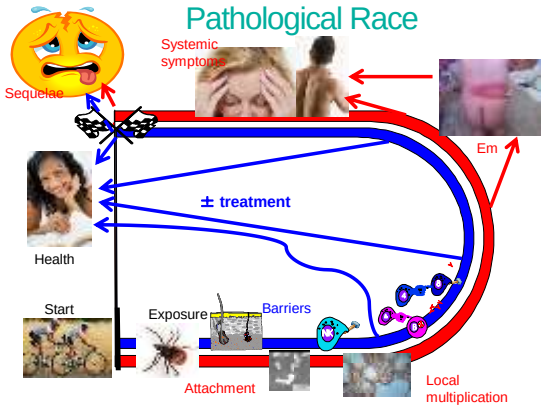
# Update on Lyme Disease

Tim Brooks

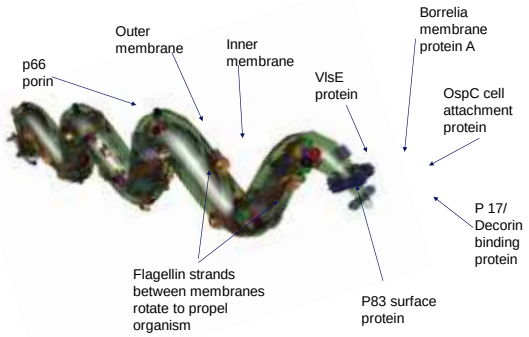
## Overview

- Infection, the host response, antibodies and diagnosis
- Test performance & quality assurance
- New test strategies:
  - Early detection & predictors of disease activity
  - PhD studentships
- Ticks & Borrelia in the UK
- Policy decisions
- Summary

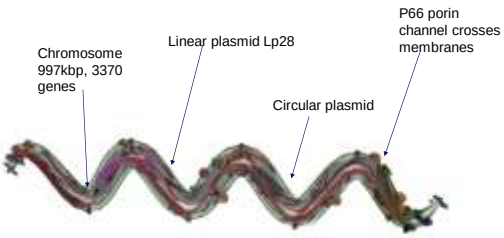
## Pathological Race




## Anatomy of a professional

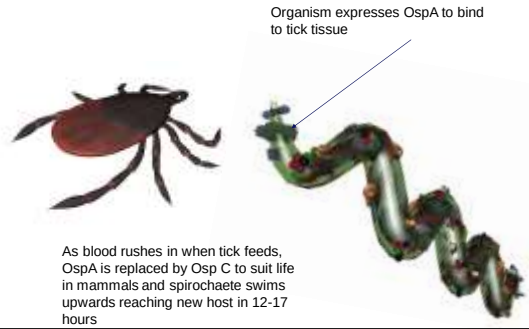



## Inside the spirochaete



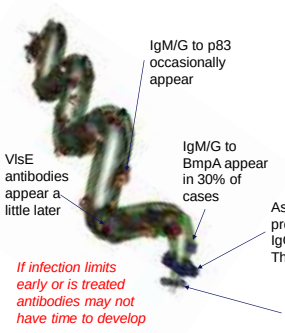

Borrelia have at least 27 plasmids which can be exchanged in whole or part between organisms

## In the tick




As blood rushes in when tick feeds, OspA is replaced by Osp C to suit life in mammals and spirochaete swims upwards reaching new host in 12-17 hours

## Early infection



IgM/G to p83 occasionally appear

VlsE antibodies appear a little later

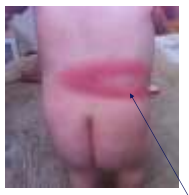
IgM/G to BmpA appear in 30% of cases

*If infection limits early or is treated antibodies may not have time to develop*

As organism starts to proliferate IgM and then IgG to OspC appear. They are short-lived

Many people develop p17 IgM/G

*Rash may be absent in up to 30% of cases*



Organism can be found in skin biopsy

## Disseminated infection

Antibody pattern

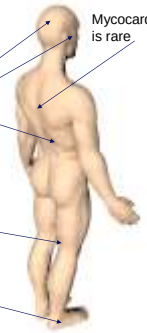
All antibodies may appear
OspC antibodies decline early
IgM slowly disappears leaving variable patterns of IgG responses
Pattern may correlate to species in some cases
VlsE dominates

