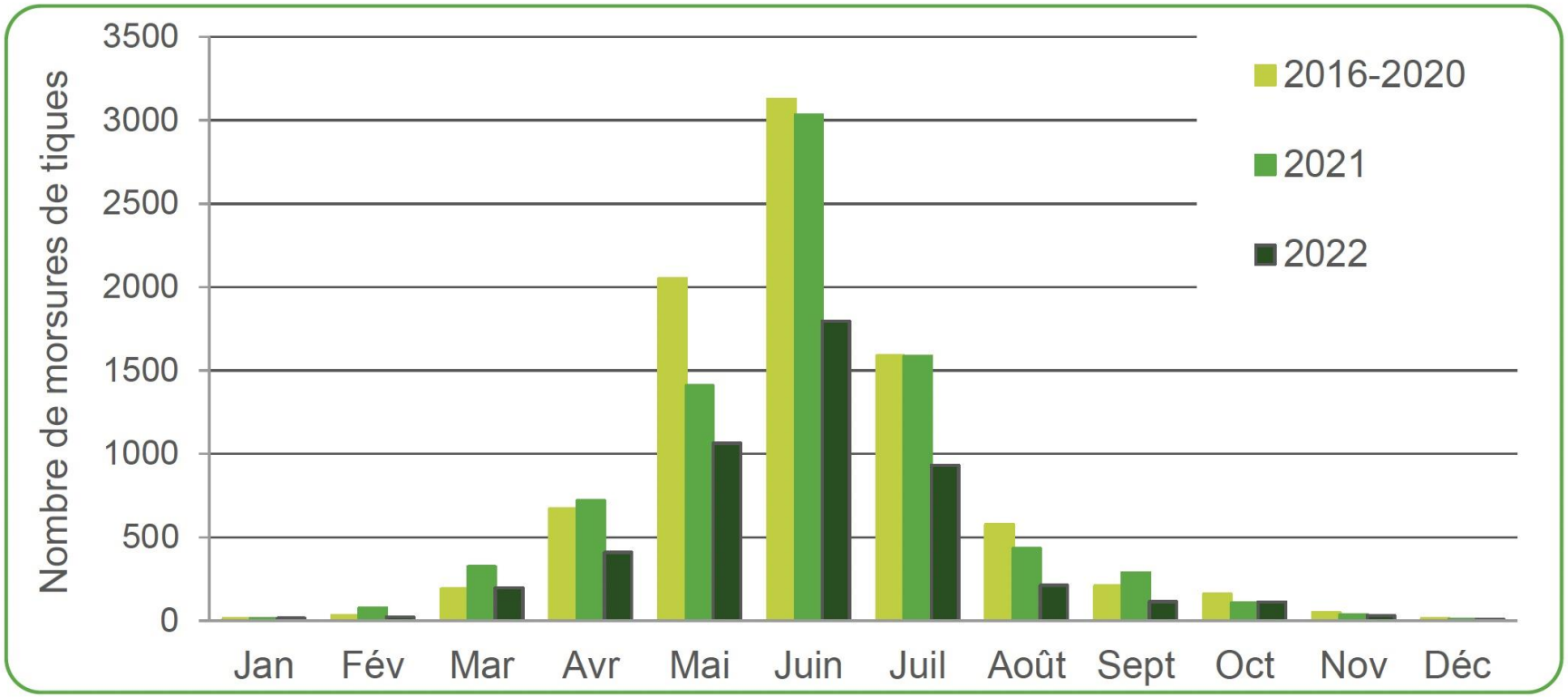


Figure 5: Number of bites per month, 2016 – 2022

https://tiquesnet.wiv-isp.be/results

UCLouvain

Province (nombre de tiques analysées)	Pourcentage de tiques infectées par agent pathogène 2021 (intervalle de confiance à 95 %)					
	Borrelia burgdorferi s.l.	*Anaplasma phagocytophilum*	*Borrelia miyamotoi*	*Neoehrlichia mikurensis*	*Babesia spp.*	*Rickettsia helvetica*
Bruxelles (n = 8)	12,5 % (1,7-53,7)	0 %	0 %	0 %	0 %	12,5 % (1,7-53,7)
Anvers (n = 107)	13,1 % (7,9-20,9)	2,8 % (0,9-8,3)	4,7 % (2-10,7)	4,7 % (2-10,7)	1,9 % (0,5-7,2)	16,8 % (10,9-25,1)
Limbourg (n = 91)	14,3 % (8,5-23,1)	5,5 % (2,3-12,5)	2,2 % (0,6-8,4)	6,6 % (3-13,9)	3,3 % (1,1-9,7)	17,6 % (11,1-26,8)
Brabant flamand (n = 108)	7,4 % (3,7-14,1)	3,7 % (1,4-9,5)	2,8 % (0,9-8,3)	2,8 % (0,9-8,3)	0,9 % (0,1-6,3)	9,3 % (5,1-16,4)
Flandre orientale (n = 121)	8,3 % (4,5-14,7)	8,3 % (4,5-14,7)	3,3 % (1,2-8,5)	1,7 % (0,4-6,4)	0,8 % (0,1-5,6)	14,9 % (9,6-22,4)
Flandre occidentale (n = 50)	6 % (1,9-17)	0 % (0-7,1)	0 %	0 %	0 %	16 % (8,2-28,9)
Flandre (n = 477)	10,1 % (7,7-13,1)	4,6 % (3,1-6,9)	2,9 % (1,7-4,9)	3,4 % (2,1-5,4)	1,5 % (0,7-3)	14,7 % (11,8-18,1)
Brabant wallon (n = 77)	13 % (7,1-22,5)	1,3 % (0,2-8,6)	2,6 % (0,7-9,8)	2,6 % (0,7-9,8)	2,6 % (0,7-9,8)	6,5 % (2,7-14,7)
Liège (n = 110)	9,1 % (5-16,1)	7,3 % (3,7-13,9)	2,7 % (0,9-8,1)	4,5 % (1,9-10,5)	0 %	11,8 % (7-19,3)
Luxembourg (n = 104)	6,7 % (3,2-13,5)	6,7 % (3,2-13,5)	2,9 % (0,9-8,6)	3,8 % (1,5-9,8)	1 % (0,1-6,5)	16,3 % (10,4-24,7)
Namur (n = 61)	18 % (10,3-29,7)	1,6 % (0,2-10,7)	3,3 % (0,8-12,2)	0 %	1,6 % (0,2-10,7)	6,6 % (2,5-16,2)
Hainaut (n = 54)	5,6 % (1,8-15,9)	3,7 % (0,9-13,6)	1,9 % (0,3-12)	0 %	5,6 % (1,8-15,9)	13 % (6,3-24,8)
Wallonie (n = 406)	10,1 % (7,5-13,4)	4,7 % (3-7,2)	2,7 % (1,5-4,8)	2,7 % (1,5-4,8)	1,7 % (0,8-3,6)	11,3 % (8,6-14,8)
Total* (n = 928)	**9,9 %** (8,2-12)	**4,7 %** (3,5-6,3)	**2,9 %** (2-4,2)	**2,9 %** (2-4,2)	**1,5 %** (0,9-2,5)	**13,3 %** (11,2-15,6)

