Is phage therapy for Lyme disease (LD) possible?

Isolation and characterisation of phages that infect Lyme *Borrelia* strains

Jinyu Shan and Martha Clokie

PHELIX RESEARCH AND DEVELOPMENT

Structure of the talk

Current problems in LD:

- What is LD, its distribution in Europe and the UK
- Introduction to the causative agent of LD disease, *Borrelia burgdorferi sensu lato*
- The current diagnostic method, and the major drawbacks

Our strategy: use of phage to treat/diagnosis of LD

- Screening for lytic phages from tick samples
- Induce phages out of their host bacteria
- Develop new diagnotic method
- Use phage-encoded enzymes to kill *Borrelia*

Facts about Lyme disease (LD), or Lyme borreliosis (LB)

- First discovered in the town of Lyme in Connecticut, USA
- Approximately 300 000 cases of LD are reported to Centres for Disease Control and Prevention (CDC) annually
- In Europe, about 85 000 cases every year
- In England and Wales, it is estimated that around 3,000 new cases of LD are diagnosed each year
- No vaccine available at present, can developed into chronic LD even after antibiotic treatment

LYME ARTHRITIS

AN EPIDEMIC OF OLIGOARTICULAR ARTHRITIS IN CHILDREN AND ADULTS IN THREE CONNECTICUT COMMUNITIES

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An epidemic form of arthritis has been occurring in eastern Connecticut at least since 1972, with the peak incidence of new cases in the summer and early fall. Its identification has been possible because of tight geographic clustering in some areas, and because of a characteristic preceding skin lesion in some patients. The authors studied 51 residents of three contiguous Connecticut communities—39 children and 12 adults—who developed an illness characterized by recurrent attacks of asymmetric swelling and pain in a few large joints, especially the knee. Attacks were usually short (median: 1 week) with much longer intervening periods of complete remission (median: 2.5 months), but some attacks lasted for months. To date the typical patient has had three recurrences, but 16 patients have had none. A median of 4 weeks (range: 1–24) before the onset of arthritis, 13 patients (25%) noted an erythematous papule that developed into an expanding, red, annular lesion, as much as 50 cm in diameter. Only 2 of 159 family members of patients had such a lesion and did not develop arthritis ($P < 0.000001$). The overall prevalence of the arthritis was 4.3 cases per 1,000 residents, but the prevalence among children living on four roads was 1 in 10. Six families had more than 1 affected member. Nine of 20 symptomatic patients had low serum C3 levels, compared to none of 31 asymptomatic patients ($P < 0.005$); no patient had iridocyclitis or a positive test for antinuclear antibodies. Neither cultures of synovium and synovial fluid nor serologic tests were positive for agents known to cause arthritis. "Lyme arthritis" is thought to be a previously unrecognized clinical entity, the epidemiology of which suggests transmission by an arthropod vector.

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Presented in part at the 40th Annual Meeting of the American Rheumatism Association, Chicago, Illinois, June 30, 1976 (1).

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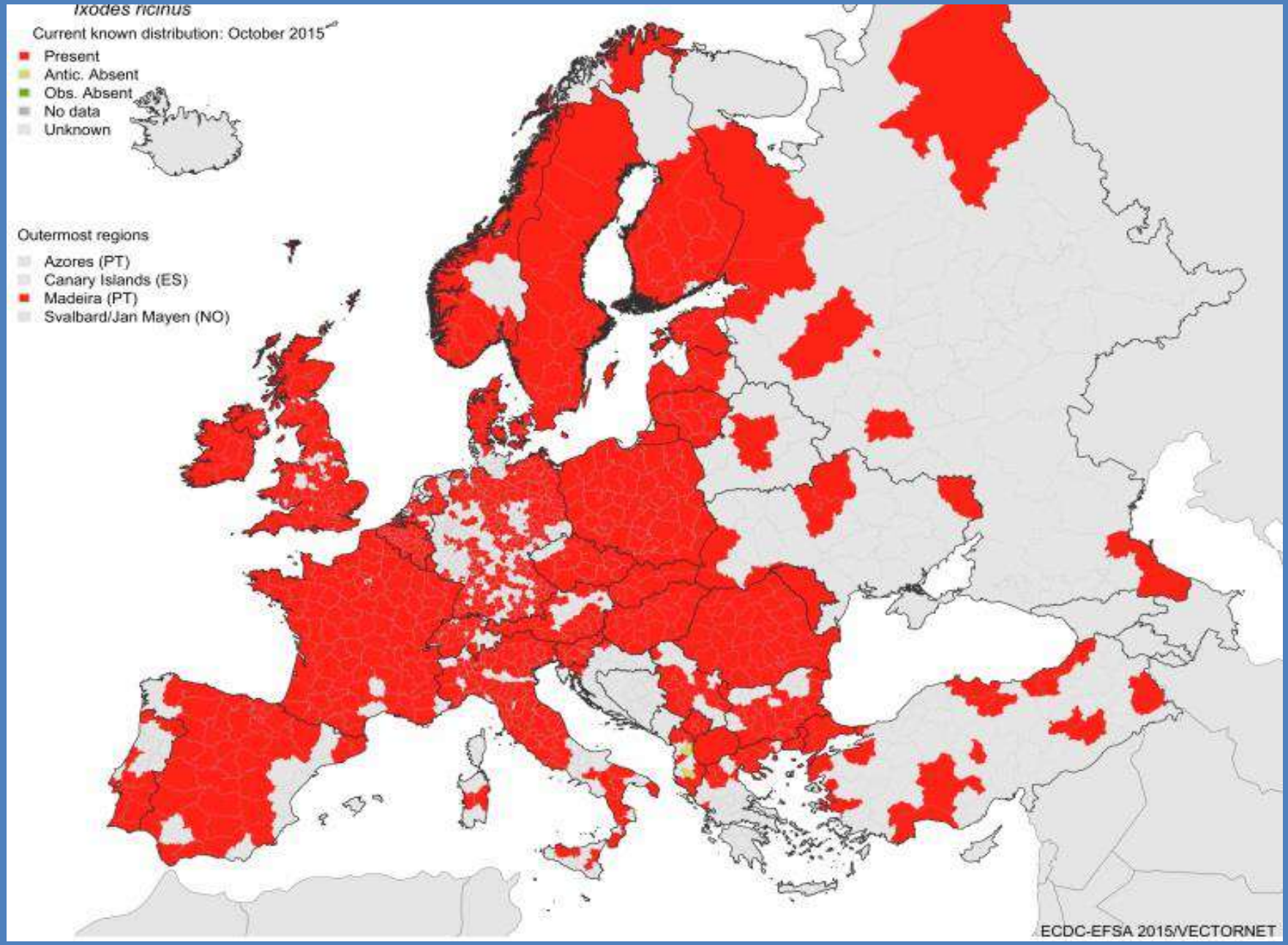
In November 1975 a mother from Old Lyme, Connecticut, informed the State Health Department that 12 children from that small community of 5,000, 4 of whom lived close together on the same road, had a disease diagnosed as juvenile rheumatoid arthritis (JRA). During the same month another mother from

