eurofins | Biomnis

Tick-borne diseases

DS20-INTGB - June 2017

With an increase in forested areas, an increase in the number of large mammals, and developments in forest use and recreational activities, the incidence of tick-borne diseases is on the rise.

In addition to Lyme disease, which has an estimated incidence of 43 cases per 100,000 (almost 30,000 new cases identified in France each year), ticks can transmit numerous infections.

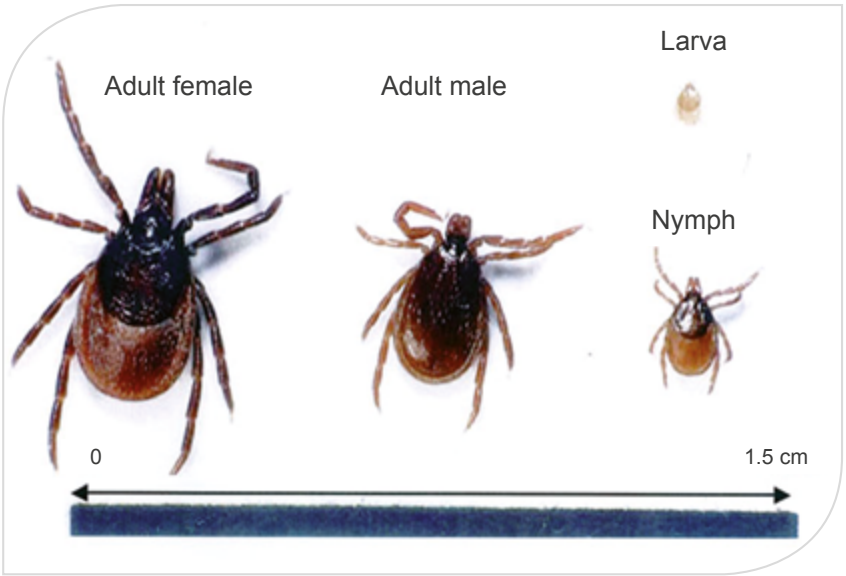
Although the initial manifestations of these diseases are often non-specific, they can become chronic and develop into severe clinical forms, sometimes with very disabling consequences. They respond better to antibiotic treatment if it is initiated quickly, hence the need for early diagnosis.

The vector: ticks

The main vector of these diseases are hard ticks, acarines of the *Ixodidae* family. In France, more than 9 out of 10 ticks removed from humans are Ixodes ricinus and it is the main vector in Europe of human-pathogenic Lyme borreliosis (LB) spirochaetes, the tick-borne encephalitis virus (TBEV) and other pathogens of humans and domesticated mammals.

It is only found in ecosystems that are favourable to it: deciduous forests, glades, and meadows with a temperate climate and relatively-high humidity. Therefore, it is generally absent above a height of 1200-1500 m and from the dry Mediterranean region.

Its activity is reduced at temperatures above 25°C and below 7°C. As a result, its activity period is seasonal, reaching a maximum level in the spring and autumn.



It is a blood-sucking ectoparasite with 3 distinct stages of development: larva, nymph, adult.

During its life-cycle, the tick climbs above the herbaceous layer on the lookout for a host, which it detects using various sensory organs on its first pair of legs; these are sensitive to mechanical, thermal and chemical stimuli generated by the host.

The blood-meal can last between 3 and 10 days. A female tick can ingest up to 150 times its weight in blood. At the end of the meal, the tick detaches from its host and falls to the ground.

Several months (between 9 and 12) are needed to pass onto the next stage.

Egg laying (about 20,000 eggs) kills the female tick.

The total duration of the life-cycle is on average between 2 and 4 years.