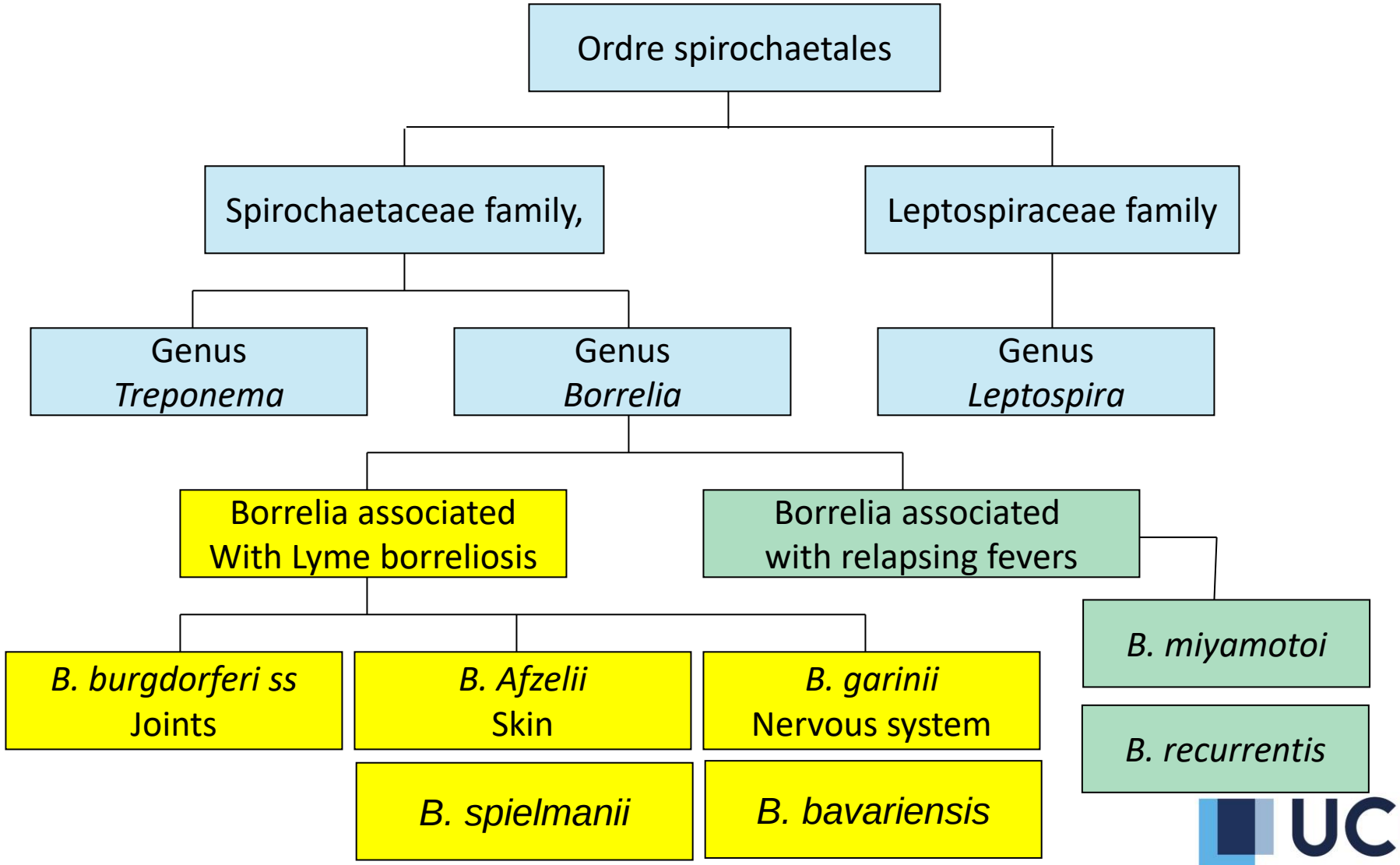
** Pour 37 tiques, la province où la morsure de tique a eu lieu n'est pas précisée*

UCLouvain

DIAGNOSTIC

UCLouvain

The causative agent: *Borrelia burgdorferi sensu lato*



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Vector: *Ixodes*

Review

CellPress

Borrelia miyamotoi: a widespread tick-borne relapsing fever spirochete

Alex Wagemakers[1], Pieter J. Staarink[1], Hein Sprong[2], and Joppe W.R. Hovius[1,3,4]

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[2] Laboratory for Zoonoses and Environmental Microbiology, National Institute for Public Health and Environment (RIVM), Antonie van Leeuwenhoeklaan 9, PO Box 1, Bilthoven, The Netherlands
[3] Department of Internal Medicine, Division of Infectious Diseases, Academic Medical Center, Meibergdreef 9, 1105AZ Amsterdam, The Netherlands
[4] Amsterdam Multidisciplinary Lyme Center, Academic Medical Center, Meibergdreef 9, 1105AZ Amsterdam, The Netherlands