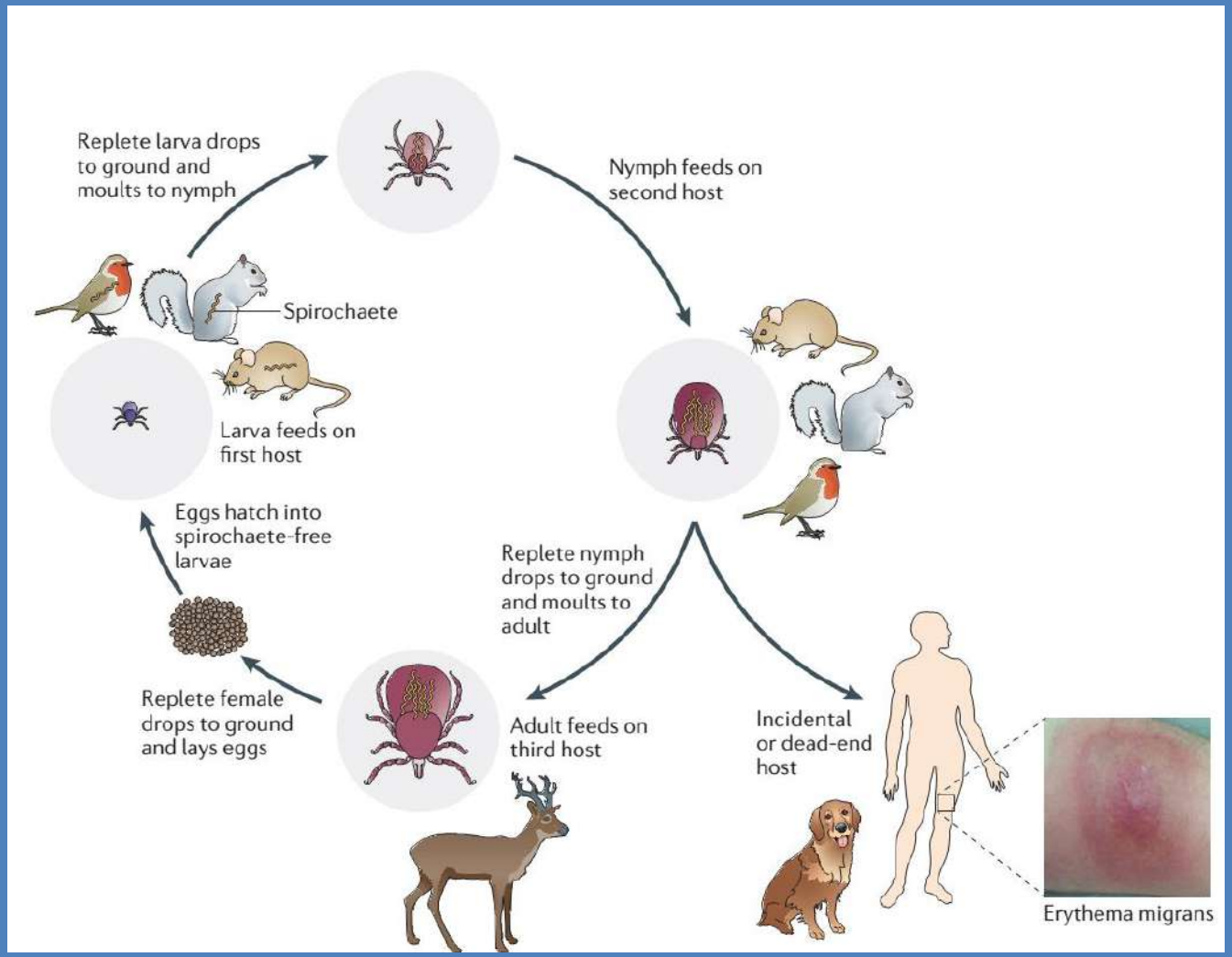
Distribution of Lyme disease (red) in Europe



(European centre for disease prevention and control)

