

**Specific PCR phages tests show *Borrelia miyamotoi*
more realistic prevalence in Europe . Covid /LD co
infection markers and shared treatments**

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***Crypto-infections Conference
DUBLIN September 26-27 2020***

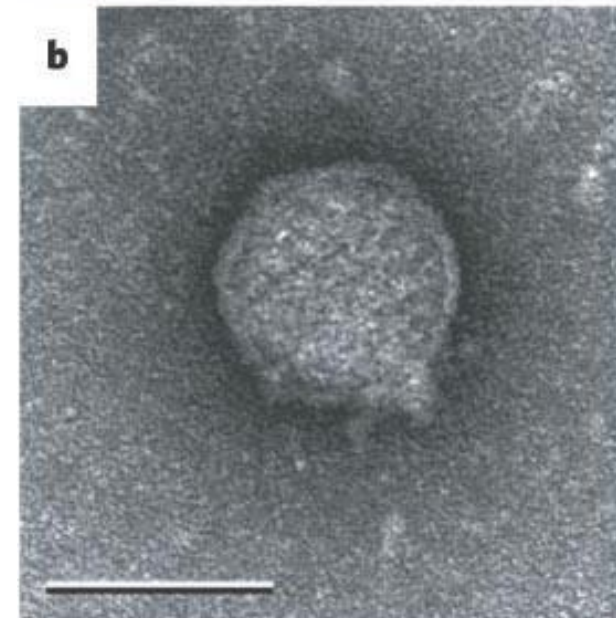
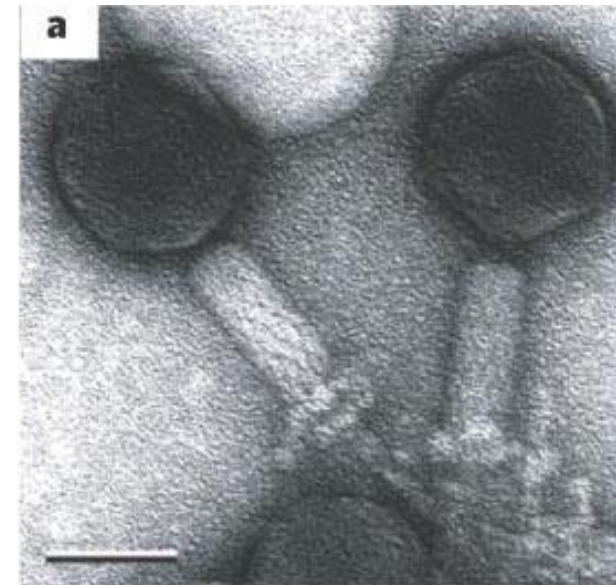
Part 1 : *Phelix Phage Borrelia detection method*
(Patent WO2018083491A1)

