Diagnosis of Leptospirosis and Austrian epidemiology

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Leptospires:

- HISTORY
- MORPHOLOGY
- TAXONOMY
- OCCURENCE
- TRANSMISSION
Leptospires: History

- Adolf Weil (1886) his account:
  “An infectious disease, accompanied by splenomegaly, jaundice and nephritis”

- 1914 - 1918 war in Europe “Weil’s disease” assumed increasing importance:

- Hübner & Reiter (1915) successful transmission of “Weil’s disease”
  to guinea pigs and the “flagella like” bodies in blood films stained with Giemsa

- Uhlenhuth & Fromme “spirochaeta interrogenes”
  - a demonstration of the spirochaete and immunisation with it

- Inada & colleagues 1914 - 1915 in Japan
  - coal-miners fell sick
  - succeeded in transmitting the infection to guinea pigs (blood transfusion)
  - to demonstrate passive protection with specific immune serum
  - in studies “mode of infection” and ……

  ➔ “Spirochaeta icterohaemorrhagiae”
Morphology

- **Order**: Spirochaetales
  - helically coiled (spiralförmig)
  - thin
  - motile
  - propelled by flagellar mechanism
  - contained wholly within an outer envelope
  - diverse in chemical and nucleic acid compositions
  - nutrition and natural habitats generally visualised by darkfield microscopy in wet preparations
  - not good visible in usual bacterial staining

leptospira is greek origin
„lepto“ = fine
„spira“ = spiralförmig

Source: www.wikipedia.org
Morphology

- Leptospires range in length
  - 10 - 20µm
  - coil amplitude of 0,1 - 0,2 µm
- two flagella, one arising at each end
- At cell division a new flagella is developed at the newly formed end after division
- In fluid media they spin rapidly on their long axis in rotational movements and move to and from in both directions

Source: www.wikipedia.org
Two methods to classify leptospires:

- **serological classification:**
  Leptospira biflexa sensu lato: non pathogenic free living leptospires (about 60 serovars)
  Leptospira interrogans sensu lato: pathogenic (about 200 serovars in 23 serogroups)

- **genomic classification:**
  16 different genospecies identification and classification of leptospires by comparing DNA fragments
Occurrence:

- Leptospirosis occurs worldwide
- Acid (pH < 7.0) and dry conditions kill leptospira
- Transmission only in wet environments
- In cold environments (+4°C) leptospires survive ~21 days
- Sources are:
  - Urine, kidneys of infected animals
  - Surface waters, mud and soil
Transmission:

- **Direct transmission:**
  - Blood or body fluids containing leptospires pass from an infected animal to other animals or humans:
  - transplacental transmission
  - sexual contacts
  - suckling milk

- **Indirect transmission:**
  - Infection of humans and animals from environmental leptospires:
  - Ponds, lakes, drain water.. contaminated by the urine from excretor animals
  - (rodents, swine, cattle, dogs, ...)

  - **Occupational infection:**
    - Exposed are drainers, slaughtermen, farmers, butchers, veterinarians, hunters, ...

  - **Non occupational infection:**
    - Infection with contaminated water by animal's urine at leisure activities, travel, ...
      (e.g. camping, boating, swimming, ....)
Transmission of leptospires
Culture methods

- **Culturing:**
  - EMJH-Medium: (Ellinghausen, McCollough, Johnson u. Harris)
  - Serum-free oleic-acid albumin medium
    - (quality control: clear medium)
  - Subculturing from and to liquid medium
    - (0,1->0,2ml)
  - Growth in liquid medium is proved by gentle shaking
  - Aseptically transferring (safety cabinet)
Culture methods

- **Temperature:**
  - Cultures are kept in dark at 29°C (5->7 days) to avoid toxic changes
  - Screw-capped tubes to prevent contamination
  - Duplicate tubes for several serovars (antigen)
  - 10ml volumes for growth
Culture methods

Culture control and preservation

- Purification of contaminated cultures
  (filtration: membrane filter – 0.22µm
   centrifugation and resuspension in fresh medium)
- Proving of strains (cultures) for identity (serologically)
- Preservation of stock cultures:
  Cryopreservation in liquid nitrogen in a liquid phase
Culture of leptospires
Contaminated culture of leptospires
Microscopic agglutination test

Equipment:

Materials:
- Antisera (reference antisera)
- Cultures of leptospires (density and growth control)
- Phosphate buffered saline
- Safety cabinet
- Tubes
- Microtiter plates (flat bottom)
- Pasteur pipettes (steril)
- Tips
Testperformance (Screening):

- 1/25 dilution of serum in phosphate buffered saline (tubes)
- Microtiterplate:
  - A special plate for every animal species (determined by the number of antigens we use for this species)
  - Human sera are screened in extra plates
  - In one plate it is possible to check 10 samples
- Platelabelling:
  - Left side: Serovars of leptospires (antigen)
  - Upper side: Identity number of sample
  - Right side - column 11: pos.controlserum (connected to serovar)
  - - column 12: only antigen = neg.control (safety cabinet)
Microscopic agglutination test

- **Test performance (Screening):**
  - 50µl serum dilution in column 1 (sample 1)
  - 50µl serum dilution in column 2 (sample 2) and so on
  - 50µl pos. control serum (corresponding to antigen) in column 11