The causative agent of Lyme disease

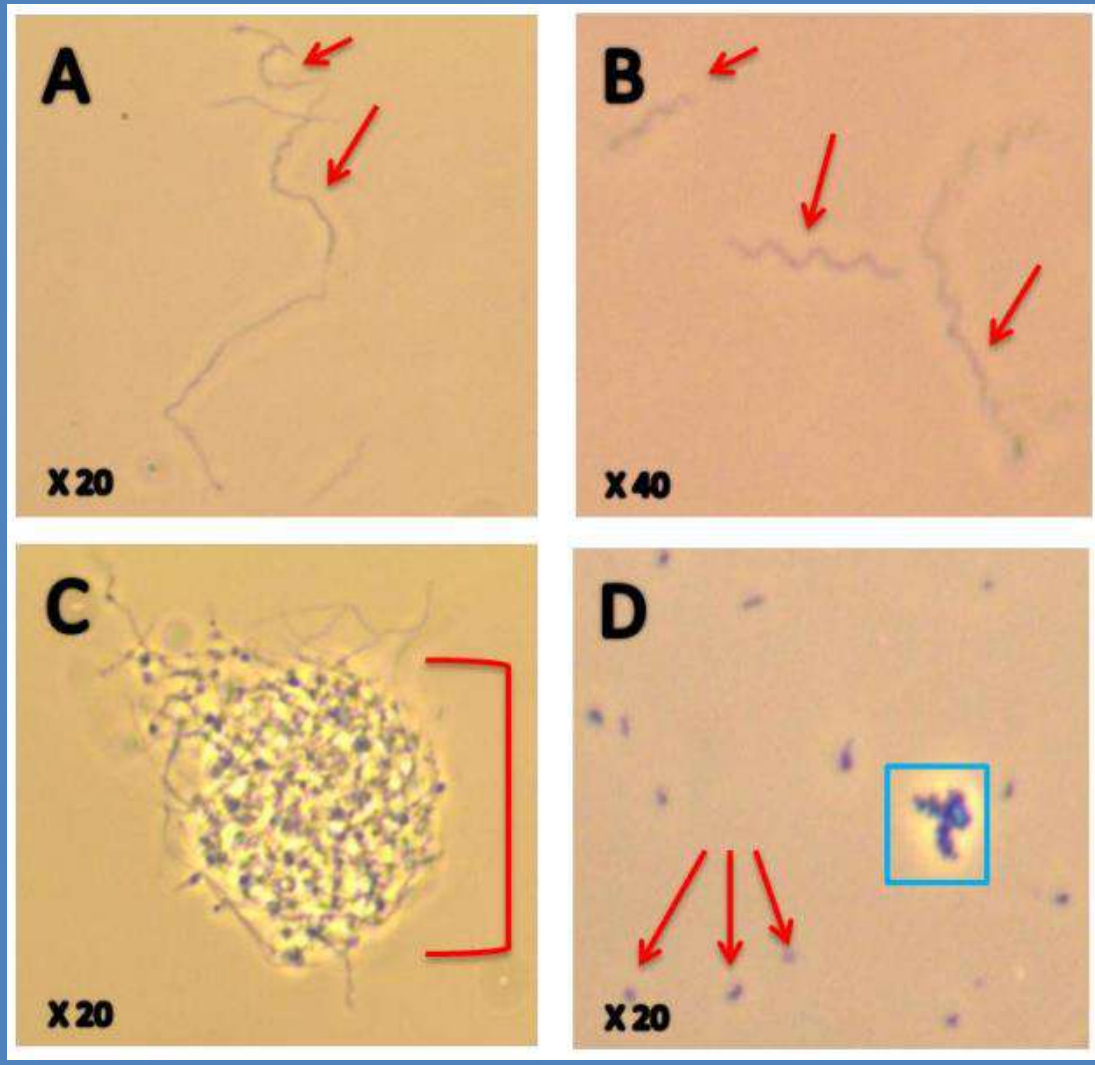
- Caused by the LD spirochaetes collectively referred to as *Borrelia burgdorferi sensu lato*
- The Lyme bacteria are spread to humans through the bite of infected ticks



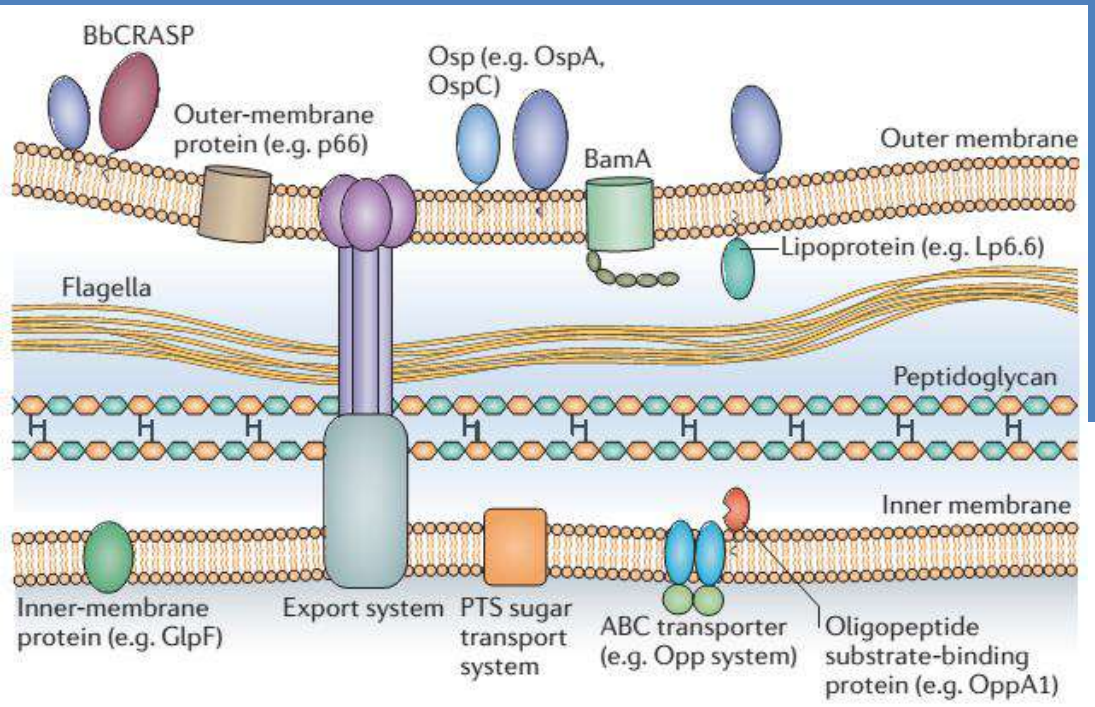
(Radolf, *et al*., 2012)

