Leptospira: The disease and its diagnosis.

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http://r6kbio.wikia.com/wiki/Leptospira_interrogans
Leptospirosa

- Are bacteria

- Most mammals can be infected

- A number of different species of leptospirosa comprising: pathogenic, non-pathogenic and some indeterminate in their nature

Disease pathway

• Enters the body through cuts, abrasions and through mucus membranes
• Make their way into the blood stream & circulate
• Colonise the kidneys
• Shed via urine back into the environment and circulate
Best thought of a as collection of diseases

- Presentation of the disease depends on the combination of host and the leptospiral strain

  e.g. Weil’s disease – the rat carrying the rat-adapted strain of lepto (Icterohaemorrhagiae) can be asymptomatic but a human that catches that strain will be very ill.
Disease symptoms

- Varies from subclinical to death depending on whether it is infected with a host adapted strain or not.

- Signs of illness can include: fever, muscle pain, vomiting and diarrhoea, loss of appetite, rash, lethargy, depression, light sensitivity.

- It can sometimes cause abortion in stock animals and blood in urine (red water) in young animals.

Steve Gurney, (NZ multisport athlete) – hospitalised with lepto
Background to leptospirosis in NZ

- NZ is in an unusual situation
- Only two native land mammals (bats)
- Pathogenic strains are limited and have come in on introduced species
- *Lepto* have a fairly confined distribution in NZ
Nothing stays the same…

**Lepto is a dynamic disease**

- Changes in incidence
- Changes in host reservoirs
- Genetic changes in the lepto themselves
  
  e.g. - adaption

- Changes in both farming practices and climate will have an impact too.
Known maintenance hosts for leptospirosis in New Zealand

*L. borgpetersenii* serovar Ballum: rodents and hedgehogs
*L. borgpetersenii* serovar Hardjo (subtype Hardjobovis*): cattle, deer, sheep
*L. borgpetersenii* serovar Balcanica: possums
*L. borgpetersenii* serovar Tarassovi: rodents and pigs

*L. interrogans* serovar Pomona*: pigs
*L. interrogans* serovar Copenhageni*: rodents

*animal vaccines available
it's tricky....

...even for those working in this area
Nomenclature: serology vs. species

- *Lepto* originally classified by serology (which is determined by cell surface antigens)
- Later by DNA homology, PCR, sequencing
- Now both systems in use and can cause confusion
Duel Classification Systems

- Serological vs DNA-based system (cell surface antigens vs DNA content)
- Little similarity between schemes i.e. two serologically closely related serovars may be completely different species.
- Both systems however are useful
Serology info to bear in mind

- Micro Agglutination Test (MAT) detects immunoglobulins. IgM peaks first followed by IgG
- Can take 2-3 weeks from infection till good serological response develops (therefore IgG more important in MAT)
- Age, genetic background of host, infecting serovar are factors
- Host-adapted strains may not create high titres – levels can be indistinguishable from vaccination titres
- N.B. Not every animal with an infection demonstrates titres (silent carriers)
Why use both classification systems?

• Both classification have their advantages.

• **Serology** – low tech. therefore useful in resource poor areas, epidemiological surveys and a provides evidence of both current and past exposure.

• **DNA** - can be strain-specific based on technique and therefore also useful in epidemiology. May detect minute amounts of DNA. Proof of current infection.
Commonly-used diagnostic tests in NZ

- Microscopic agglutination test (MAT) (serology)
  (ELISA sometimes used as a pre-screen in hospitals)
- Polymerase Chain Reaction (PCR)
  – chemical reaction that amplifies minute quantities of DNA
- Bacterial culture
- Dark field microscopy (DFM) (not generally used in commercial labs, but we use it for research purposes).
Diagnostic sample types for various tests

• **Culture:** blood, urine, CSF, organ tissue (e.g. kidney)

• **MAT:** serum

• **PCR:** blood/serum, urine, tissues

• **DFM:** blood, urine, tissue slurries
Serology- what is it based on?

- Serology is based on bacterial cell surface antigens - immunological

*Think of a jacket...*

- Two genetically very different bacteria can “wear” the same jacket (e.g. Hardjobovis and Hardjoprajitno). = serovar Hardjo
  Different DNA species, same jacket (serovar) – hence two disparate classification systems for *Leptospira*.

- Genetically closely related *Lepto* with the almost identical jackets - look the same to the immune system (e.g. Hardjobovis and Balcanica. 
  Same DNA species, almost identical jackets – serologically indistinguishable.

- Either same species or different species of *Lepto* with similar looking jackets
  Same serogroup and get cross-reactivity.
Why use serology - what does it tell us?

- Not as informative in acute stages of disease when the body is learning to recognise the antigen

- Screening for exposure to disease

- Often it tells us the serovar in NZ - epidemiologically useful!

- ...but some animals are “silent carriers” – no titres but they do carry Lepto

- Can’t always distinguish between vaccination and exposure titres
Dark field Microscopy

• Takes time, practice and skill

• Not easy to see if concentration is low

• Easier if alive – not so easy to identify if dead.
PCR: is DNA-based

- Theoretically can amplify 1 gene copy (therefore very sensitive)
- Organism does not have to be alive

(Protocols now available to distinguish live from dead lepto)

- Routine Vet. Path Lab. methods identify it only as *Lepto* (not the strain) (researchers currently working on PCR schemes to try and identify strain type as well)
Bacterial Culture

• **Pros:** - Concrete evidence of the presence of live *Lepto*
  - MAT and DNA analysis can both be performed on cultures
  - if you get a culture, whole genome sequencing can be performed providing identification, as well as fine detailed epidemiological information

• **Cons:** - exceedingly slow
  - also often contamination problems, therefore low overall sensitivity is an issue
Course of the disease – choice of test

Lepto colonising organs

PCR – not too predictable
Culture – not reliable
DFM - unreliable

Lepto shed, sometime intermittently in urine

PCR- generally reliable – minute quantities DNA needed
& serovar info missing usually
Culture - possible but contamination issues, very slow
DFM – not used in Vet/human Path labs usually, can be sensitive

Urine

PCR and culture may detect “silent shedders”. PCR
& culture success depends on whether the organism
has been cleared from the body – can be serovar –
dependent.

Lepto circulating in the blood stream

Blood

PCR – good , not necessarily serovar-specific
DFM - variable sensitivity
Culture – reasonably good but very slow
MAT – insensitive

Disadvantage – narrow window of opportunity

Serum

PCR – not too predictable
Culture – not reliable
DFM - unreliable

Lepto circulating in the blood stream

PCR- generally reliable – minute quantities DNA needed
& serovar info missing usually
Culture - possible but contamination issues, very slow
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0                          7                        14                       21                       28                      35                       42                      49                       56
rising titres         Days post infection                                                                             falling titres

PCR and culture – more sensitive, serovar info
Culture -not suitable
PCR – not so sensitive in blood now

MAT serology – can indicate past exposure.
Low titres may be confused with vaccination titres. Can
have “silent shedders” i.e. MAT negative carriers
Diagnostic Test Choice: Summary

- No single test meets all diagnostic needs
- Different tests more suitable for different sample types at various stages of the disease
- All have benefits and drawbacks
- The limitations of each test, and the choice of test at the stage of disease at which the patient presents, means many cases can go undiagnosed. A combination of tests types may be a good approach.