Borrelia miyamotoi **is a relapsing fever spirochete that has only recently been identified as a human pathogen.** ***Borrelia miyamotoi*** **is genetically and ecologically distinct from** ***Borrelia burgdorferi*** **sensu lato, while both are present in** ***Ixodes*** **ticks. Over 50 patients with an acute febrile illness have been described with a** ***B. miyamotoi*** **infection, and two infected immunocompromised patients developed a meningoencephalitis. Seroprevalence studies indicate exposure in the general population and in specific risk groups, such as patients initially suspected of having human granulocytic anaplasmosis. Here, we review the available literature on** ***B. miyamotoi,*** **describing its presence in ticks, reservoir hosts, and humans, and discussing its potential impact on public health.**

Borrelia miyamotoi is a relapsing fever spirochete present in *Ixodes* ticks

Glossary

Babesiosis: human tick-borne disease caused by *Babesia* parasites.
***Borrelia burgdorferi* s.l.:** phylogenetic clade comprising at least 19 species transmitted by *Ixodes* ticks, with distinct geographic locations. Lyme borreliosis is caused by at least eight different genospecies, including *B. burgdorferi* sensu stricto in North America, and *Borrelia afzelii* and *Borrelia garinii* in Europe.
Erythema migrans (EM): expanding erythematous skin lesion, often the primary symptom of Lyme borreliosis.
Glycerophosphodiester phosphodiesterase (GlpQ): antigenic protein in TBRF spirochetes (absent in *B. burgdorferi* s.l.).
Human granulocytic anaplasmosis (HGA): tick-borne disease caused by *Anaplasma phagocytophilum.*
Human monocytotropic ehrlichiosis (HME): tick-borne disease caused by *Ehrlichia chaffeensis.*
***Ixodes* ticks:** hard ticks, including *Ixodes scapularis* (deer tick) in North America, *Ixodes* ricinus (sheep tick) in Europe, and *Ixodes persulcatus* (taiga tick) in Asia. *Ixodes* ticks have three stages (larva, nymph, and adult) and molt into a successive stage after a blood meal that lasts several days. *Ixodes* ticks are able to transmit several pathogens to humans.
Lyme borreliosis: disease caused by *B. burgdorferi* s.l. that often presents with

Trends Parasitol. 2015 Jun;31(6):260-9. doi: 10.1016/j.pt.2015.03.008.

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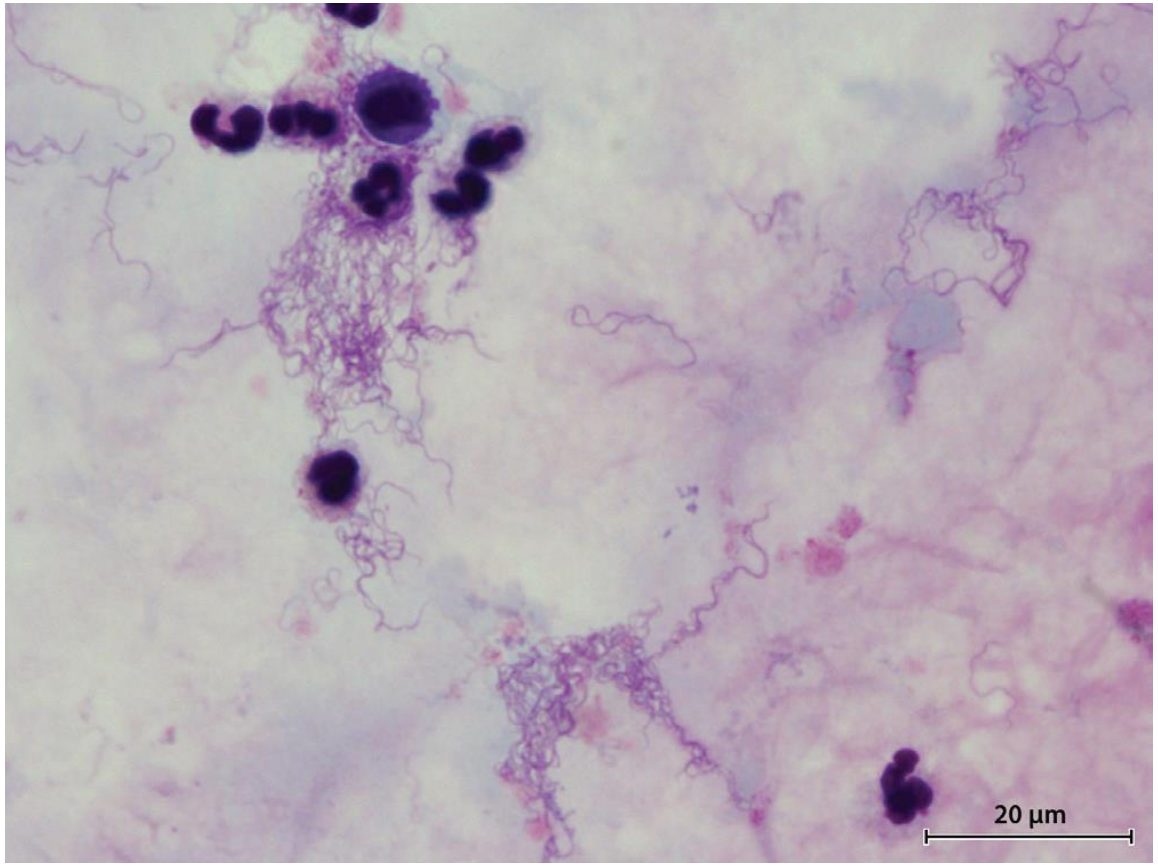
Borrelia associated with relapsing fevers