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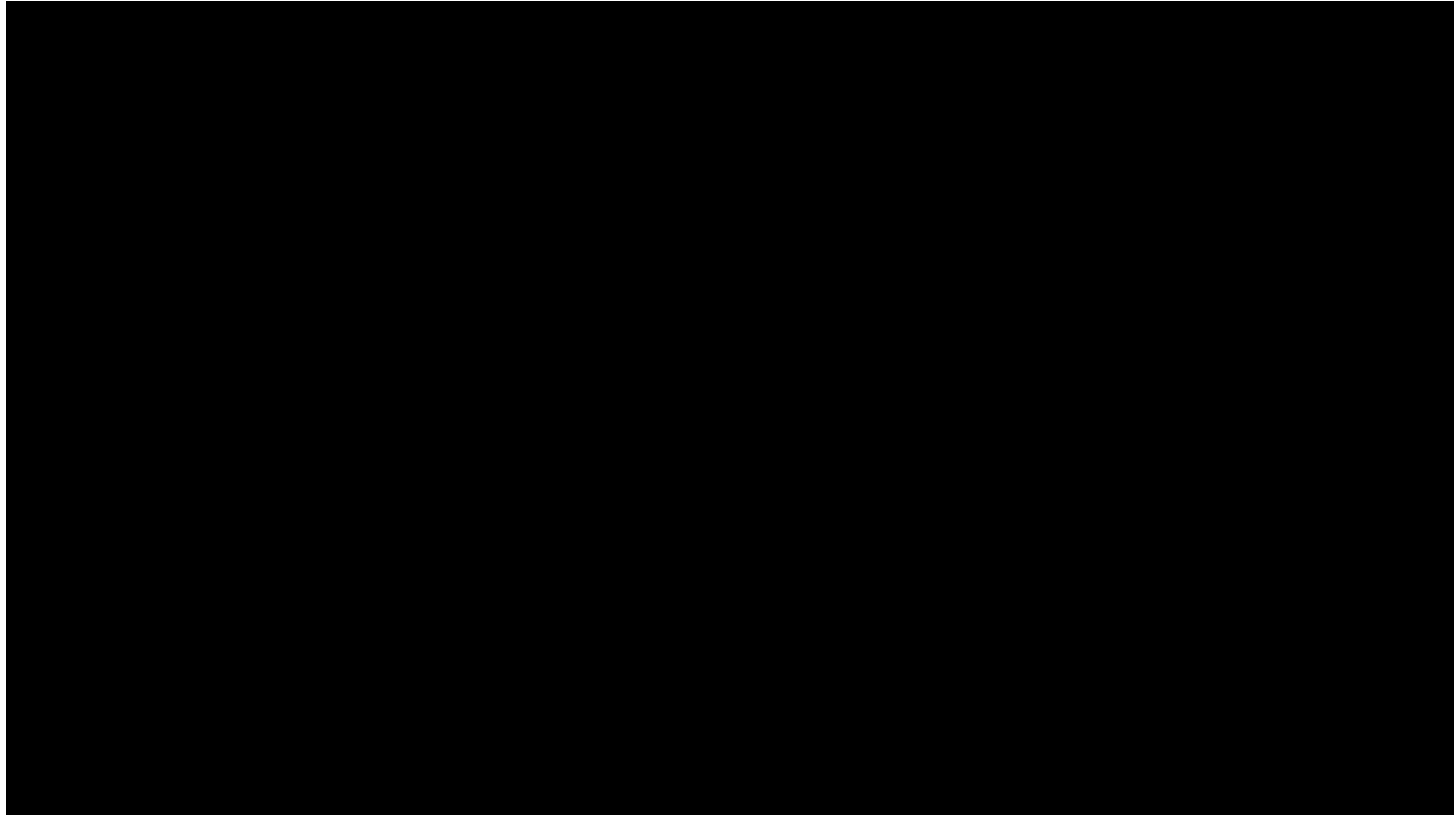
How phages look like?