Lyme *Borrelia* strains

- *Borrelia* is an obligate parasite with a minimal genome
- *Borrelia* rely on the host to acquire building blocks
- In the human body, glucose is the primary energy source for *Borrelia*
- *Borrelia* in the blood is very low 100 cells/ml
- *Borrelia* can be outside human cells or inside human cells, you need antibody to stain the *Borrelia* and observed the location of *Borrelia*
- Intracellular *Borrelia* can survive inside human cells, showed no cell cytotoxicity

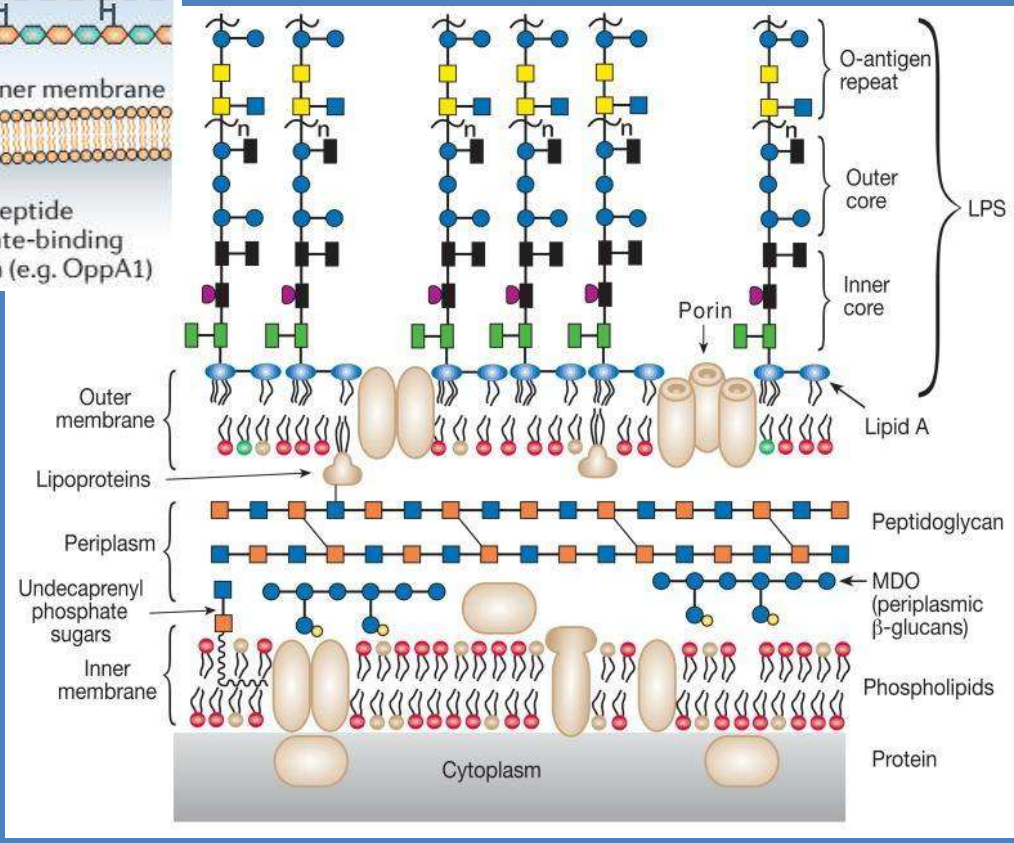


What is special about *Borrelia* spp.?



Borrelia cell wall

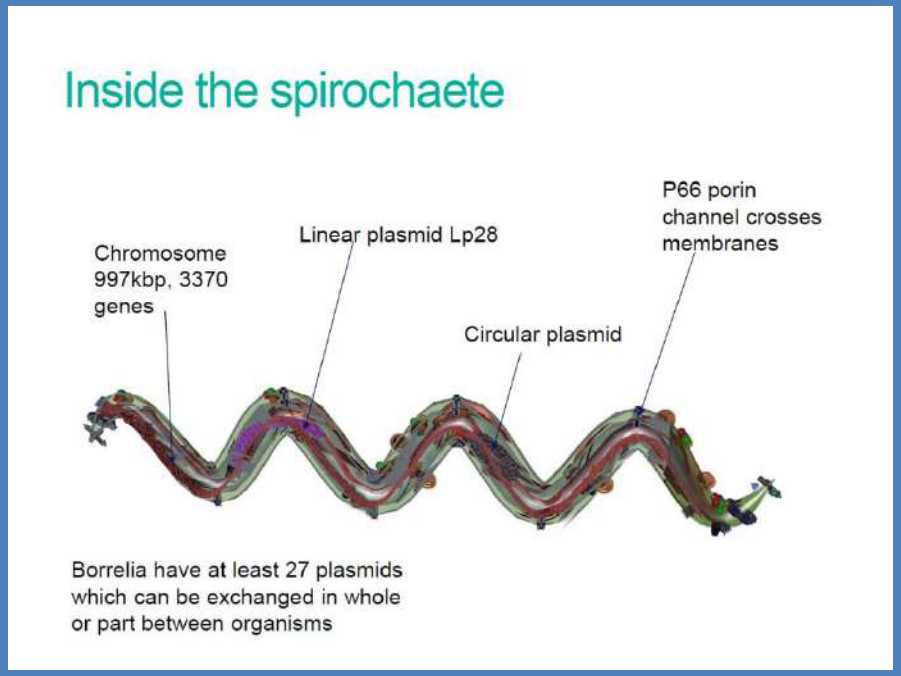
Gram negative bacterial cell wall →



What is special about *Borrelia* spp.?