(B.recurrentis) giemsa staining

Detection in blood by giemsa staining or by PCR targeting 16s gene



Wilting et al. Euro Surveill. 2015

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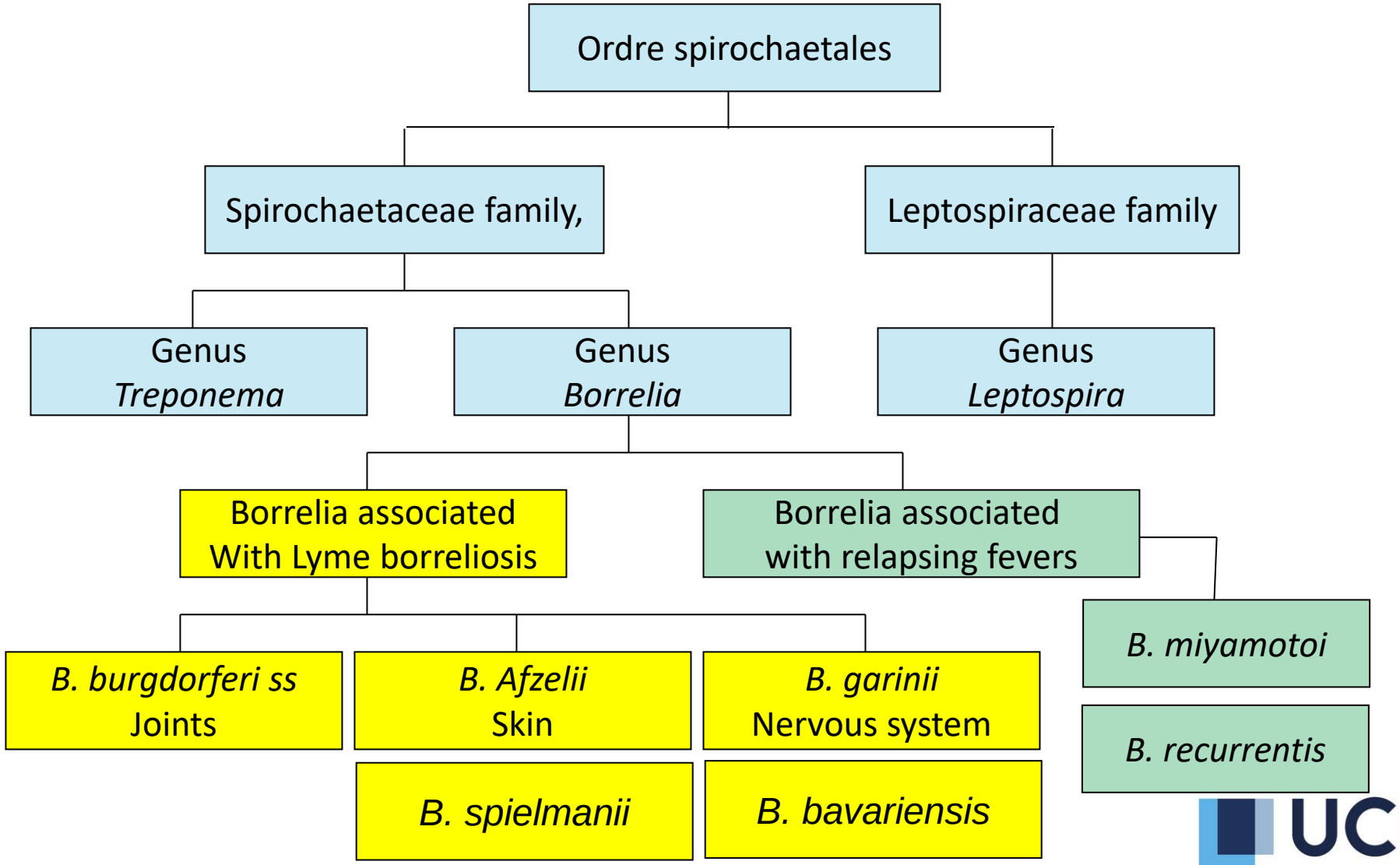
The causative agent: *Borrelia burgdorferi sensu lato*



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Lyme borreliosis : diagnostic tests

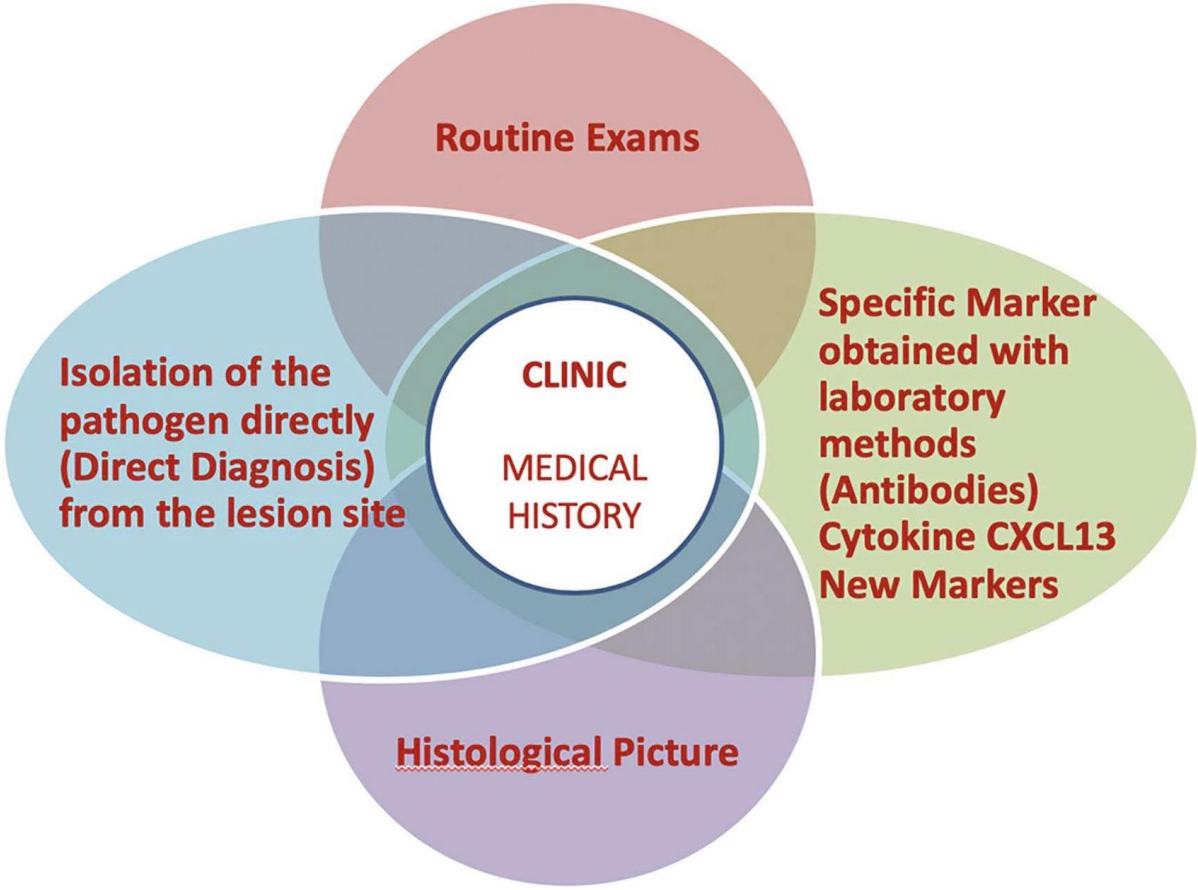

FIGURE 1 | Overview of LB management.

