Information zu Schistosomiasis-Cluster Südkorsika

Sehr geehrte Damen und Herren!


Da in der Vergangenheit Bulinus-Wasserschnecken, die als Wirt von Schistoma spp. gelten, in Südkorsika gefunden wurden, kann vermutet werden, dass ein lokaler Parasitenzyklus existiert. Umweltuntersuchungen werden veranlasst, um das aktuelle Vorkommen von Bulinus Wasserschnecken zu bestätigen und gegebenenfalls das Risikogebiet für die Übertragung von Urogenital-Schistosomiasis (Blasen-Bilharziose) zu ermitteln.

Es wird ersucht diese Information bei der reisemedizinischen Beratung und medizinischen Betreuung von Reisern zu berücksichtigen.
Leishmania and Leishmaniasis

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Neglected tropical diseases (NTD)

**Virus**
- Dengue/Severe dengue
- Rabies

**Protozoa**
- Chagas disease
- Human African trypanosomiasis (sleeping sickness)
- Leishmaniasis

**Bacteria**
- Buruli ulcer
- Leprosy (Hansen disease)
- Trachoma
- Yaws (treponematoses)

**Helminth**
- Cysticercosis/Taeniasis
- Dracunculiasis
- Echinococcosis
- Foodborne trematodiases
- Lymphatic filariasis
- Onchocerciasis (river blindness)
- Schistosomiasis
- Soil-transmitted helminthiases
NTD: Fast facts

- are endemic in 149 countries
- affect >1 billion people worldwide
- kill ~534,000 people/year
- low-income populations in developing regions of Africa, Asia, & the Americas
- 100% of low-income countries are affected by at least 1 NTD
- individuals are often affected with more than one parasite or infection
- treatment cost for mass drug administration programs is < $0.5/person/per year
Global Overlap of Six of the Common NTDs

guinea worm disease, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminths, trachoma
Leishmaniasis: Key Facts

- caused by obligate* intracellular protozoa of the genus *Leishmania*
- occurs in >90 countries
- endemic in areas of the tropics, subtropics, & the Mediterranean basin
- on every continent except Australia and Antarctica
- the ecologic settings: rain forests to deserts
- *Vector-born disease*: parasites are transmitted by the bite of infected female *phlebotomine* sandflies (~30 different species)
- currently affects 12 million people
- ~1.3 million new cases/year; 20,000 - 30,000 deaths/year

*by necessity
3 main forms of Leishmaniases:

a) **visceral** (kala-azar) - the most serious form
b) **cutaneous** - the most common
c) **mucocutaneous**
Leishmania: The parasite I

- trypanosomatid protozoa
- Order: Trypanosomatida
- Genus: *Leishmania*
- named in 1903 after the Scottish pathologist W. B. Leishman
- two morphological forms:
  - *promastigote* (with an anterior flagellum) in the insect host
  - *amastigote* (without flagella) in the vertebrate host
Leishmania: The parasite II

- 21 species are known to cause disease in humans:
  - *L. tropica*; *L. major*; *L. aethiopica*
  - *L. donovani* complex
    - (L. donovani, L. infantum = L. chagasi)
  - *L. mexicana* complex
    - (L. mexicana, L. amazonensis, and L. venezuelensis)
  - the subgenus *Viannia*
    - (L. (V.) braziliensis, L. (V.) guyanensis, L. (V.) panamensis, & L. (V.) peruviana)

- different species: morphologically indistinguishable
- they can be differentiated by molecular methods or monoclonal antibodies
Epidemiology

- widely distributed around the world
- tropical zones of America, Africa, South America, southern Europe & Asia
- geographical distribution depends on sand fly
- clime changes-increase in temperature-expand the distribution of vectors/leishmaniasis

- In 2009: sandflies in Austria


- 2 human cases of leishmaniasis with assumed Autochthonous infection in Austria are documented