- a main chromosome (911 kb for the type strain B31), and 20 or more smaller plasmids ranging from 5-50 kb

- Cp32 plasmid family of *Borrelia burgdorferi* has been demonstrated to be a bacteriophage

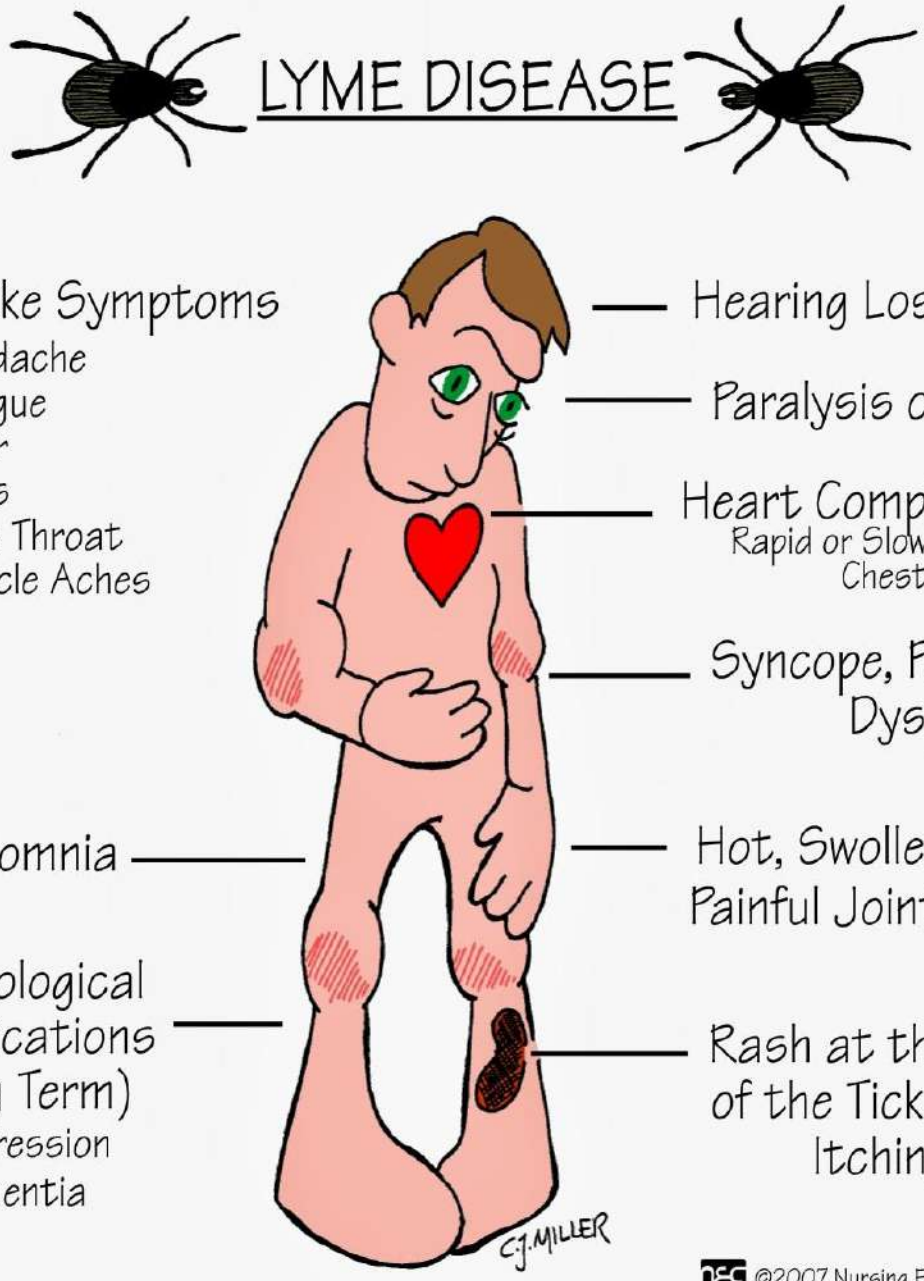


(Tim Brook, HPE)

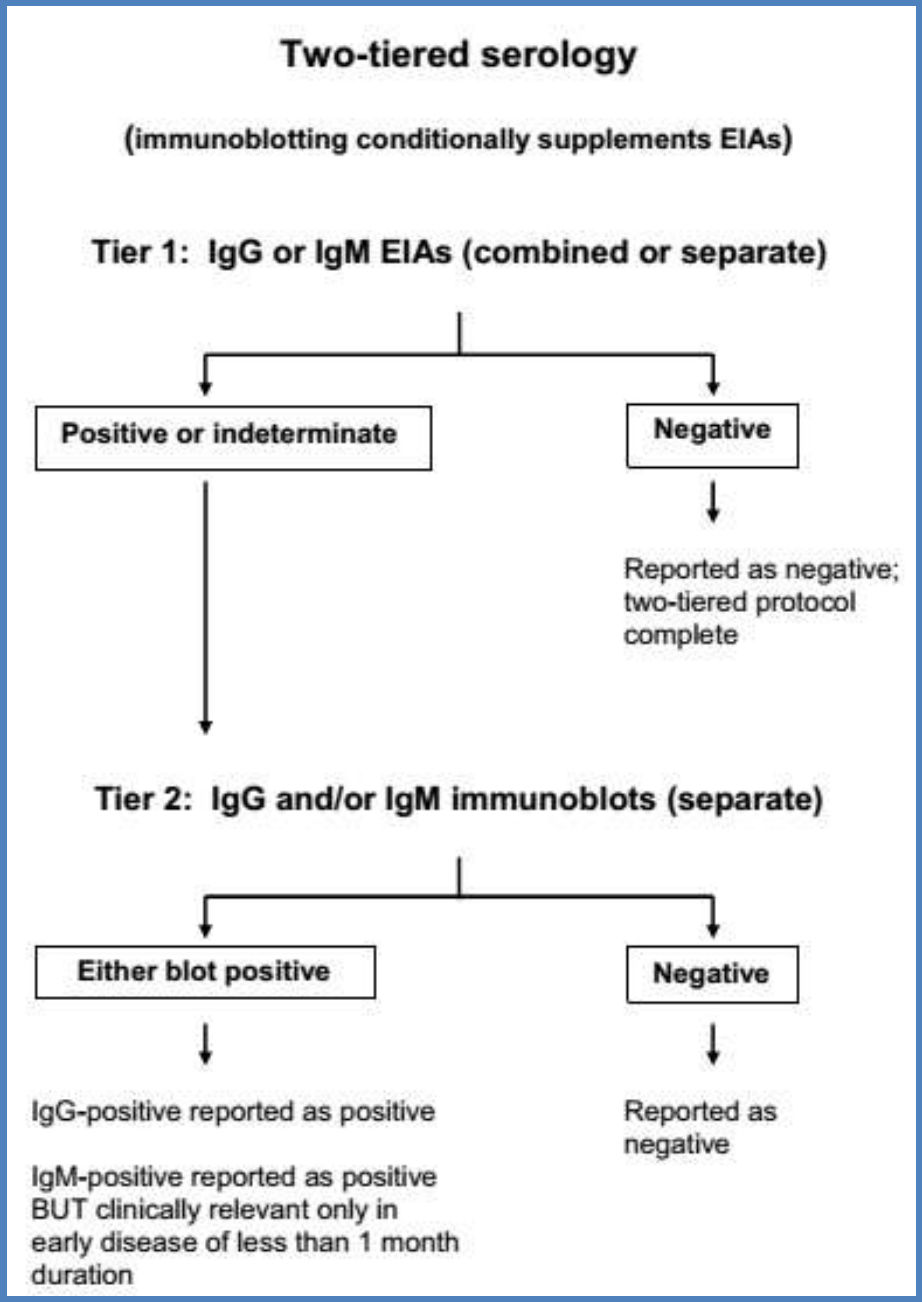
The current diagnostic method of LD: clinical diagnosis