Trevisan et al. Front. Med., 23 July 2020

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Lyme borreliosis in Belgium: diagnostic tests

- Clinical and epidemiological data are essential
- Serology is the preferred diagnostic method
  - Useful in later stages of the disease
  - Reflects the immune response, humoral and cellular
  - Measures immunity, not disease activity
  - One should take this into account when interpreting serological results.

- Performance depends on the Ag(s) set(s)
  - Recombinants
  - C6 or VlsE for IgG and OspC for IgM
  - IgM to be interpreted only within 6 to 8 weeks following exposure

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Direct diagnosis: Molecular testing

	Erythème migrant		*Méningoradiculites Formes compliquées précoces*		*Arthrites*		*Acrodermatite chronique atrophiante ACA*	
	Serum	*peau*	*Serum*	*LCR*	*Serum*	*Liquide* articulaire*	*Serum*	*Peau*
Culture	NR	38-80%	NR	≤10%	NR	<5%	NR	20-60%
PCR	NR	60-90%	NR	25%	NR	50-70%	NR	60-90%
ELISA	20-50%	NR	70-90%	50-90%	90-100%	NR	95-100%	NR

*NR : non réalisable * : meilleurs résultats avec les biopsies synoviales*

Dr Marc V Assous- Hopital Cochin

Sensitivity increases over time

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Direct diagnosis: Molecular testing

- PCR can help with diagnosis, as a complementary test to serology:
  - In case of skin and joint manifestations
  - In EM, the sensitivity is about 68%
  - Regarding fluid and joint biopsy the sensitivity is 76-85%
  - Each doubtful or positive result is confirmed by sequencing, thus allowing a specificity of 100%
- PCR has little place in neurological manifestations
  - Sensitivity is between 10 to 50%
  - The index of intrathecal antibody synthesis is more sensitive (+ CXCL13)
- The diagnostic value of PCR for blood/serum/plasma or urine is not established and the test is not recommended.

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Direct diagnosis: Molecular testing

Type of test	**Purpose of the test**	**Number of test**	**Positive PCR**	**Doubtful PCR**	*B.afzelii*	*B.burgdorferi*	*B.garinii*	*B.sp*
PCR Borrelia flagelline or OspA	Confirmation of diagnosis	1 755	56 (3,2%)	98 (5,6%)				
SEQ Borrelia flagelline or OspA	**Species identification**	**41 (2,3%)**	**29 (1,7%)**	**11 (0,6%)**	**11 (0,6%)**	**14 (0,8%)**	**8 (0,5%)**	**8 (0,5%)**
5S-23S rDNA Real Time PCR	Detection of infected ticks, Borrelia species determination (epidemiology, strain collection)							
Borrelia culture	Diagnosis							

Data from 2018 to 2022: the vast majority of positive PCRs are articular fluids, almost no CSF.

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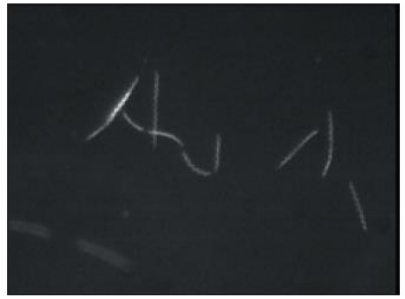
Direct diagnosis: bacterial culture

- Culture
  - has a sensitivity between 40 and 80% for skin biopsies, articular fluids
  - Very low: 1.2-13% for blood and CSF,
  - Culture on specific BSK medium (liquid)
  - Lasts 2 to 6 weeks
  - Demand for this test is very low.

- Direct fresh examination or after staining has very low sensitivy



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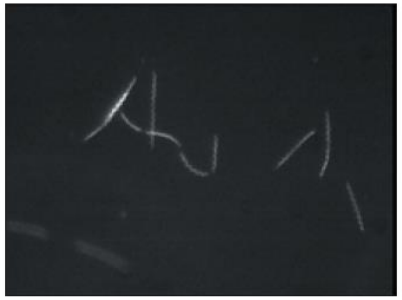
Direct diagnosis: bacterial culture

Results form for first European QC trial for Borrelia burgdorferi sensu lato culture			
Laboratory:	NRC/UCLouvain/Belgium		
Medium used for culture:	BSK-H medium, complete, 6% rabbit serum		
Sample number	Culture result (positive/negative)	Number of days incubated until positive	Species
1	Positive	6	*B.Afzelii*
2	Negative	/	/
3	Positive	6	*B.garinii*
4	Positive	6	*B.Burgdorferii*
Remarks			
Culture was made until days 24			
Sample 1 : BSK-H medium does not appear optimal for bacterial growth, loss of motility (days 18)			



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Serology: standard two-tiered testing algorithm versus modified two-tiered testing algorithm

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Guideline	Year
French infectious disease society; SPILF	2006
IDSA	2006
British Infection association	2011
Swiss Infectious Diseases Society	2006
Canadian Public Health Laboratory Network	2006
Committee for infectious diseases and vaccinations of the German academy for pediatrics and adolescent health	2012
German Borreliosis Society	2010
EFNS	2010
Polish Society of epidemiology and infectious diseases 2015	2015
Belgian Society of Infectious Diseases and Clinical Microbiology	**2016**
ESGBOR	2017
NICE	2017
Rheumatology Society and German Association	2013
German Society of Hygiene and Microbiology	2017
German dermatology Society	2016
German Neurology Society	2012