- **Safety cabinet:**
  - 50µl of the corresponding antigen in row 1 (well 1->12)
  - 50µl of the corresponding antigen in row 2 and so on
  - -> final dilution 1:50

- Column 12 = only antigen (leptospira) = neg. control

- Shaking softly (plateshaker)
- Incubation 29°C (2->2,5 hours) in a humid chamber
- Examination of each well by dark-field microscopy for agglutination
- 50% agglutination at one or more antigens is examined by titration
Microscopic agglutination test

- Test performance (Agglutination)
  - Tubes:
    - 1/25 dilution of serum in phosphate buffered saline
  - Microtiterplate:
    - An extra plate for each antigen
    - Platelabelling:
      - Left side: Seradilutions 1:50 -> 1:6400
      - Upper side: Identity number of sample (serum dilution)
      - Right side (column 11):
        - pos.controlserum for corresponding antigen
      - Right side (column 12): antigen control
Microscopic agglutination test

Testperformance (Agglutination):  
Row A: 100µl dilution in the corresponding well of row A  
- 50µl phosphate buffered saline in row B ->H  
- Dilution from row A ->H (volume 50µl)  
- Dilutionsteps: 1:50 –> 1:6400

- Safety cabinet:  
  - 50µl of the corresponding antigen in each well  
  - Shaking softly (microplateshaker)  
  - Incubation 29°C (2-2,5 hours)  
  - Examination of each well by dark-field microscopy for agglutination (row A-H)  
- As endpoint is defined this serum dilution that shows 50% agglutination of leptospires
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<thead>
<tr>
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<th>1</th>
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<th>11</th>
<th>12</th>
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<tbody>
<tr>
<td><strong>Pat.1</strong></td>
<td><strong>A=ict.</strong></td>
<td>50µl Pat 1+L.ict.</td>
<td>wie Pat.1</td>
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<td><strong>H=hard.</strong></td>
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Platelabeling for Screening:
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<th>Pat.6</th>
<th>Pat.7</th>
<th>Pat.8</th>
<th>Pat.9</th>
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<th>-Ko</th>
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<td>wie Pat.1</td>
<td>wie Pat.1</td>
<td>wie Pat.1</td>
<td>+Ko</td>
<td>-Ko</td>
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<tr>
<td>B=1: 100</td>
<td>50µl + 50µl PBS</td>
<td>wie Pat.1</td>
<td>wie Pat.1</td>
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<td>+Ko</td>
<td>-Ko</td>
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<tr>
<td>C=1: 200</td>
<td>50µl + 50µl PBS</td>
<td>wie Pat.1</td>
<td>wie Pat.1</td>
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<td>+Ko</td>
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<td>+Ko</td>
<td>-Ko</td>
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<td>-Ko</td>
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<td>+Ko</td>
<td>-Ko</td>
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<tr>
<td>H=1:6400</td>
<td>50µl + 50µl PBS</td>
<td>wie Pat.1</td>
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<td>+Ko</td>
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Microscopic agglutination test:

positive control

negative control
Leptospirosis: Serological examination of veterinarians

- 137 blood samples tested
- 10 veterinarians had antibodies against leptospira – 4 of them with positive titer
- 1 veterinarian with high antibody titers against L.saxköbing and L.bataviae - he had to stay in hospital
- 9 veterinarians reported about different clinical signs
- 2 veterinarians with positive titers were practising with swine and all of them practising in slaughtering
- Summary: veterinarians are exposed with infections to zoonosis (example: leptospirosis)
Blood samples from 147 hunters and 108 wild boars were tested.
11 serovars of Leptospira
15 hunters (10%) had antibodies with positive, 26 hunters suspect titers
(see first diagramm)
A control group (50 persons – mainly city dwellers) no leptospira antibodies

Samples of 36 wild boars (30%) antibody positive, 16 samples suspect titers
(see second diagramm)

Conclusion: High risk of leptospira infection in hunters compared to other occupational groups.
Leptospirosis 2009 (Human samples):

- 45 human blood samples tested
- 41 samples/styria, 3 samples/upper Austria, 1 sample/Vienna
- 16 serovars of leptospires
- In 26 samples antibodies against leptospira found

- **Organigram 1:** Antibody titer in 22 samples 1:50 (=suspect):
  - L.icterohäm., L.bratislava, L.canicola

- **Organigram 2:** Antibody titer in 6 samples >1:100 (=positive):
  - L.iterohäm., L.bratislava, L.autumnalis
Plate for Microagglutination test

[Diagram of a 12x8 grid with letters A to H on the left and numbers 1 to 12 on the bottom]
Leptospirosis 2008 (Human samples):

- 85 human blood samples tested
- 76 samples/styria, 6 samples/upper Austria, 3 samples/Vienna
- 16 serovars of leptospires
- In 41 samples antibodies against leptospira found

- **Organigram 1**: Antibody titer in 34 samples 1:50 (=suspect):
  - L.icterohäm., L.bratislava, L.grippotyphosa, L.ballum

- **Organigram 2**: Antibody titer in 7 samples >1:100 (=positive):
  - L.bratislava, L.icterohäm., L.grippotyphosa