Neuroborreliosis
Pcr occasionally positive
IgM in CSF

Arthritis
PCR Negative

Acrodermatitis chronicum atrophans
Pcr may be positive

Mycocarditis is rare



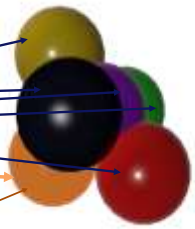
## Making the VlsE protein

Linear plasmid LP28

Variable region creates variable parts of protein

Conserved region creates conserved protein

A 6-mer peptide C6 is part of this region



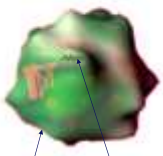
Conserved regions stay constant and C6 peptide stimulates antibodies across all variants

As VlsE changes many people are anti-VlsE negative when tested on a single protein

VlsE constantly changes so organism keeps ahead of immune system. At least 15 variants exist

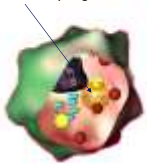
Pattern of antibodies to VlsE epitopes is linked to persistence of symptoms

## Antigen presentation



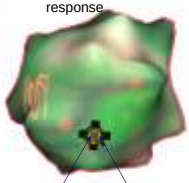
Macrophage recognises Borrelia and locks on

Borrelia in phagosome



Macrophage engulfs Borrelia and processes it into protein fragments

Antigen is presented on macrophage surface in MHC complex and triggers CD4 T cells to help generate immune response



Antigen fragment

MHC and T-cell trigger complex

## Quality control schemes: NEQAS

Provided from Switzerland as a cross-Europe scheme

Screening tests

| Screening Kit declared for this survey | IgG / Ig total | | | IgM | | |
|---|---|---|---|---|---|---|
| | P | E | N | P | E | N |
| AESKU SEVEN-UP | 1 | – | – | 1 | – | – |
| ALLTRAG LYMECHECK | 1 | – | – | 1 | – | – |
| bioMerieux VIDAS IgG / IgM | 41 | – | – | 34 | – | – |
| DiaMex Optiplex IgG / IgM | 2 | – | – | 2 | – | – |
| DiaMex Optiplex Screening IgG / IgM | 1 | – | – | 1 | – | – |
| Dusann LIAISON | 7 | – | – | 5 | – | – |
| EUROIMMUN ELISA | 1 | – | – | 1 | – | – |
| MIKROGEN recomWell | 1 | – | – | 1 | – | – |
| NOVALISA IgG | 1 | – | – | 1 | – | – |
| R-BIOSCREEN | 3 | – | – | 3 | – | 1 |
| SERION ELISA classic IgG / IgM | 2 | – | – | 1 | – | – |
| Siemens Enzygnost | 2 | – | – | 1 | – | – |
| ACCUDIAG III burgdorfer-FA | 1 | | | | | |
| Immunetics C6 Lyme (Ig total) | 2 | – | – | | | |

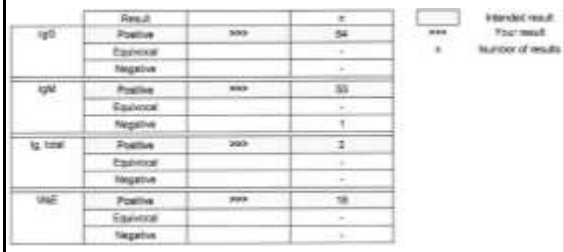
Some participants use several screening tests

P = positive, E = equivocal, N = negative

## NEQAS

| Confirmatory kit declared for this survey | IgG | | | IgM | | |
|---|---|---|---|---|---|---|
| | P | E | N | P | E | N |
| EUROLINE WB | 7 | - | - | 3 | - | - |
| EUROLINE IfN-AT | 8 | - | - | 3 | - | - |
| EUROLINE IfN-AT-adv (IgM) | | | | 1 | - | - |
| MIKROGEN recomLine | 7 | - | - | 4 | - | - |
| DiaMex Optiplex IgG / IgM | 3 | - | - | 3 | - | - |
| Virotrad Virastrips | 3 | - | - | 2 | 1 | - |

## NEQAS 3



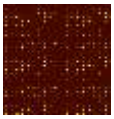

## Next Generation Assay Development - Genomics

- Current PCR not sensitive for detection of Borrelia in blood
- Numbers of organisms in blood is very low
- Current developments in progress:
  - Look at different gene targets to improve sensitivity of PCR
    - Cover wide range of genotypes for all three Lyme species
  - Investigate high volume blood extraction methods to improve sensitivity of PCR for blood samples
    - Extraction from whole blood sample – up to 20ml