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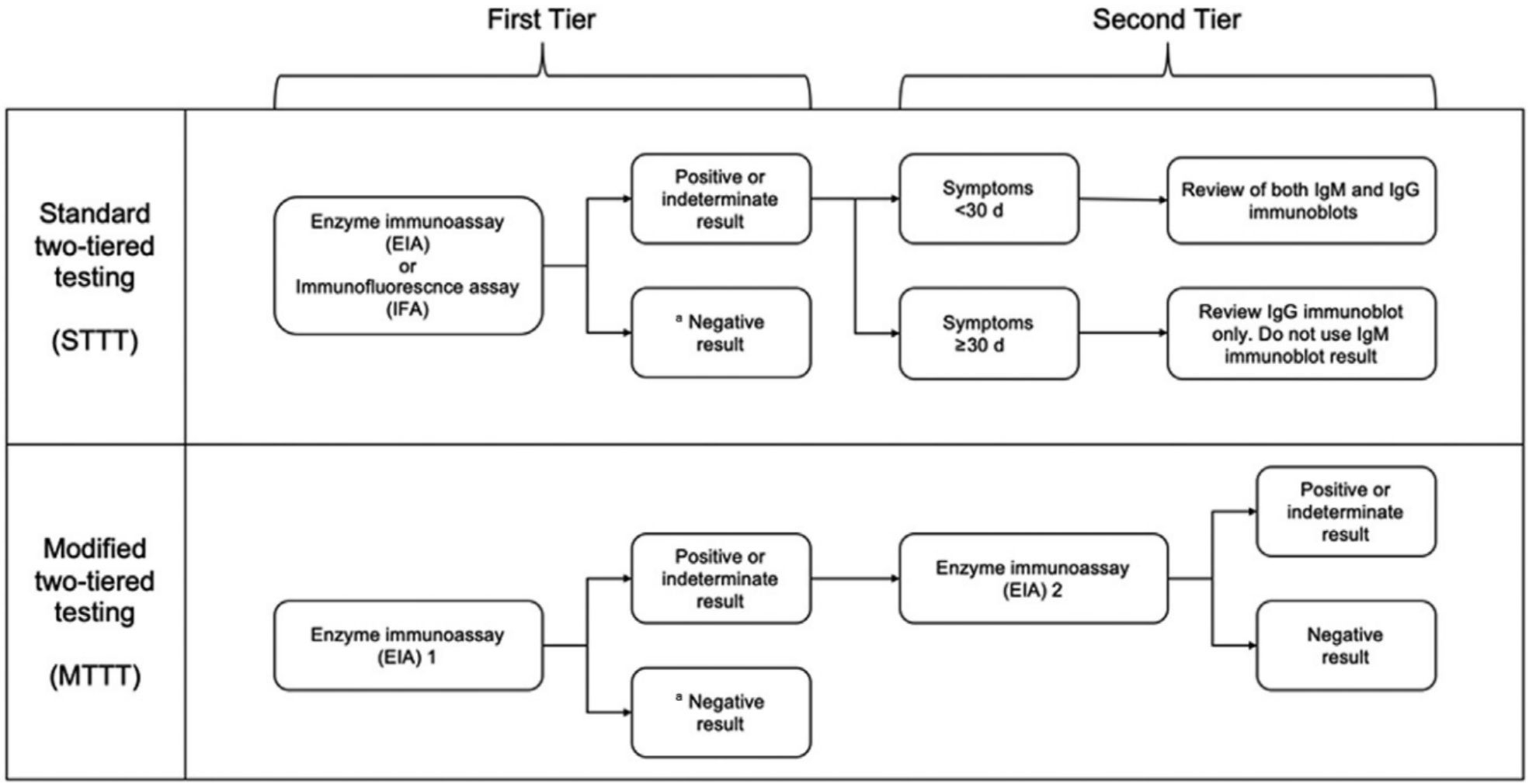
Evaluation of serology workflow



Adapted from Kabyashi et al (2022). Diagnostic Testing for Lyme Disease. Infect Dis Clin N Am

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Standard Two-Tiered Testing (STTT) Algorithm

- **2-Tiered**
  - Sensitive enzyme immunoassay (EIA) (OR immunofluorescent assay (IFA))
    - Goal: Maximize sensitivity
  - Standardized Western immunoblot when positive or equivocal by EIA/IFA
    - Goal: Maximize specificity

- Second National Conference on Serologic Diagnosis of Lyme Disease
  - Declared as reference method in 1994…for 25 years

- Limitation
  - Challenges with immunoblot result interpretation
    - ➢ Concerns about specificity of the bands
  - Low sensitivity (35%-60%) during acute disease
    - ➢ Primarily due to limited immunoblot sensitivity

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Modified Two-Tiered Testing (MTTT) Algorithm

- Food & Drug Administration (FDA)
  - July 2019: Clearance of several (4) Lyme disease serologic assays for use as second test in a Lyme disease testing algorithm
- CDC recommendations for serologic diagnosis of Lyme disease
  - August 2019: Update of 1994 guidance to allow approved EIA in place of western immunoblot assay
- Sequential or concurrent use of 2 (or more) EIA's
  - Without the use of immunoblots.