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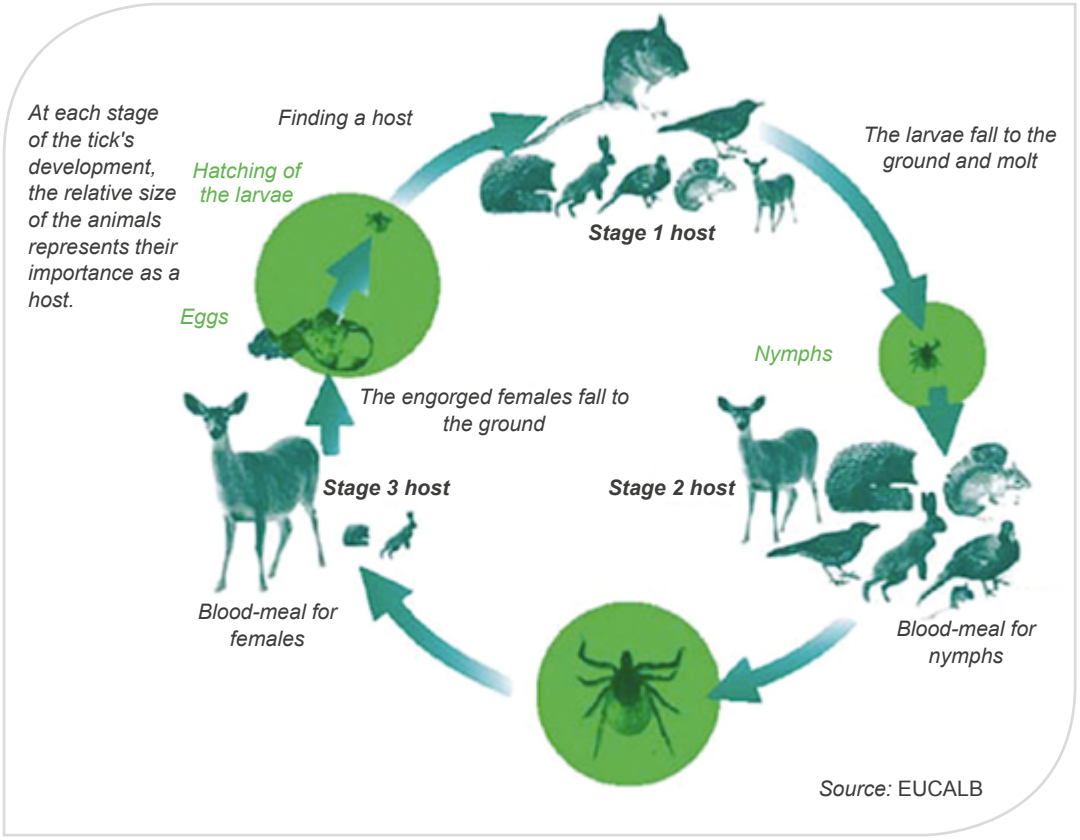

Man is an accidental host: it is a stage 2 host from which the nymphs (approx. 2 mm in size) feed.

The distribution of ticks throughout France shows regional disparities, with some areas having higher densities of nymphs, but vector studies are incomplete (data from JF Chapuis, BEH 2010):

1. Alsace (146 nymphs/100m^2)
2. Limousin (121 nymphs/100m^2)
3. Lower Normandy (111 nymphs/100m^2)
4. Île-de-France (Essonne) (73 nymphs/100m^2)

Other ticks involved in the spread of disease are:

- *Dermacentor sp* (mainly *reticulatus*): found outside forests, including in urban areas, with activity period that extends until winter; it is the adult, which is more readily identifiable, that can feed on humans.
- *Rhipicephalus sanguineus:* found in the southern and Mediterranean regions, it is the tick most commonly found on dogs (at every stage), and it can also bite humans, especially if the infestation is heavy.

Lyme disease

Lyme disease is the most common vector-borne zoonotic disease in the Northern Hemisphere. Monitoring of the disease is a priority because of its emerging nature and its potential severity.

The agent responsible is a spiral bacterium of the group collectively known as *Borrelia burgdorferi sensu lato*, which comprises many species. *B. garinii, B. afzelli* and *B. burgdorferi sensu stricto* are the main European species.

The distribution throughout France is consistent with the distribution of the vector.

The incidence of Lyme disease is estimated at 43 cases per 100,000 inhabitants.

