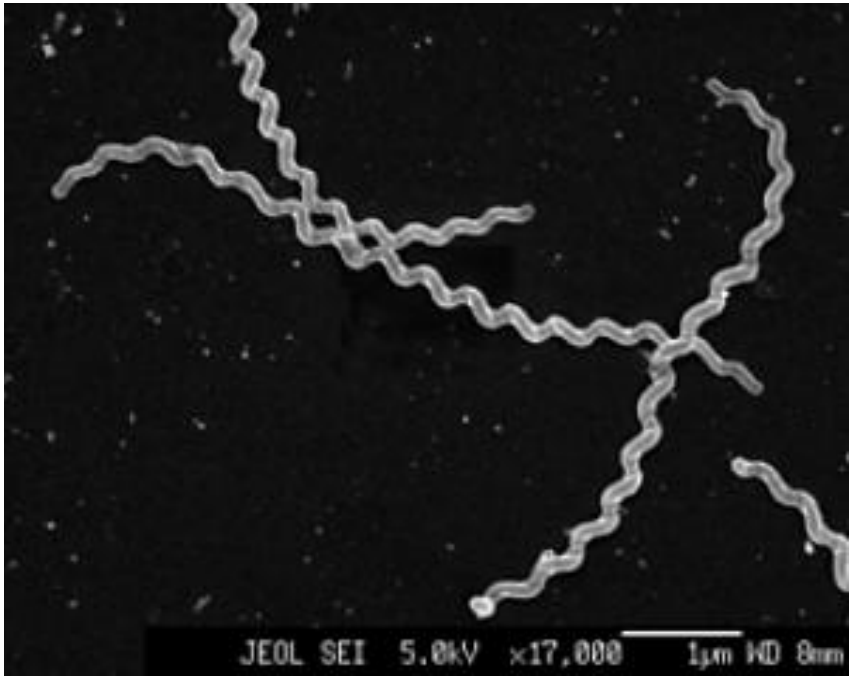
Leptospira: The disease and its diagnosis.

Julie Collins-Emerson

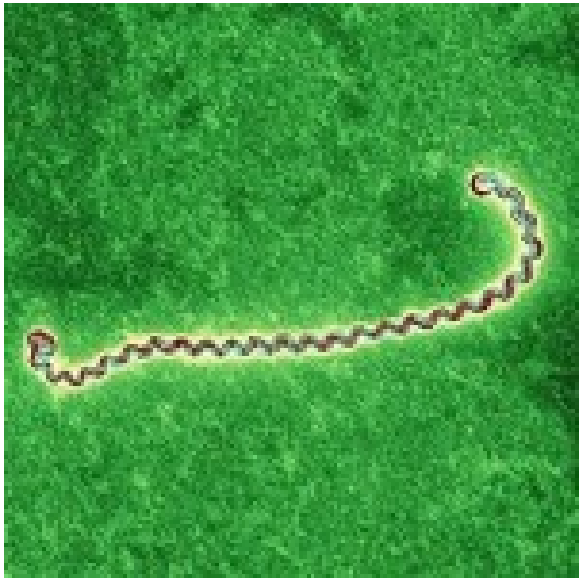
Lepto forum 06 March 2017



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

Leptospira

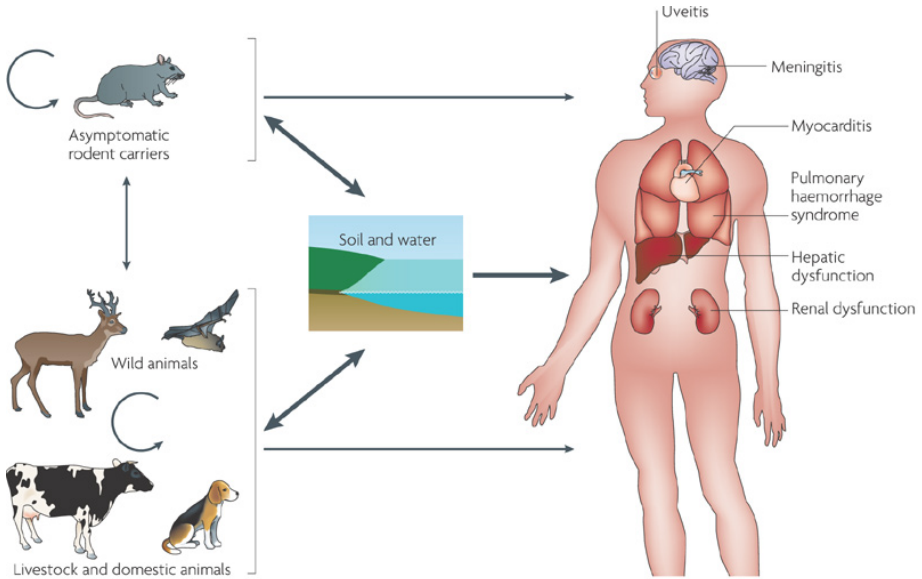
- Are bacteria
- Most mammals can be infected
- A number of different species of lepto comprising: pathogenic, non-pathogenic and some indeterminate in their nature