realizze health



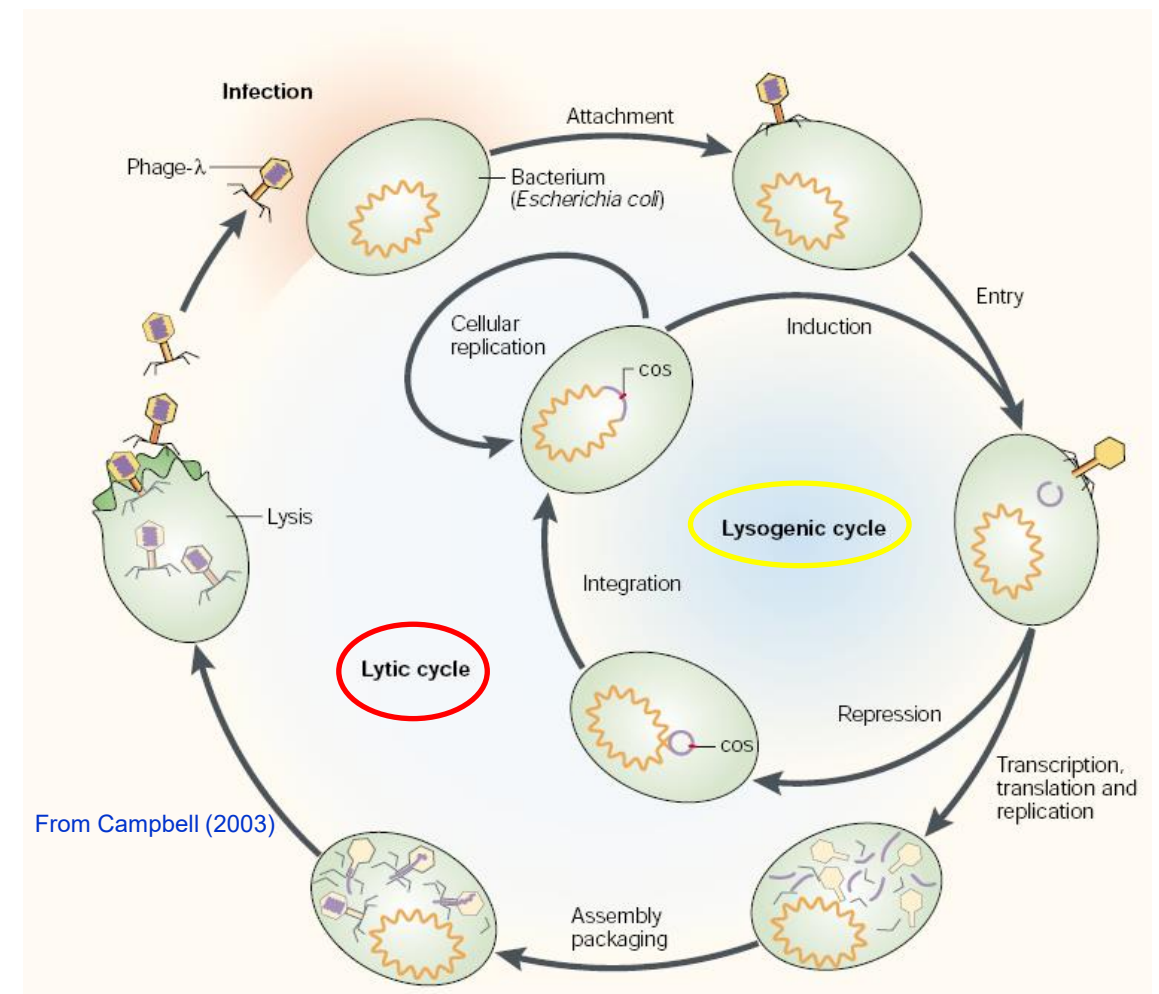
Phage life cycle



Phelix Phage Borrelia Test

Phages are specific viruses. Inserting their genetic material in their host, they can either produce numerous copies that further destroy the bacteria, or stay in a dormant lysogenic stage and be expressed under specific circumstances.

Phelix Charity and Leicester University developed a *Borrelia burgorferi* SL, RF and a specific *B. miyamotoi* Phage PCR tests already validated in early and late stage patients and healthy volunteers, showing >90% sensitivity and 100% specificity due to confirmatory sequencing.