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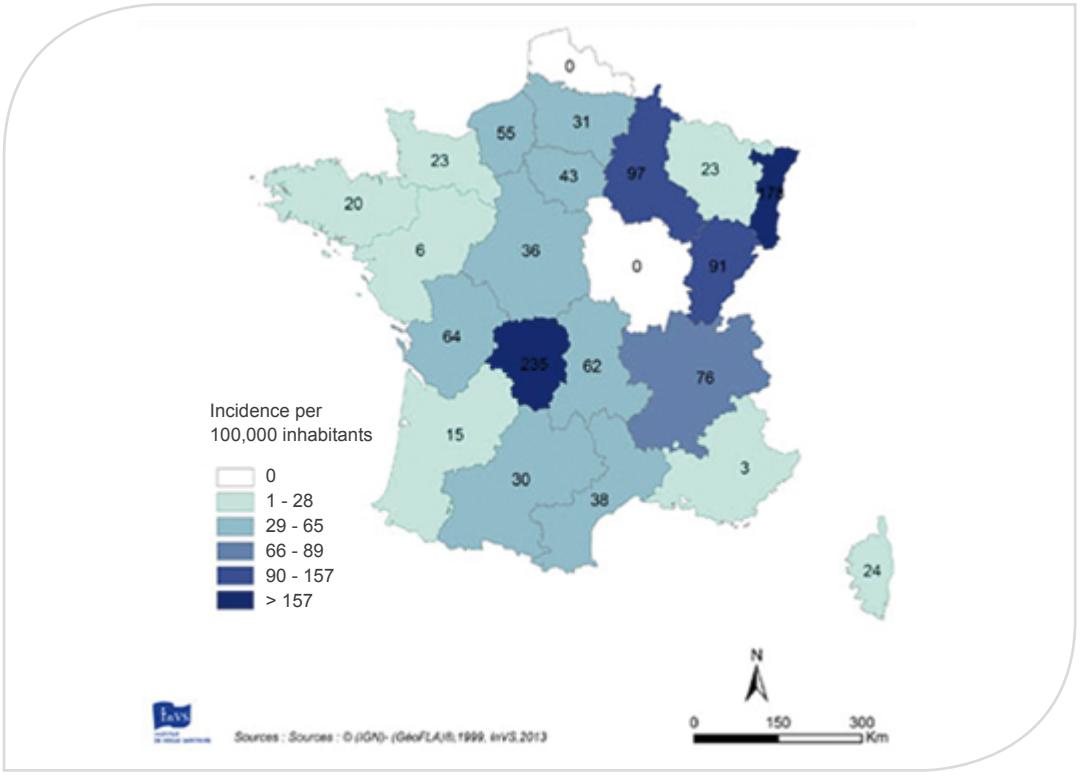
Estimated mean annual incidence of Lyme disease in France by region 2009-2011 (Sentinelles Network, source: InVS)



1. Transmission of *Borrelia* to its host

Approximately 10 to 20% of ticks are infected with Borrelia, and the bacterium can be found in all stages of the tick's development.

When the bacterium is ingested by the tick, it produces an outer surface protein (OspA) which interacts with a protein in the gut of the tick (TROSPA): this interaction enables the bacterium to attach within the intestine of the vector and protects it from the tick's immune system.

The bacterium multiplies, then migrates from the intestines to the salivary glands.

Saliva is an essential element in the transmission phase and for the initiation of the infection. Various mechanisms then contribute to the transmission of *Borrelia* to the vertebrate host. Firstly, suppression of the production of OspA and expression of OspC constitute a major virulence factor to enable the transmission of the spirochetes. Secondly, the interaction between the salivary protein Salp15 and OspC results in protection from the bactericidal effects of anti-OspC antibodies. Suppression of the expression of OspC, which is highly immunogenic, and the over-expression of the protein VlsE, which has high antigenic variation, constitute the essential mechanisms to evade the host's immune response: the bacterium is not destroyed by the very early anti-VlsE antibodies.

Thus, all of these mechanisms allow infection of the host and dissemination to its target organs.

The risk of *Borrelia* transmission increases with the time that the tick is attached to the host. The risk is very low with an attachment time of less than 7 hours, and very high after 48 hours: primary and secondary preventive measures are therefore essential to prevent the transmission of *Borrelia*.