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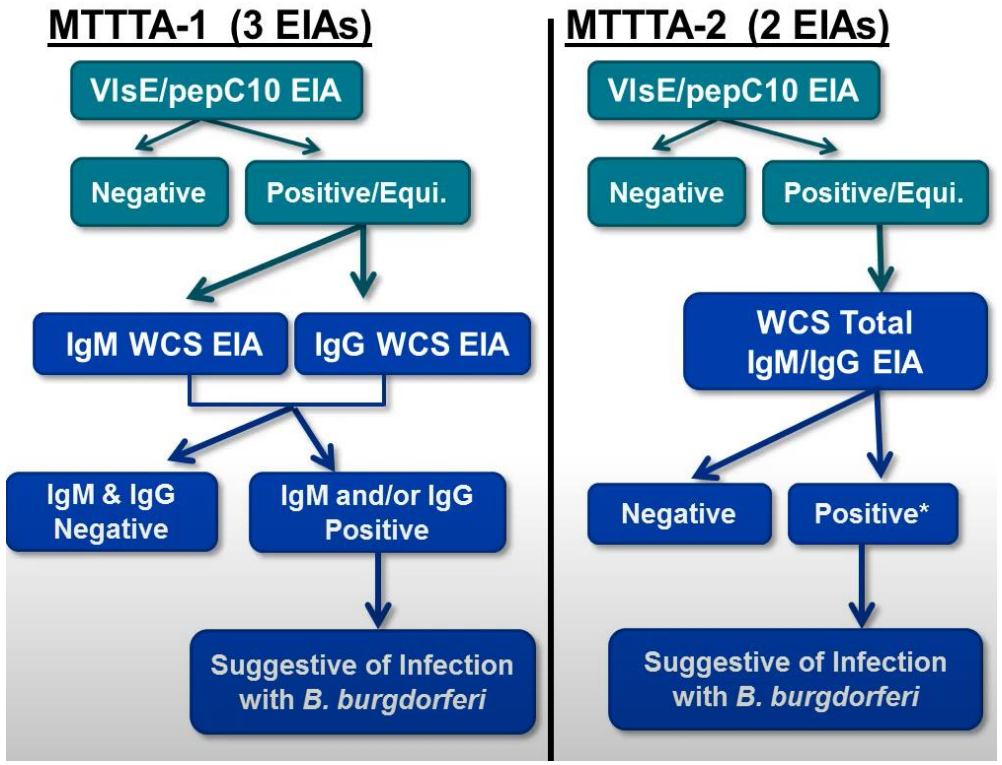
MTTTA

- MTTTA is entirely EIA-based
- 1st and 2nd tier EIAs differ in the B. burgdorferi antigens used
- Tier 1:
  - VLsE/pepC10 antigen (recombinant)
  - Total IgM/IgG
- Tier 2:
  - Whole Cell Sonicate (WCS) material from cultured Borrelia spirochetes
  - IgM and IgG separately or Total IgM/IgG



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MTTTA vs STTTA

Stage of Lyme disease	% positive by the STTTA	% positive by a 2-EIA MTTTA
Erythema migrans	42%-58%	53%-67%
Early disseminated Lyme	73%-95%	85%-100%
Late Lyme	97%-100%	100%
All stages	**57%73%**	**68%-88%**

- MTTTA more sensitive vs. the STTTA across studies and EIA in patients with EM and overall
- Equivalent sensitivity during later stages of Lyme disease

- False positivity rate remains unchanged

- One study performed in Europe (Baasma et al. 2020, Netherlands) -> comparable results

PMID: 21865190
PMID: 29743307
PMID: 27558183
PMID: 32632699

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MTTTA: (dis)advantages

Advantages	Disadvantages

• Greater sensitivity in early Lyme disease
  • BUT still 'only' +/- 60% => Clinical presentation remains important
• More cost-effective and less labour intensive (vs immunoblot)
• Shorter turnaround time and less technically demanding
  • Ability for smaller laboratories to perform all stages of testing
• Easier interpretation of results (nobanding pattern)
	• Inability to distuingish active versus past infections
	• Loss of information on specific antigen epitopes
	  • Loss of insight in the extent and maturity of the antibody response and possible reinfections
	• Limited research performed in European context

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Immunoblot: Step 2 in STTTA

- Antibody response is evaluated against the different antigens which are separated and fixed on a solid support, generally nitrocellulose strips.
- **Western blot:** whole cell antigens with proteins separated by electrophoresis according to molecular weight
- **Line blot:** purified proteins (recombinant or mixture of native and recombinant)

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Western blot

- Different guidelines (Dressler et al. 1993, CDC 1995, Engstrom 1995 et al., Hauser et al. 1999)

- **USA:** BBSS 297 strain from a patient with neuroborreliosis
  - IgM: at least two bands: OspC, p39 (BmpA) and p41
  - IgG: at least 5 bands (p18, OspC, p28, p30, p39, p41, p45, p58, p66 en p93)

- **Europe:** B. afzelii strain Pko (originally isolated from human erythema migrans lesion in Germany)
  - IgM: at least one band: strong p41, p17 (DbpA)
  - IgG: at least 2 bands (p14, p17, p21, OspC, p30,p39,p43, p58 and p83/100)

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Line blot

- Improvement sensitivity without loss of specificity in the early disseminated stage by adding **recombinant VlsE and DbpA**

- Because the diversity of genospecies in Europe, combinations of recombinant antigens from strains belonging to different species

- Recently: Miniaturised immunoblots in 96-well microplate format
  - Microplate wells contain test strips that are printed with purified, pathogen-specific antigens

PMID: 32509603

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Early and late antigens

Early antigens	Early/Late antigens	Late antigens
OspC (p21-p25, Major outer surface lipoprotein C)	VlsE (vmp-like sequence E)	OspA (p31)
BbK32 (Fibronectin-binding protein)	Dbpa (p17-p18, Decorin-binding protein A)	p30
Flagellin (p41)	OppA-2 (p58, Oligopeptide-binding protein)	p66
	BmpA (p39)	p83/100
	p14	p93
	p28	
	p43	
	p45	

Antigen	Characteristics
P41	- cross-reactivity with other spirochetes (e.g. Treponema) - IgM antibodies against p41 appear very early after infection
OspC	The first detectable antibodies after infection
P17/Dbpa	Lyme arthritis

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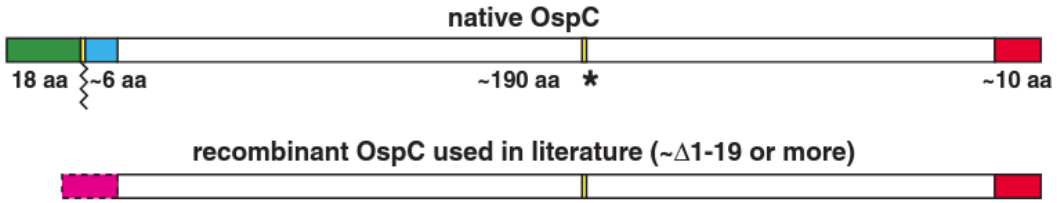
Poor specificity IgM (1)

- Isolated positive IgM: careful attention
- Specimens collected > 6 weeks after onset of symptoms -> 'false positive'