Bull's eye rash (erythema migrans)

LYME DISEASE



Immune response-based serologic tests

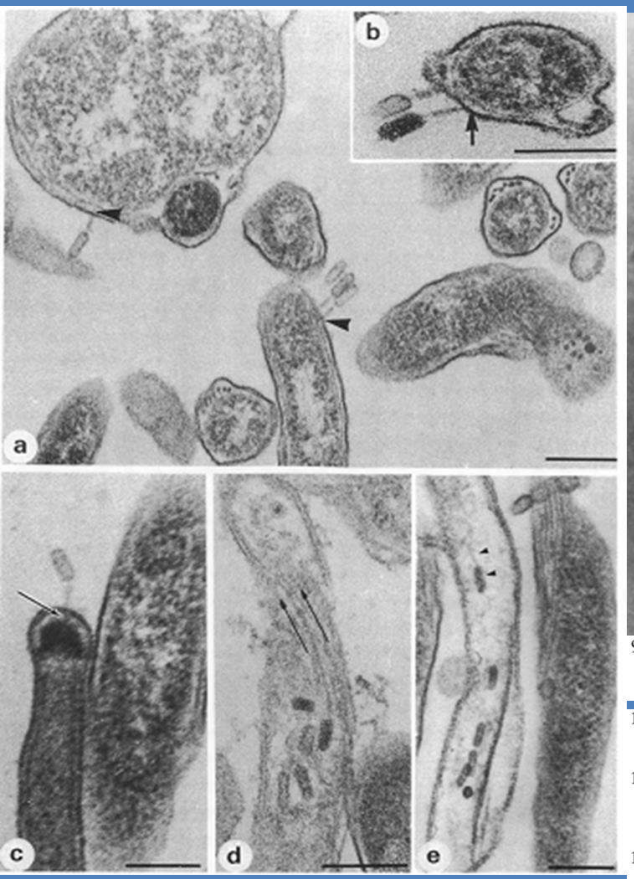


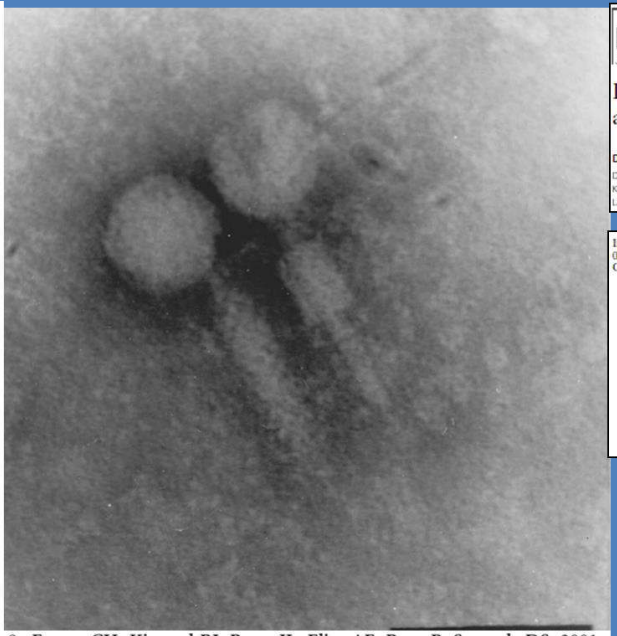
- The current antibody-based standard diagnostic tests for LD are unreliable because during the early stage of LD disease, the antibodies are either not yet developed or being suppressed by the bacteria.
- Consequently, the current diagnostic system has a low sensitivity, and is estimated to miss around 54% of patients.

PCR diagnostic method

- Since the antibody-based diagnosis may not be positive within the first 2-4 weeks of LD, a PCR-based diagnostic is a promising alternative for early LD diagnosis.
- Currently, PCR has been used in detecting *B. burgdorferi* DNA from skin biopsies, synovial and cerebrospinal fluid (CSF), but these PCR testing result is not clinically useful.
- For example, PCR testing of skin biopsy samples is generally used in research and not recommended for diagnosis because the skin rash (erythema migrans) can be established clinically.
- PCR testing of synovial fluid is not reliable and not well established. While only one third of LD patients in the USA showed positive PCR result against CSF samples.
- Additionally, there has rarely been a successful story in PCR detecting *Borrelia* DNA in the blood.
- In fact, half of patients in the early stage of LD showed PCR negative in blood samples. This is due to the extreme low number of the *Borrelia* circulating in the blood.