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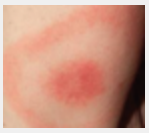
2. Clinical information: the 3 stages of infection and how to diagnose them - European consensus

Stages of infection	Clinical forms	Recommended essential tests for diagnosis, and their respective results	Optional tests
Early localised stage or primary phase  Incubation 7 to 21 days	**Erythema migrans:** - Bull's-eye rash (≥ 5 cm in diameter), with or without a clear centre, developing in a ring, with elevated edges - Spontaneous resolution (up to 6 months without treatment, in a few days on antibiotic therapy) **Antibiotic therapy helps prevent complications and progression to the other stages**	NO TESTS = diagnosis based on clinical examination	None if lesion is typical
Early disseminated stage or secondary phase - Some weeks or months after the primary phase - Dissemination of the pathogen via the blood	**Neurological Lyme disease** In adults: - mainly meningoradiculitis, meningitis; - in rare cases: encephalitis, myelitis; - in very rare cases: cerebral vasculitis In children, mainly meningitis and facial paralysis.	- Lymphocyte reaction in CSF and/or elevated protein levels in CSF. - Positive IgG serology in CSF; sometimes delayed in the blood. - Intrathecal synthesis of specific IgG. In early cases, intrathecal synthesis of specific antibodies may still be absent at the time of the initial sample.	PCR on CSF
	Lyme arthritis - Arthritis of one or more large joints - Possibility of recurrence	- Positive serology in the blood, normally with elevated levels (IgG) - Inflammatory synovial fluid	- Analysis of synovial tissue or fluid - Detection of Bb sl using PCR
	Borrelial lymphocytoma - Painless erythematous or purplish blue nodular swelling - Usually on the lobe or helix of the ear, nipple or scrotum - More common in children (especially on the ear)	- Seroconversion or positive serology - Histological confirmation in ambiguous cases	- Histological confirmation - Detection of Bb sl using PCR on skin biopsy
	Multiple erythema migrans lesions	- Specific serology	- Histological confirmation
	Cardiac manifestations (rare) Atrioventricular conduction disorders (first, second or third degree AV block), arrhythmias, sometimes myocarditis or pancarditis	- Specific serological testing	- Detection of Bb sl using PCR on myocardial biopsy
Late disseminated stage or tertiary phase Several months and years after the onset of the infection	**Joint symptoms,** neurological and cutaneous symptoms Acrodermatitis chronica atrophicans (ACA)	- Specific IgG serology with elevated levels	- Histological confirmation - Detection of Bb sl using PCR on skin biopsy

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3. Natural course of Lyme disease

Lyme disease occurs following a bite from an infected tick: the risk of transmission of the bacterium is estimated at 1 or 2%.

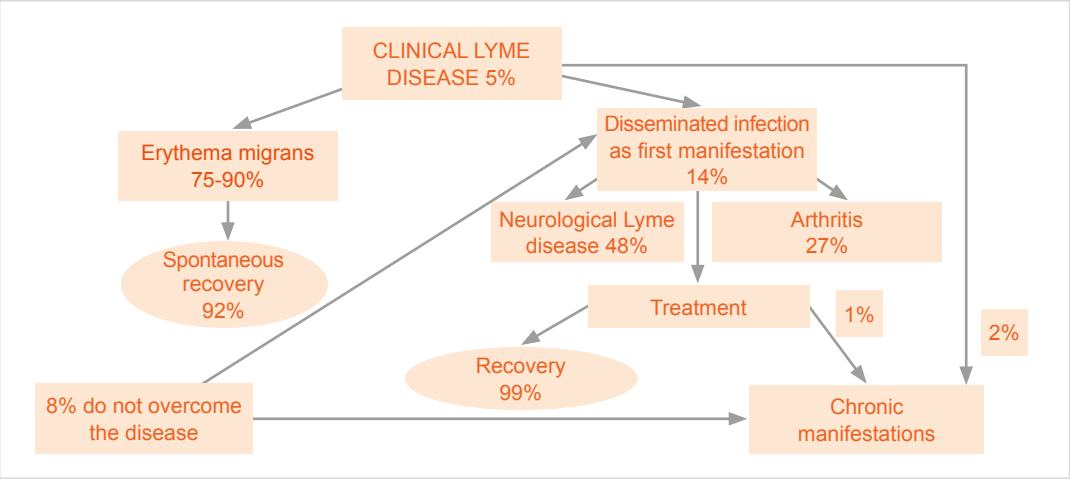
If transmission takes place, clinical manifestation of the disease is seen in 5% of cases. Lyme disease develops in three phases:

- the primary phase, in which erythema migrans is the most common manifestation (77-90% of cases);
- the secondary phase of dissemination, in which neurological manifestations are the most frequent in France (48%), followed by joint manifestations (about 27%).