## Next Generation Assay Development - Serology

- In-depth antigen/epitope investigation
  - Bioinformatic analysis of proteins and antigens to discover those that are:
    - Shared with other *Borrelia spp.*
    - Shared with other spirochates
    - Specific to either *B. burgdorferii s.s.*, *B. afzelli*, *B. garinii*
  - Development of protein microarrays and multiplex serology methods
    - Methods to be investigated include:
      - line blots
      - Luminex technology
      - Improved ELISA
  - Analysis with serum samples to determine performance of assays and their ability to differentiate Borrelia genotypes and be used to determine disease progression





## Host markers

Collaborative study Liverpool HPRU & PHE with Ceske Budejovice

Prospective study with early and late cases

Aims to look at host markers

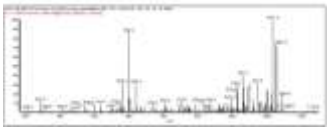
Based on proteomics and immune signals

Oxford Immunotech have trials on a revised T-cell test

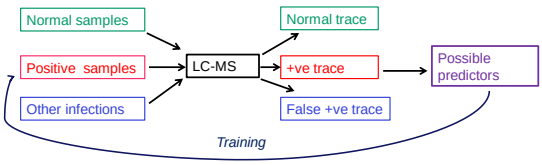
PHE review T-cell tests regularly but not practical at present to do studies in UK

## Metabolomics

Liquid chromatography - mass spectrometry



Illustrative trace of molecules



## Colorado State University results

Development of a Metabolic Biosignature for Detection of Early Lyme Disease

Molins *et al* Clinical Infectious Diseases **20 (6)**:1767-75 (2015)

95 markers distinguished Lyme patients from normal controls

44 of these markers were specific for Lyme as opposed to other infections

Sensitivity 88% (84-95%) for positive detection

Specificity 95% (90-100%)

Potentially may also allow indication of active infection in the *very* long term.

## Tick studies: Scott Layzell

### Four areas sampled

Cirencester Park, Gloucester

West Dean, South Downs National Park

King's Forest, Thetford, Norfolk

Wytham woods, Oxfordshire

### Four sites at each

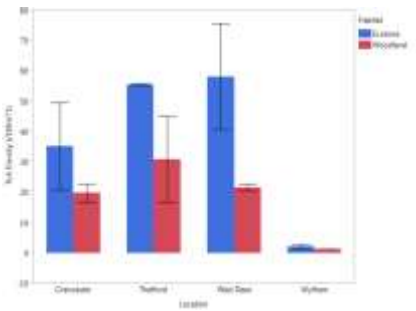
2 Ecotone (richer biological diversity)

2 Woodland

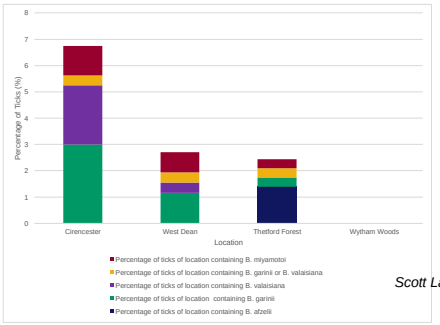
*Ecotone: transition from woodland to open grassland*

*Woodland*

## Distribution of ticks collected



## Proportion of ticks carrying *Borrelia spp*




*Scott Layzell*

## Prevalence rates of *Borrelia* in ticks

| Location | Habitat | Nymphs tested | Prevalence |
|---|---|---|---|
| Coed y Brenin, Gwynedd | Mixed woodland | 40 | 0 |
| Dalby, Yorkshire | Mixed woodland | 237 | 8.02 |
| Glisland, Northunmbria | Conifer/grassland | 55 | 0 |
| Gisburn Forest, Lancashire | Conifer/grassland | 253 | 0.79 |
| Graig-fechan, Clwyd | Moorland | 212 | 0.94 |
| Hampsfell woos, Cumbria | Decid woodland | 245 | 7.75 |
| Harwood, Northumberland | Conifer/grassland | 167 | 0 |
| Kielder Forest, Northumberland | Conifer/grassland | 185 | 0.54 |
| Loch Doon, Ayrshire | Conifer/grassland | 8 | - |
| Madie Forest, Dumfries | Decid woodland | 232 | 8.19 |
| Mell Fell, Cumbria | Decid woodland | 28 | 3.57 |
| Naddle Forest, Cumbria | moorland | 249 | 0 |
| | | | |
| 4 woodlands, Wiltshire | Woodland | 215 | 1.4 |
| Exmoor, Somerset | Woodland | 120 | 9.9 |
| 4 woodlands, New Forest, Hants | Woodland | 120 | 0.8 |
| Richmond Park, London | Parkland | 83 | 0 |
| Swinley, Surrey | Pine woodland | 94 | 2.12 |
| Salisbury, Wiltshire | Urban fringe | 111 | 6.3 |
| 3 sites, Dartmoor, Devon | Woodland/moorland | 107 | 8.41 |
| | | | |
| Cirencester, Glos | Woodland/ edge | 252 | 5.62 |
| South Downs, Suffolk | Woodland/ edge | 252 | 1.93 |
| Thetford, Norfolk | Woodland/ edge | 253 | 2.08 |
| Wytham, Oxon | Woodland/edge | 9 | 0 |