Current knowledge about *Borrelia* phages





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Bacteriophage in the *Ixodes dammini* Spirochete, Etiological Agent of Lyme Disease

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Demonstration of Cotranscription and 1-Methyl-3-Nitroso-Nitroguanidine Induction of a 30-Gene Operon of *Borrelia burgdorferi*: Evidence that the 32-Kilobase Circular Plasmids Are Prophages

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Distribution of cp32 Prophages among Lyme Disease-Causing Spirochetes and Natural Diversity of Their Lipoprotein-Encoding *erp* Loci

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The Relapsing Fever Spirochete *Borrelia hermsii* Contains Multiple, Antigen-Encoding Circular Plasmids That Are Homologous to the cp32 Plasmids of Lyme Disease Spirochetes

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Systematic effort is needed to investigate both temperate and lytic phages infecting *Borrelia* species

Results

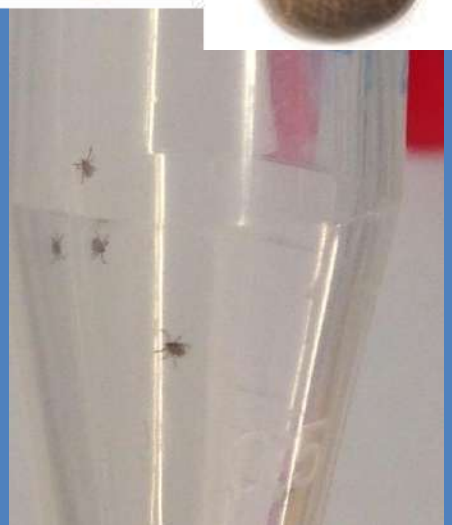
Professor Sven Bergström, Department of Molecular Biology, Umea University, Sweden

Strain collection in the lab

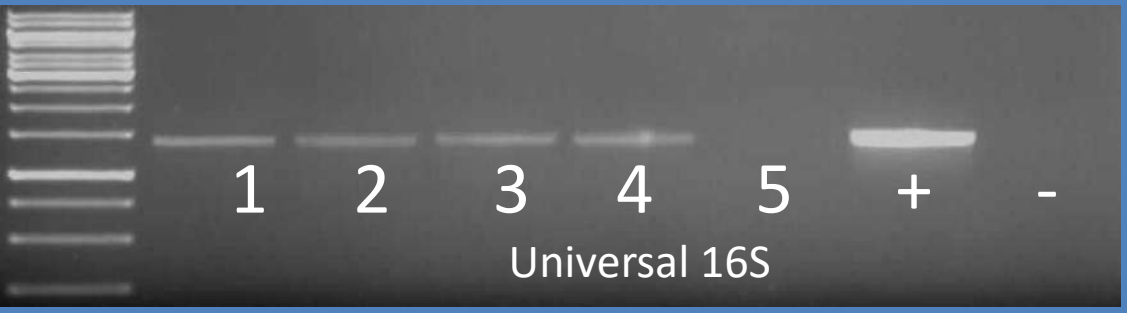
	Isolate names	Scientific names
1	1120	*Borrelia duttonii*
2	Her HS1	*Borrelia hemsii*
3	VS185 P9	*Borrelia burgdorferi*
4	NE218	*Borrelia valaisiana*
5	ACA1	*Borrelia afzelii*
6	UK filtered	*Borrelia burgdorferi*
7	190 P9	*Borrelia garinii*
8	China23	*Borrelia burgdorferi*

Current strategy for obtaining Borrelia phages

Tick sampling/dissection



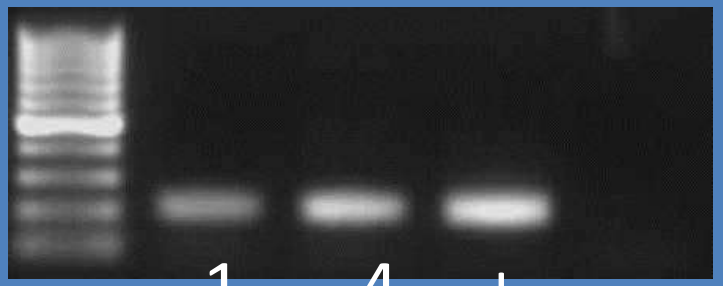
Tick dissection, enrichment and PCR detection of *Borrelia* and phages



Universal 16S

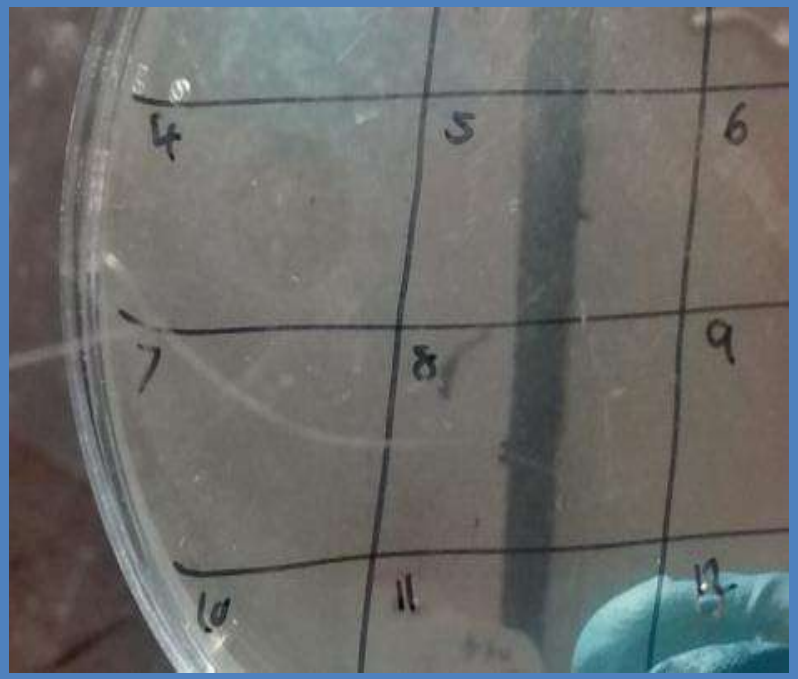
	Sample
1	Tick gut suspension (Martha's tick)
2	Tick blood from a well fed tick
3	Tick body ground suspension (Martha)
4	Tick ground suspension (Bradgate park)
5	Deer Poo