In these two phases, the clinical manifestations are directly linked to the presence of the bacterium.

- the tertiary phase; this phase develops late in the absence of treatment, but it can also be the inaugural phase of the disease. It is caused by pathophysiological mechanisms where the role of *Borrelia* is indirect; the clinical manifestations observed are the result of autoimmune-like reactions associated with the presence of bacterial molecules forming antigenic communities with joint, neurological and cutaneous tissue components.

The clinical expression of Lyme disease is highly variable. The different phases may overlap, and progression from one to the next is not systematic. Furthermore, the early phase may be asymptomatic.



Cases of neurological Lyme disease

In France, cases of Lyme disease in the disseminated stage are most commonly discovered as the result of neurological manifestations.

Typically, meningoradiculitis in the form of very severe pain at the site of the bite, increasing at night, develops after one month. This responds poorly to pain relief and non-steroidal anti-inflammatory drugs, and is often accompanied by paraesthesia.

Aseptic meningitis, a frequent and early manifestation (within two weeks), presents in the form of a headache without meningeal stiffness.

Cranial nerve involvement is frequently found in children, manifesting as facial paralysis.

Laboratory diagnosis is based on positive serological testing (80-95% of cases) in either serum or CSF (presence of IgG).

Negative results in blood serum do not rule out the diagnosis of neurological Lyme disease in the acute phase: investigation for IgG in CSF is essential.

Investigation for intrathecal synthesis of specific antibodies based on Reiber's hyperbolic formula is required to secure the diagnosis: this makes it possible to distinguish between local synthesis and passive transudation of serum antibodies through the blood-brain barrier. The intrathecal synthesis index is calculated by comparing the levels of specific antibodies between CSF and serum collected on the same day and by adjusting the two compartments based on total IgG levels and albumin levels in serum and CSF.

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4. Laboratory diagnosis

In accordance with the recommendations from the SPILF ([French Society for Infectious Diseases] and the EUCALB), serological diagnosis is performed in two steps:

- Sensitive quantitative detection methods
  - Using automated immunoenzymatic techniques
  - Separate detection of IgG and IgM
- Specific qualitative methods for confirmation
  - In the event of a positive or ambiguous result
  - Using IgG or IgM Western blot

Medical pathologists are often confronted with difficulties regarding the interpretation of the laboratory tests, especially if these are prescribed in situations where the tests are invalidated (primary phase, after use of antibiotics). In addition, it is necessary to take into consideration a certain level of prevalence of IgG in healthy subjects in endemic areas. As demonstrated by Stanek (*The Lancet*, 2012), the presence of IgG is an indicator of contact with *Borrelia*, but it is not possible to distinguish between a serological scar and an active infection, even in the presence of IgM. The antibodies secreted are not protective.

Good practice for serological diagnostics

Serological testing is not to be performed:

- in asymptomatic subjects
- for systematic screening of exposed subjects
- in the event of a tick bite without clinical manifestations
- in the event of typical erythema migrans: do not expect antibodies to be present
- for systematic serological monitoring of treated patients

The importance of serological testing lies in the diagnosis of the secondary and tertiary phases of Lyme disease, possibly in combination with detection of the bacteria using PCR (synovial fluid, skin biopsy, CSF) in cases where there is a doubt about the diagnosis.

5. Treatment

The aim of treatment is to eradicate the spirochetes present at the lesion sites and to prevent progression to late forms of the disease.

Treatment is based on the recommendations of the French Society for Infectious Diseases (SPILF) and involves the use of antibiotics which exert their effect at skin, joint or meningeal level.

Example:

First-line treatment of erythema migrans in adults: amoxicillin 1g 3x/day for 14 to 21 days.

Boutonneuse fever

This disease is caused around the world by approximately twenty species of Rickettsia. It is characterised by fever, rash and eschar.

In France, Boutonneuse fever (also known as Mediterranean spotted fever) is caused by *Rickettsia conorii* and is transmitted in the summer by the dog tick, *Rhipicephalus sanguineus*.

After 7 days of incubation, a black mark appears at the site of the bite, accompanied by fever with headache and myalgia. Three days later, a maculopapular rash develops over the entire body.

Although usually a benign disease, it has potentially serious complications in 5% of cases.