<table>
<thead>
<tr>
<th>Type</th>
<th>Pathogen</th>
<th>Location</th>
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<tr>
<td><strong>Visceral leishmaniasis</strong></td>
<td><em>L. donovani</em> complex, <em>L. donovani</em>, <em>L. infantum</em> = <em>L. chagasi</em></td>
<td>Bangladesh, Brazil, India, Nepal, Sudan, &amp; China</td>
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| **Cutaneous leishmaniasis**   | Old World: *L. major*, *L. tropica*, & *L. aethiopica*  
New World: *L. mexicana* | Afghanistan, Brazil, Iran, Peru, Saudi Arabia, & North Africa |
| **Mucocutaneous leishmaniasis** | *L. braziliensis* | Bolivia, Brazil & Peru |
Geographic distribution

B González-Llavona Actas Dermosifiliogr. 2007
Major risk factors

• affects the poorest people on the planet
• is associated with malnutrition, population displacement, poor housing, a weak immune system, & lack of resources
• is linked to environmental changes such as deforestation, building of dams, irrigation schemes, & urbanization
Poverty as a potentiator of leishmaniasis morbidity and mortality

Alvar et al 2006
http://www.who.int/leishmaniasis/en/
The Reservoir

- dog, fox, wolf, opossum, small forest rodents: hydrax
- In Europe, the most important reservoir host: dog
- in Mediterranean areas ~50% of the stray dogs are infected
- many of these animals have been brought to Central Europe by tourists
- tourists increasingly take their own dogs abroad
- ~20 000 dogs infected with *Leishmania* spp. in Germany
- In Austria, up to 50% of diseased imported dogs are infected with *Leishmania* spp.
The Vector

- 2-3 mm long blood-sucking sandflies
- *Phlebotomus* spp. in the Old World
  - Europe, Asia, and Africa
- *Lutzomyia* spp. in the New World
  - America
- Specific – can support the grow of only one species
- Permissive – support the grow of more than one species
- Specificity caused by the need for appropriate binding sites on the sand fly gut cells for the specific ligand expressed by promastigotes
The Vector

- ~500 known phlebotomine species
- ~30 of them have been positively identified as vectors of the disease
- ♀ sandfly lays its eggs in the holes of certain rodents, in the bark of old trees, in ruined buildings, animal shelters, household rubbish - the larvae find the organic matter, heat & humidity
- dusk till down: search for blood - covers a radius of ~100 m around its habitat
Transformation to amastigotes

Ingestion by macrophage

*L. donovani* carried to deeper organs, causing visceral leishmaniasis

Some parasites in peripheral blood

*L. tropica* remains in skin, causing cutaneous leishmaniasis

Skin

Phlebotomus lutzomyia

Infection through bite

5 days

Amastigote

Promastigote infective after fly feeds

Transformation to promastigote in gut

Human

Transformation to amastigotes
Intracellular mechanism of infection

• The strategy: to avoid destruction by the immune system: 'hiding' inside phagocytic cell

• avoid the action of the humoral immune response

• Neutrophils:
  – are short-lived
  – intermediate host cells
  – “Trojan horses”: to silently enter macrophages - avoiding cell activation

• Macrophages:
  – long-lived
  – the final host cells for proliferation
Cutting Edge: Neutrophil Granulocyte Serves as a Vector for *Leishmania* Entry into Macrophages

Ger van Zandbergen, Matthias Klinger, Antje Mueller, Sonja Dannenberg, Andreas Gebert, Werner Solbach, and Tamás Laskay

42h after infection apoptotic neutrophil granulocytes (NG) contain viable *L. major* promastigotes

Transmission electron micrograph of parasites inside a parasitophorous vacuole
Phagocytosis of infected neutrophil granulocytes results in survival & multiplication of the parasite
Visceral Leishmaniasis (VL)

- Kala-Azar (Black Fever)
- most severe form of leishmaniasis
- second-largest parasitic killer in the world (after malaria)
- untreated - fatal
- ~200 000 - 400 000 new cases/year
- Over 90% of new cases in: Brazil, Ethiopia, Sudan, South Sudan, Bangladesh, and India
Highly endemic in Brazil, Sudan; South Sudan, Ethiopia, Iraq, India; Bangladesh
Visceral Leishmaniasis (VL)