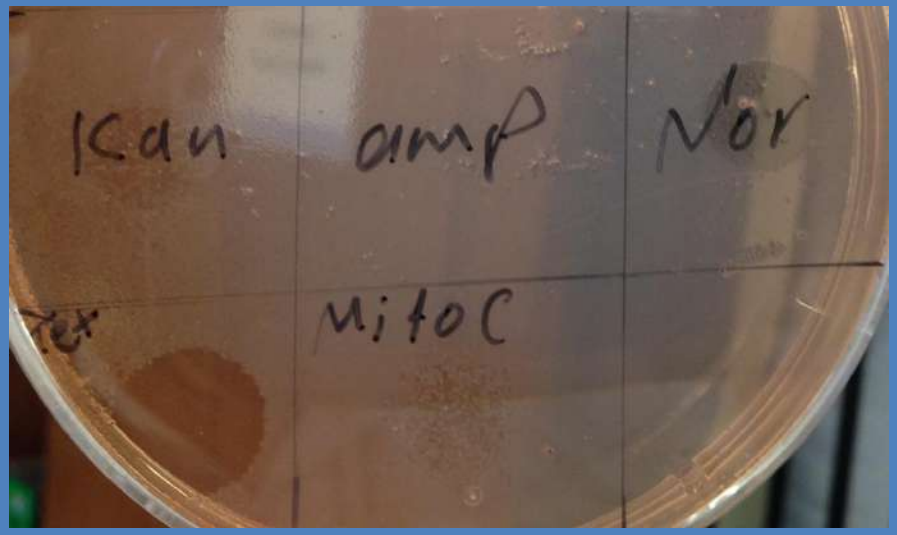
Borrelia-specific 16S



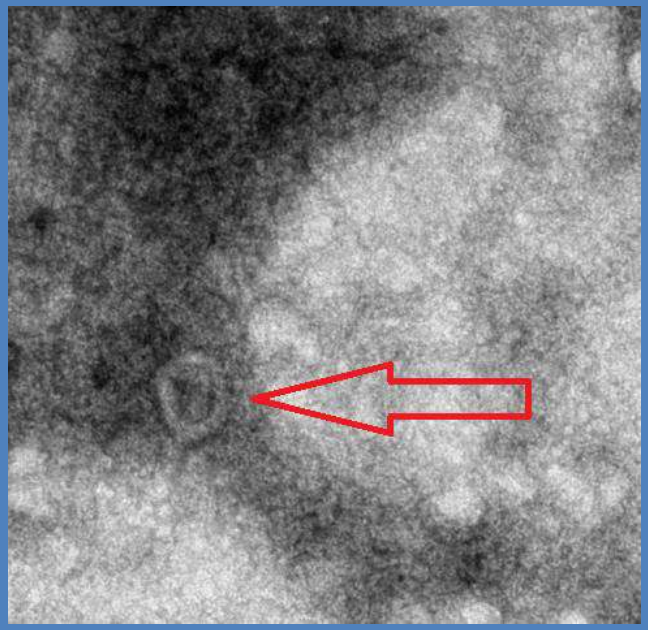
Phage-specific nested PCR

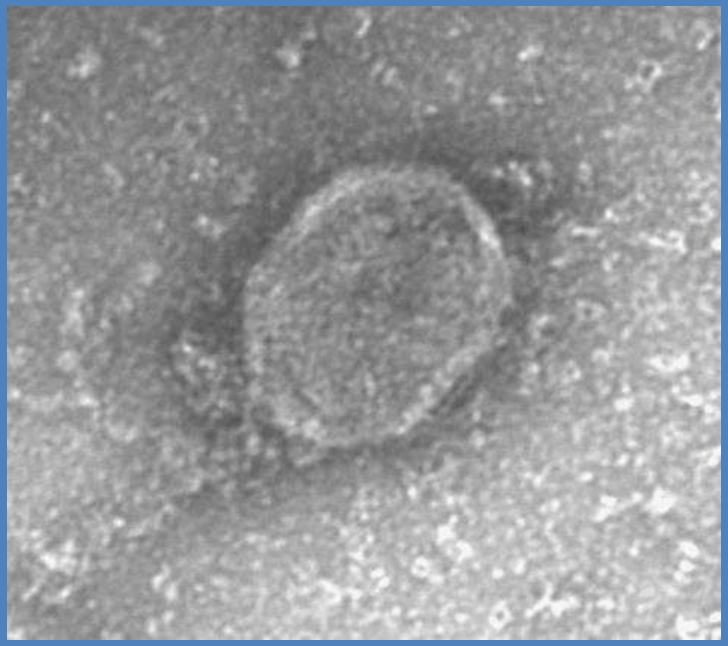
Grow *Borrelia* on a petri dish





• Temperate phages can be induced from Lyme *Borrelia* strains (table below). Currently working on increasing the phage concentration and phage purification. At least two different phages can be seen under transmission electron microscope (TEM)





Ongoing work in the lab

- Phage induction from different strains
- Optimisation of spot test and plaque assay
- Large screening using 96-well plate based live/dead assay against enriched tick samples, induced phage samples
- Over-expression of potential Holin and endolysin (identified from *Borrelia* genomic sequences)