AGES
Österreichische Agentur für Gesundheit
und Ernährungssicherheit GmbH

Our Concern.
Diagnosis of Leptospirosis and
Austrian epidemiology

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**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, ...)

AGES — Health, Nutrition, Safety. Our Concern.
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
Microscopic agglutination test

Test performance (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

- **Testperformance (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**

**Test performance (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)
- Dilution steps: 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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Platelabeling for Screening:
# Agglutination:

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A=1: 50  100µl
B=1: 100  50µl + 50µl PBS
C=1: 200  50µl + 50µl PBS
D=1: 400  50µl + 50µl PBS
E=1: 800  50µl + 50µl PBS
F=1:1600  50µl + 50µl PBS
G=1:3200  50µl + 50µl PBS
H=1:6400  50µl + 50µl PBS

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 were identified. 15 hunters (10%) had antibodies with positive, 26 hunters suspect titers (see first diagramm). A control group (50 persons – mainly city dwellers) had no leptospira antibodies. Samples of 36 wild boars (30%) were antibody positive, 16 samples had 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
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