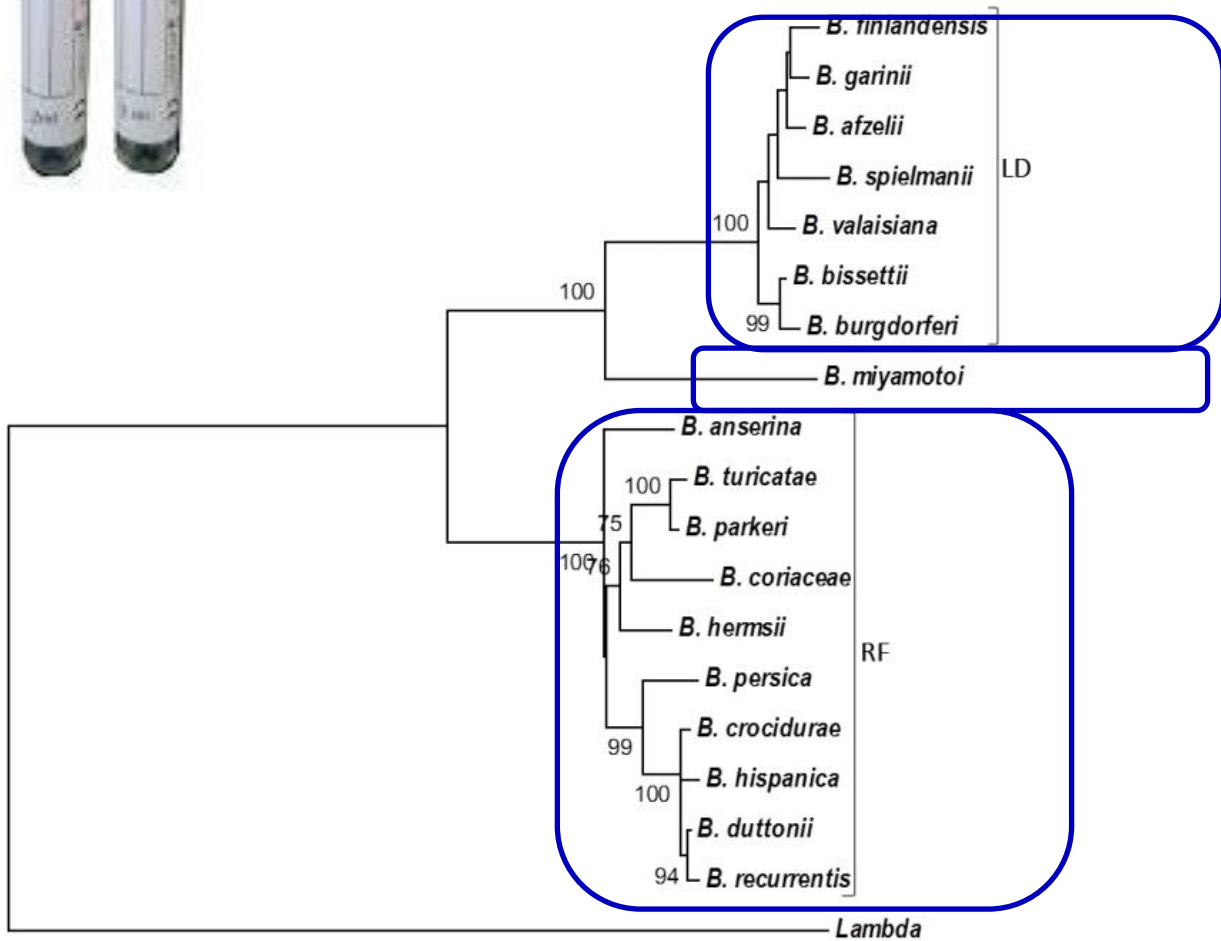
Phelix Phage Borrelia Test



**Manual DNA
Phenol/Chloroform
extraction**



3 different real-time PCRs



**Confirmatory sequencing for
positive-like samples**

LD Diagnostic Methods Comparison

| Diagnostics | Remit |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibody-based | <ul style="list-style-type: none"> • Low sensitivity Can't distinguish active and non-active <i>Borrelia</i> presence • Some difficulties in identifying <i>Borrelia</i> sub-types |
| Bacterial DNA-based | <ul style="list-style-type: none"> • Direct evidence of <i>Borrelia</i> presence Can be able to tell different <i>Borrelia</i> sub-types • Low sensitivity, as Bacteria do not circulate. Possible antibiotic interference . <i>Borrelia</i>(fragments can give false positive if cycles >35 and no sequencing confirmation |
| Lymphocyte transformation test | <ul style="list-style-type: none"> • Provide indirect evidence Can only detect Lymphocytes that have been in contact with <i>Borrelia</i> within 45±15 days, • Variable sensitivity (depends of the immune system state and factors affecting it , time of exposure • Difficult identification of <i>Borrelia</i> sub-types and active <i>Borrelia</i> |
| Phage test | <ul style="list-style-type: none"> • Direct evidence by in vivo “ amplification “ of <i>Borrelia</i> presence • High sensitivity and specificity • No antibiotic interference • Can distinguish Lyme from Relapsing fever <i>Borrelia</i> strains and specific miyamotoi |

Results of the Tests Done on Late stage patients

Methods

Borrelia burgd si and a specific B. miyamotoi and Relapsing fever Phages PCR Tests were performed on over 2200 samples (all late stage) from various European countries and from USA. All results were verified by sequencing

Results:

34 % of the tested samples were negative, 66% positive among which 64 % Borrelia miyamotoi; 25% RF

Performance of the Phage Test in clinical cases

Case 1. Male age 70. Lives in Mexico. Tick bites in USA 2015 Non typical rash Since 2017 : Fatigue, loss of sight, Fever (mild)muscular pains and legs strength loss; Urinary problems; Brain fog

| Phage PCR October 2019 | Bacterial qPCR USA and Mexico | OTHER Tests |
|---------------------------------------|----------------------------------|-----------------------------------------------------------|
| B. miyamotoi: Positive | | |
| Borrelia burgd SL Negative | Borrelia negative | Elisa and WB IgM negative IgM negative Elisa IgG doubt |
| Borrelia RF Negative | Babesia negative | BARTONELLA NEG |