- Infectious agent:
  - *L. donovani* & *L. infantum/L. chagasi*
- transmission: the bite of infected sand fly (through blood transfusions and needle sharing)
- reservoir: dogs
- incubation period: 10 days - 1 year
- migrates to liver, spleen, lymph nodes, & bone marrow
- fever, weight loss, enlargement of the spleen and liver, low red blood cell count (anemia), low white blood cell count (leukopenia), & low platelet count (thrombocytopenia), darkening of the skin (India)
- Untreated results in the death of the host
Disease

Clinical picture of kala-azar in Kenya
- Increased enlargement of the spleen and liver
- Deepening skin pigmentation—kala-azar “black sickness

Infantile kala-azar
Chronic, irregular fever, anaemia, moderately enlarged liver, greatly enlarged, firm spleen
If untreated, the infection is often fatal, commonly from secondary infections
VL & HIV co-infection

- opportunistic infection associated with HIV
- both pathogens infect macrophage
- mutually reinforcing:
  - HIV-infected people are vulnerable to VL (↑ risk 100 - 2320 X)
  - VL accelerates HIV replication and progression to AIDS

- In southern Europe ~70% of cases of VL in adults are associated with HIV infection

- co-infected patients
  - can serve as human reservoirs
  - numerous parasites in their blood - a source of infection for the insect vector
  - may have atypical manifestations: involvement of the gastrointestinal tract

- Diagnosis:
  - Indirect methods (serological tests) for VL frequently fail
  - direct methods (aspirations BM; LN or splenic) are reliable but are invasive, require skilled microscopy
VL Diagnosis

• the clinical presentation lacks specificity

• Differential Diagnosis:
  - typhoid fever, military tuberculosis, brucellosis,
  - malaria, tropical splenomegaly syndrome,
  - schistosomiasis, leukemia and lymphoma

Good test:
• highly sensitive
• highly specific
• distinguish acute disease vs asymptomatic infection
• simple
• affordable
Puncture of bone marrow

Splenic puncture
Formol-gel test

Large quantities of IgG are produced by kala-azar patients, increased levels of globulin in the sample leads to formation of gel at RT/20min
Diagnosis VL: Conventional parasite detection techniques

- demonstration of amastigotes in tissues by light microscopic examination of the stained specimen (Giemsa’s stain)
- *in vitro* culture: each species will grow only in certain media
- diagnostic sensitivity is highest for splenic aspirates; life-threatening hemorrhage
- bone marrow aspiration is much safer
- other sources of specimens: liver, lymph node or blood
Diagnosis VL: rK39

immunochromatographic test strip

- ready-to-use
- immunochromatographic strip test
- based on rK39 antigen
- a rapid test for use in difficult field conditions
- inexpensive (∼1 to 1.5 $)
- easy to perform
- no additional equipment needed
- remains positive long after treatment (up to 3 years)
Diagnosis VL : DAT (Direct Agglutination Test)

- highly specific and sensitive
- inexpensive
- very practical diagnostic tools under rural conditions
- does not require sophisticated equipment, a cold chain
- very simple to perform
- used on urine samples: noninvasive
Diagnosis VL: Immunological methods

– **ELISA**
  - Screening of large number of samples, rapid, greatly influenced by the antigen used

– **Indirect Fluorescent Antibody**
  - Used for detection of antibodies using fixed promastigotes
  - Based on antibodies in early stage of infection
  - Undetectable 6-9 months after cure

**Drawbacks**
- Not all infected (=) positive develop clinical disease or require treatment
- Up to 32% of the healthy population may test positive
- Serological tests look for an immune response and not for the organism itself,
- The test does not become negative after the patient is cured,
- It cannot be used as a check for cure, or to check for re-infection or relapse
- Patients with abnormal immune systems (e.g., HIV+) - false-negative tests
Diagnosis VL: Molecular methods

- detection of parasite DNA in tissue samples
- the serum PCR showed a sensitivity of 97% and a specificity of 95%
Treatment I

- **Pentavalent antimonials sodium stibogluconate:** first-line treatment for over 70 years (cheaper generic forms available)
- **Antimonials:** toxic drug with frequent, life-threatening, adverse side effects (cardiac arrhythmia and acute pancreatitis), failure rates >60%
- **Amphotericin B:** replaced antimonials, side effects: fever, chills, rigor, anaphylaxis, costly, complicated regimen
Treatment II