http://yourpetsneedthis.com/leptospirosis-in-dogs-florida/

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

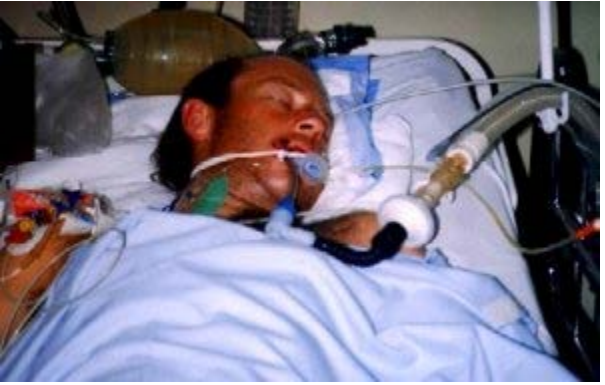


Nature Reviews | Microbiology

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

Steve Gurney, (NZ multisport athlete) – hospitalised with lepto

- 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 .

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

mEpiLab.massey.ac.nz

Classification

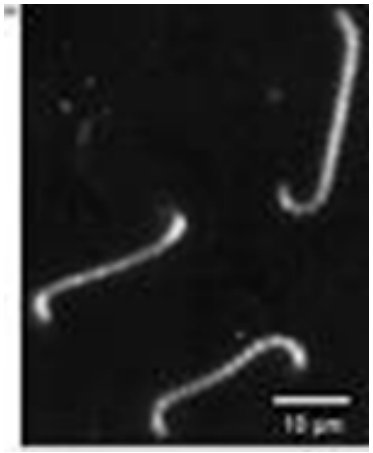
it’s tricky….

…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

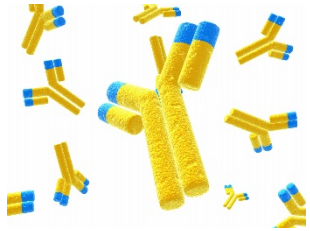


- Serological vs DNA-based system
  (cell surface antigens vs DNA content)

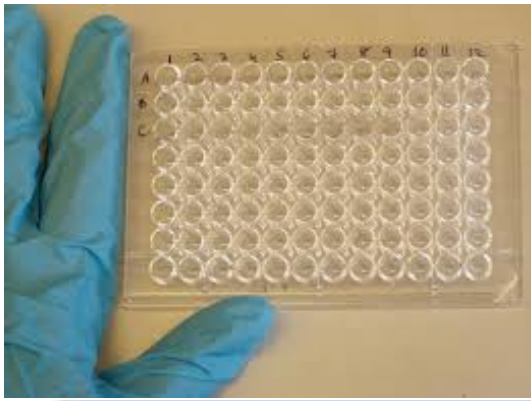
- Little similarity between schemes
  i.e. two serologically closely related serovars may be completely different species.

- 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)



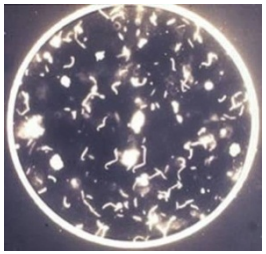
Why use both classification systems?

- 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

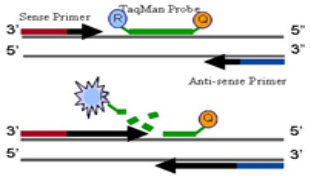
- Microscopic agglutination test (MAT) (serology)

  (ELISA sometimes used as a pre-screen in hospitals)



by Becca Chandler

- 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?

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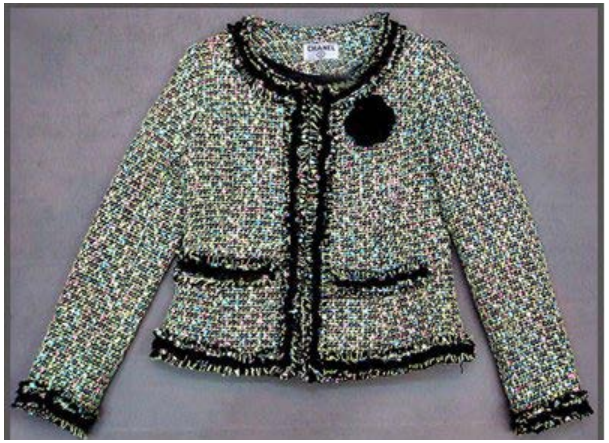
- Serology is based on bacterial cell surface antigens - immunological



Chanel original

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.*

Chanel knock-off

•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

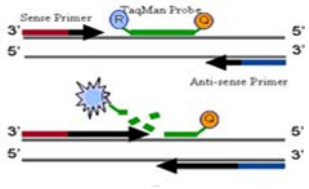
Dark field Microscopy

- Takes time, practice and skill
- Not easy to see if concentration is low
- Easier if alive – not so easy to identify if dead.

Roche.com

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)

- <u>Routine</u> 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

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

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

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 colonising organs

Lepto shed, sometime intermittently in urine

Urine

Blood

serum

Lepto circulating in blood stream

0 7 14 21 28 35 42 49 56

rising titres

Days post infection

falling titres

PCR – good , not necessarily serovar-specific
DFM - variable sensitivity
Culture – reasonably good but very slow
MAT – insensitive
Disadvantage – narrow window of opportunity

MAT serology – 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

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- No single test meets all diagnostic needs
- Different tests more suitable for diff. 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.