In terms of laboratory results, typical findings are elevated parameters of inflammation, thrombocytopenia, and increased LDH. Serological testing confirms the diagnosis with the presence of antibodies or elevated serum concentrations of IgG and IgM.

The standard treatment is doxycycline, or a quinolone in the event of contraindications.

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Human granulocytic anaplasmosis

Formerly known as HGE or human granulocytic ehrlichiosis, this disease was originally described in cattle and is caused by *Anaplasma phagocytophilum*, a coccus of the order Rickettsiales.

In spring or summer, 5 to 21 days after a tick bite, the disease is characterised by the onset of acute flu-like illness. It is usually benign, but breathing, bleeding or renal complications can occur.

Laboratory results are typically altered, with neutropenia, thrombocytopenia, and moderate hepatic cytolysis. Careful examination of blood smears stained with MGG can reveal intragranulocytic morulae.

Serological testing involves 2-3 samples: one early and 2 later at 1 and 2 months. IgG and IgM are investigated.

Apart from in the event of the aforementioned complications, recovery is usually spontaneous, however the time to recovery can be shortened by taking doxycycline (or rifampicin in the event of contraindications).

Babesiosis

Babesiosis, or piroplasmosis, is well known in veterinary medicine, where it affects dogs and livestock, causing fever, haemolysis and haematuria. In humans, the clinical course of the disease is severe in asplenic or immunodeficient patients, with intravascular haemolysis and multiple organ failure. In the rest of the population it can cause a persistent fever.

It is caused by a protozoa which has a pear shape or Maltese cross formation in red blood cells and there are approximately one hundred different species. In humans, *B. microti* is most common in the USA, and *B. divergens* is most common in Europe. The prevalence of the disease is 1.5% in areas where the vector and the parasite are present; it is greater than 10% in patients with Lyme disease.

Serological testing makes it possible to detect contact with the pathogen, but investigation of IgM is not validated, and dating of the infection is impossible.

Other diseases potentially transmitted by ticks

Tularemia is caused by *Francisella tularensis* and is most often associated with skin contact with a hare. It can present in the form of ulceration and swelling of lymph nodes.

TIBOLA (*tick-borne lymphadenitis*) is a ricksettial disease caused by *R. slovaca* and *R. raoultii*, and manifests as flu-like illness with enlarged lymph nodes.

Cat-scratch disease or inoculation lymphoreticulosis is caused by *Bartonella henselae*; it causes a lesion at the site of initial infection and regional lymphadenopathy.

Q Fever, caused by *Coxiella burnetii*, is usually transmitted by inhalation of contaminated dust. Ticks can be a vector of Q Fever between humans (or pets) and wild animals, which constitute an important reservoir for the disease.

Tick-borne encephalitis is caused by tick-borne encephalitis virus, a member of the genus *Flavivirus*. It can be found in France in the regions of Alsace and the Vosges. It initially manifests as flu-like illness.

Serological testing can be used to confirm these diagnoses with good sensitivity and specificity.

Co-infections with Lyme disease, babesiosis, anaplasmosis and TBE are possible. These are to be suspected based on clinical signs.

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Prevention and action to be taken in the event of a tick bite

Prevention

During an "at risk" activity (for example, a walk in the forest), it is recommended to wear clothing that covers bare skin and that gathers at the wrists and ankles. Light-coloured clothing is preferable, in order to be able to spot the ticks easily.

Insect repellent for the skin or insect-repellent clothing can be used (except for pregnant women and children).

Upon returning from an "at risk" activity, careful examination of the body and scalp is essential, in order to detect and remove any ticks as quickly as possible.

What to do

Ideally, tick removal should be performed with a tick-removal tool. Any use of alcohol or other chemical products is to be avoided, because these encourage the tick to regurgitate.

Once the tick has been removed, the bite area should be disinfected and monitored for 4 weeks in order to detect possible erythema migrans.