23 Bettridge et al. 2013; Hansford et al. 2014; Layzell et al.

## Genospecies prevalence

Bettridge et al. 2013

71 ticks sequenced

- 58% *B. valaisiana*
- 33% *B. garinii*
- 3% *B. afzelii*

Hansford et al. 2014

24 ticks sequenced

- 50% *B. garinii*
- 29% *B. afzelii*
- 20% *B. valaisiana*

Layzell et al. (unpublished)

Glos

- ~5.5% Bbsl
- ~3.2% *B. garinii*
- ~2.3% *B. valaisiana*

South Downs

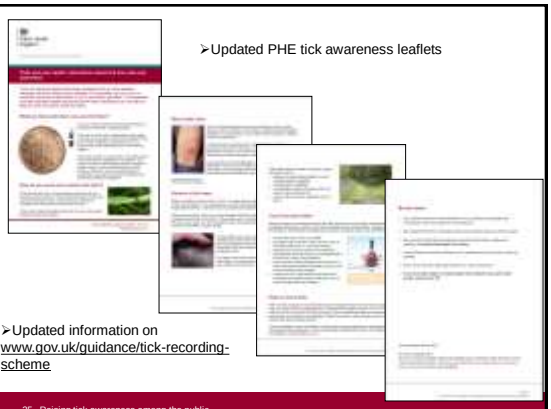
- ~2% all *B. garinii or B. valaisiana*

Norfolk

- ~2.2% Bbsl
- ~1.5% *B. afzelii*

Huge variation in genospecies dominance geographically
-> Implications for clinical presentation

24 Genospecies prevalence



- Updated PHE tick awareness leaflets
- Updated information on www.gov.uk/guidance/tick-recording-scheme

25 Raising tick awareness among the public


www.bathnes.gov.uk/services/public-health/latest-health-messages#bta

www.wiltshire.gov.uk/news/articles/tick-awareness-wiltshire-countryside-summer

26 Raising tick awareness among the public

## Lyme disease incidence & policy

Number of lab-confirmed cases

Varies yearly, around 1200

Depends on weather, economy, animal populations…….

PHE is working on getting disambiguated data out more quickly

Lyme as a notifiable disease

DH is considering case for making Lyme notifiable

Would give a "feel" for number of cases in general practice

Lyme guidance

Now in NICE portfolio of work

Lyme clinical services

Not commissioned: possibly a network?

## Open days and publicity

NHS Choices is the official site for patients

PHE information for patients is posted on NHS Choices

https://www.gov.uk/government/publications/lyme-disease-signs-and-symptoms

Some medical information is on .GOV.UK

https://www.gov.uk/government/collections/lyme-disease-guidance-data-and-analysis

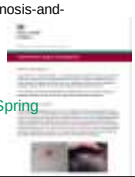
https://www.gov.uk/government/publications/lyme-disease-diagnosis-and-treatment

See also NICE

http://cks.nice.org.uk/lyme-disease

The next PHE Open Day will be held in London in early Spring

Social media releases: in progress



## Summary

Currently 1100-1200/yr laboratory confirmed cases in England & Wales
- Does not include clinically diagnosed EM

PHE participates in Europe wide EQA

New test methodologies under investigation
- Host & Pathogen markers
- Proteomics, metabolomics, molecular techniques

Tick distribution varies widely across UK
- From woodland to gardens
- So does carriage rate in ticks and Borrelia species distribution

Trials need infrastructure & sufficient numbers

Publicity and policy changes underway

## Acknowledgements

RIPL staff

Jolyon Medlock & Maaike Pietzsch MEZE

Liverpool University Health Protection Research Unit

Scott Layzell