- Native dimeric '**Outer Surface Protein C**' (OspC) is the most important serological marker (sensitivity up to 90%)
  - Cultivation of this antigen is complicated, therefore recombinant Ag is used.
  - C-terminal epitope of OspC occurs in certain other human proteins/bacteria/environment
  - **Lower specificity**



- Same for p41 (sequence identitiy with flagellin proteins of Treponema)

- BmpA (IgM and IgG): cross-reacts with Syphilis, CMV, B19, RF

PMID: 22309852
PMID: 34937165
PMID: 37873860

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Overview cross-reactivity

	p41	BmpA (p39)	VlsE	OspC
RFB	IgM+/IgG+	IgM+/IgG+	IgM+/IgG+	IgM+/IgG+
Syphilis	IgM+/IgG+	IgM+/IgG-	IgM-/IgGnd	IgM+/IgG-
Yersinia	IgM+/IgG+	IgM+/IgG+	Nd	IgM+/IgG+
EBV	IgM+/IgGnd	Nd	Nd	IgM+/IgG+
CMV	IgM+/IgG-	Nd	Nd	IgM+/IgG-
B19	Nd	IgM+/IgGnd	IgM+/IgGnd	Nd
RF	IgM-/IgG-	IgM+/IgG+	Nd	IgM-/IgG-

Nd: no data

PMID: 37873860

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Poor specificity IgM (2)

- Isolated positive IgM for specimens collected more than 6 weeks after the onset of the symptoms
  - considered as false positive (CDC criteria)

- 4 criteria should be sought in case IgM+, IgG-
  I. Verification of the positivity criteria for serology
  II. High probability of tick exposure?
  III. Symptoms and clinical signs highly evocative of early Lyme borreliosis
  IV. IgG seroconversion on retesting more than 4 weeks later

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CXCL13, a biomarker for Lyme neuroborreliosis

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Introduction to CXCL13

- B-cell-attracting chemokine
- B. burgdorferi induces release of CXCL13 into the CSF -> B cells are recruited to the site of infection -> B-cell enriched pleocytosis
- Promising assay
- **Transportation: 2-8°C**
  - **PRO**:
    - CXCL13 levels are detectable early in the disease process, days to weeks before AB production
  - **CONTRA**:
    - elevated in other neuroinfectious and neuroinflammatory diseases
    - Falls rapidly after start of antibiotic therapy

Pooled sensitivity	Pooled specificity
89% (95% CI 85%-93%)	96% (95% CI 92%-98%)

PMID: 29674128

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Study at UZ Leuven (2016-2019) for LNB

2016-2019				
	Confirmed (n=64)		Not Confirmed (n=566)	
	#	%	#	%
Negative < 20 pg/ml	7	10.9	488	86.2
Indeterminate 20-30 pg/ml	1	1.6	21	3.7
Positive 30-100 pg/ml	9	14.1	26	4.6
Strongly positive > 100 pg/ml	47	73.4	31	5.5

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Take home messages

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Serology is not indicated

I. For asymptomatic patients following a tick bite, as asymptomatic seroconversion is possible, in which treatment is not indicated

II. For the follow-up of patients with Lyme borreliosis

III. For screening, as antibodies only reflect an exposure to Borrelia and not the disease itself

IV. For erythema migrans

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Borrelia burgdorferi

Keypoints

Clinical Microbiology and Infection xxx (2017) 1–7

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Review

To test or not to test? Laboratory support for the diagnosis of Lyme borreliosis: a position paper of ESGBOR, the ESCMID study group for Lyme borreliosis

R.B. Dessau [1,*], A.P. van Dam [2], V. Fingerle [3], J. Gray [4], J.W. Hovius [5], K.-P. Hunfeld [6], B. Jaulhac [7], O. Kahl [8], W. Kristoferitsch [9], P.-E. Lindgren [10], M. Markowicz [11], S. Mavin [12], K. Ornstein [13], T. Rupprecht [14], G. Stanek [11], F. Strle [15]

[1] Department of Clinical Microbiology, Slagelse Hospital, Region Sjælland, Denmark

- Patients with a typical erythema migrans should be diagnosed clinically and treated promptly without serological testing (insensitive)
- The use of Borrelia serology in patients with non-specific subjective symptoms is discouraged!!
- In patients with disease duration > 6 weeks a specific IgG response is a prerequisite, but an isolated IgM response is of no diagnostic relevance.
- Detection of antibodies to B. burgdorferi cannot discriminate between active or past infection (clinical symptoms?)

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Case report

Woman 73 years old. Red-purple discoloration left ankle. Lyme serology is requested by dermatologist.

Serology:

Lyme Ig total (C6-peptide EIA): 9.0 / positive

Lyme IgM blot (RecomLine) : negative

Lyme IgG blot (RecomLine) : positive

IgG blot: p100 -,VlsE +,p58 +,p41 +,p39 -,OspA -,OspC -,p18 +

Is there an active Lyme disease?

Yes, late manifestation of Lyme -> Acrodermatitis chronica atrophicans

In patients with late presentation of Lyme-borreliosis, such as arthritis or ACA only the detection of IgG antibodies should be considered diagnostic.

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Important to complete the NRC request form

Please fill in the request form!!

Diagnosis of Lyme borreliosis is based on *a complete* diagnostic workup, including medical history with compatible clinical symptoms and possible exposure to tick bites

REFERENTIECENTRUM VOOR LYME BORRELIOSIS

GELIEVE HET STAAL SAMEN MET DIT INGEVULD FORMULIER OP TE STUREN N**

UZ Leuven, Dienst Laboratoriumgeneeskunde
Accreditatiecertificaat 215-MED
Herestraat 49, 3000 Leuven
Tel : 016/347000
E-mail : melissa.depypere@uzleuven.be

***KLINISCHE GEGEVENS**

Datum van aanvang symptomen : …….

Symptomen:

❑ Erythema migrans
❑ Arthritis
❑ Neuroborreliose
❑ Andere, specificeer: ……...

***EPIDEMIOLOGISCHE GEGEVENS**

❑ Tekenbeet. Datum : ……
❑ Vermoedelijke provincie van besmetting : ……
❑ Behandeling reeds gestart voor staalname.
❑ Vroegere Lyme borreliose. Jaar : ……..
❑ Andere epidemiologische gegevens :
……