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Summary

In the event of a tick bite followed by the presence of clinical signs, the following laboratory tests can be proposed:

	Suggestive clinical and biological signs	Tests to request / Expected results / Comments
Lyme disease	Primary phase: erythema migrans Biological signs only present in the secondary and tertiary phases of Lyme disease	Serological screening for IgG/ IgM + confirmatory tests if positive detection Intrathecal synthesis index (CSF + blood) for neurological Lyme disease PCR if in doubt
Boutonneuse fever	Fever, rash, eschar, elevated parameters of inflammation, thrombocytopenia, elevated LDH	Rickettsia serology for IgG and IgM: 2 serums samples at intervals of 2 weeks. In France, Mediterranean spotted fever is caused by R. conorii. Antibody present or elevated IgG and IgM concentrations.
Human granulocytic anaplasmosis	Flu-like illness Neutropenia, thrombocytopenia, hepatic cytolysis	Anaplasmosis serology for IgG and IgM: 2 to 3 serum samples: one early, then after 1 and 2 months. Antibody present or elevated IgG and IgM concentrations.
Babesiosis	Fever Intravascular haemolysis / multiple organ failure in immunocompromised/ asplenic patients.	Serology for babesiosis confirms contact.
Tularemia	Flu-like illness Ulceroglandular form.	Serological testing for Francisella tularensis IgG and IgM: 2 serum samples at 2-week intervals Antibody present or elevated IgG and IgM concentrations.
Cat-scratch disease	Inoculation lesion, regional lymph node enlargement	Serological testing for Bartonella IgG: antibody present or elevated IgG concentration PCR
Q Fever	Fever Headache, myalgia, hepatosplenomegaly	Serological testing for *Coxiella burnetti:* 2 serum samples at an interval of 2-3 weeks Antibody present or elevated IgG and IgM concentrations.
Tick-borne encephalitis (Alsace and Vosges)	Flu-like illness	Serological testing for tick-borne encephalitis virus (TBEV) IgG/IgM at 2-3 week intervals. Antibody present or elevated IgG and IgM concentrations.

Practical details for testing at Biomnis

Test	Sample	Storage and Transport	Biomnis test code[1]
Lyme disease - serological screening IgG/IgM	1 mL serum	Refrigerated	BOR
Borreliosis - serological confirmation IgG[2]	1 mL serum, CSF	Refrigerated	WBLYG
Borreliosis - serological confirmation IgM[2]	1 mL serum	Refrigerated	WBLYM
Lyme disease - serological screening - CSF	1 mL CSF	Refrigerated	BORPL
Lyme disease - intrathecal synthesis index - IgG - CSF + serum	1 mL CSF + 1 mL serum	Refrigerated	BOINT
Borreliosis - direct diagnosis - PCR	Skin biopsy, joint biopsy, synovial fluid, pericardial fluid, 0.5 mL CSF (minimum), 2 mL (minimum) EDTA whole blood or blood derivatives (serum/ plasma)	Refrigerated - Put the biopsies in a dry tube or with transport medium (M4RT)	BORBM
Human babesiosis - serology - serum	1 mL serum	Refrigerated	PIRO
Ricksettial diseases - direct diagnosis - PCR	Skin biopsy, eschar at the inocculation site	Frozen	TMP507
Ricksettial diseases - R. conorii, R. typhi - serology - serum	1 mL serum	Refrigerated	RICCT
Ricksettial diseases - *R. helvetica* - serology - serum	1 mL serum	Refrigerated	RICHE
Ricksettial diseases - *R. prowazecki* - serology - serum	1 mL serum	Refrigerated	RIPRO
Ricksettial diseases - *R. slovaca* and *R. africae* - serology IgG/IgM - serum	1 mL serum	Refrigerated	RICAF
Tularemia - *Francisella tularensis*	1 mL serum	Refrigerated	TUL
Tick-borne encephalitis - TBE - serology IgG/IgM - serum	1 mL serum	Refrigerated	TBE
Coxiella burnetii - phase 1 and 2 - serology - serum	1 mL serum	Refrigerated	RIB12
Coxiella burnetii - serology - serum	1 mL serum	Refrigerated	RICBU
Human granulocytic anaplasmosis - serology - serum	2 mL serum	Refrigerated	EHRLI
Bartonellose - *B. henselae - B. quintana* - IgG serology - serum	1 mL serum	Refrigerated	GRIF
Bartonellosis - direct diagnosis - PCR	Lymph node biopsy or aspiration, 2 mL EDTA whole blood, serum or plasma	Réfrigéré	GRIFB

[1]You can find all information about the tests offered by Biomnis in the Test Guide at www.biomnis.com; use the code assigned to each test for a quick and easy search.

[2]Code cannot be combined

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