Treatment : since September 2019 Ceftriaxone IM/IV 2g + doxycyclin 200mg+EO Pause 3 w ; Ceftriaxone IM/IV 2g + Azythromycine 500mg Metronidazol every other day: 14 days cures. Improvements : brain fog and fever stopped. Muscle and legs pains reduced. Urinary and fatigue remain (till now). APPROPRIATE DIAGNOSIS

Performance of the Phage Test in clinical samples

Case 2 male age 38. Tick bite in June 2016; No EM. Symptoms =fatigue +diffuse muscle pains + Depression. Elisa WB negative. PCR PHAGE TEST (blood) positive : treatment

| | |
|---------------------------------------------|-------------------------------------------------------------------------------------------|
| Phage PCR results September 2019 | Elisa WB results 2016 negative Serotonine Dopamine Zn CortisolDHEA low |
| B. miyamotoi positive | |
| | |

**Antibiotics (+ metabolic) treatment. 2 x10 days sequences :*cefuroxime + doxycycline* , (3 weeks pause), *azithromycin every other day + doxycylin* .
Quick positive outcome. Appropriate (good) usage of drugs**

Performance of the Phage Test in clinical cases

Case 3. Female age 46. Lives in Europe. No tick bites memories; No EM. Since 2010 : Stiff Man Syndrome (back), muscular and neurological pains left arm and legs strength loss. No fever but chills.

| Phage PCR August 2019 | Bacterial qPCR (vet lab) | OTHER Tests: Anti GAD antibodies positive EMG negative 2018 Yersinia enterocolitica positive (July 2019) |
|----------------------------------------|--------------------------------|-----------------------------------------------------------------------------------------------------------------------|
| B. miyamotoi: Positive | | |
| Borrelia burgd. SL Negative | Borrelia negative | Elisa IgM negative IgG positive WB negative 2016 |
| Borrelia RF Negative | Babesia negative | |

Treatment : Benzodiazepin baclofen since June 2018. September 2019 Antibiotics (same as case 1)
improvements : able to stand & some steps; stiffness remains; arms spasms stopped

TREATMENT MONITORING

Performance of the Phage Test in clinical samples

Case 4. Female age 10. Tick bite in 2018; EM. ELISA and WB positive. Treated 3 weeks amox. No symptoms until August 2019: flu like symptoms and joint (ankles) pains

| Phage PCR results September 2019 | Other tests |
|--------------------------------------------------------------|-----------------------------------------------------------|
| Borrelia burgd SL, RF & B. miyamotoi Negative | |
| | ASLO ASD; Mycoplasma &Chlamydia pneumoniae negative |
| | EBV Positive |

No antibiotics treatment. Appropriate (good) usage of drugs

Performance of the Phage Test in clinical samples

Case 5. male age 8. Tick bite in June 2020; No EM. Tick analysis PCR PHAGE Positive. No symptoms. PCR PHAGE TEST (Urine) Negative : No treatment

| Phage PCR results June 2020 | Phage PCR results July 2020 |
|---------------------------------|--------------------------------|
| TICK : B. miyamotoi positive | PATIENT (urine) Negative |
| | |

No antibiotics treatment. Appropriate (good) usage of drugs

Part 2: Discussion

**B miyamotoi resistance to antibiotics,
HEAT AND CLIMATE FACTORS and
predominant information exchange via plasmids may explain the
greater prevalence of miyamotoi type persisters**

GENERAL CONCLUSIONS

1) Phages being an evidence of bacterial activity, this technology is an in-vivo amplification system for active infections.

Clinicians can evaluate their treatments and discriminate the active disease from the post-lyme syndrome.

2) This is the first large scale report on prevalence of *B. miyamotoi* in late stages of borreliosis in Europe. Results were permitted by the high sensitivity of Phages PCR tests

3) *Borrelia* phage-based PCR experience is now used to develop *Bartonella* spp., *Rickettsia* spp and other « co-infections » testing

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THANK YOU!

Please send your questions by e-mail

A longer presentation is available on demand at

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