- **Liposomal amphoterin B**: first-line treatment in Europe and US, WHO reduced the price from $2,800 to $200
- **Miltefosine**: originally anticancer drug, oral, teratogenic: forbidden in pregnant women; parasite resistance is easily induced *in vitro*
- **Paramomycin**: antibiotic, excellent efficacy and safety; active against wide variety of pathogens, low costs ($5 per treatment)
- **Combination therapy**: to increase treatment efficacy, prevent the development of drug resistance, reduce treatment duration, and decrease treatment cost
Prevention

• there are no vaccines or preventive drugs
• the most effective: **Don’t not get bitten!!!!**
• avoid outdoor activities, especially from dusk to dawn, when sand flies generally are the most active
• minimize the amount of exposed skin
• wear long-sleeved shirts, long pants & socks
• apply insect repellent to exposed skin
• the most effective repellents contain DEET (N,N-diethyl-meta-toluamide)

Indoors:
- Stay in well-screened or air-conditioned areas
- Keep in mind that sand flies are much smaller than mosquitoes
- Spray living/sleeping areas with an insecticide to kill insects
- Use a bed net soaked in or sprayed with a insecticide
Prevention : Vaccine candidates I

Leishmanisation

- inoculation with live, virulent parasites- practiced successfully in the former Soviet Union, Middle East and Israel - abandoned in most countries because of logistical problems and safety concerns, due to some individuals developing non-healing lesions & immune suppression

Whole-killed (autoclaved) promastigotes

- in Brazil in the early 1940s (alone or in combination with adjuvant BCG)
- could reduce the incidence of disease by 18–78%
- showed decreasing potency with time
- concerns remain:
  - variation
  - difficulties in producing such a product to good clinical manufacturing standards
Prevention : Vaccine candidates II

DNA vaccines
• low costs of production
• stability of materials
• efficient generation of effector & memory immune responses
• more than one antigen can be produced by a single construct

Recombinant proteins
• clinical trial launched in 2012 - a recombinant form of two fused Leishmania parasite proteins with an adjuvant
Cutaneous leishmaniasis (CL)

- Baghdad Ulcer, Delhi Boil
- is the most common form of leishmaniasis
- Incubation period: a 1-12 weeks to a couple of months depending on the species
- Old World: *L. tropica*, *L. major*, and *L. aethiopica*, as well as *L. infantum* and *L. donovani*
- New World: *L. mexicana* complex *L. Viania braziliensis* complex
- Reservoir: dogs, rodents, hyraxes
Animal reservoir

Great gerbil

Fat-tailed sand rat
• About 95% of CL cases occur in the America, the Mediterranean basin, and the Middle East and Central Asia
• >2/3 of new cases in six countries: Afghanistan, Algeria, Brazil, Colombia, Iran, & Syria
Cutaneous leishmaniasis (CL)

- the parasite is restricted to the skin
- a painless papule or pimple develops at the site of the insect bite
- the papule grows & turns into an ulcer with an adherent crust of dried exudate
- Infection of the lymphatic system- lesions along the lymphatic channels
- secondary bacterial infection is common
- most lesions heal spontaneously
- life-long scars, with a de-pigmented center & a pigmented border with skin thinning
Treatment of cutaneous leishmaniasis

before (A), 3 weeks (B), and 12 months (C) after completion of treatment with liposomal amphotericin B
• a 25-year-old man with a 1-year history of skin lesions on his nose and arm
• painless papules & nodules with overlying crust
• from Iran: area endemic for cutaneous leishmaniasis
• a biopsy of the nodule revealed intracellular amastigotes
• tissue culture together & PCR confirmed infection with *L. tropica*
• successful treatment with sodium stibogluconate
• skin lesions resolved with minimal scarring
Leishmanin (Montenegro) test

- Intradermal injection of an antigen from cultured promastigotes
- Typical cell-mediated delayed hypersensitivity response in most cases of active cutaneous disease
Mucosal leishmaniasis

- Espundia
- *L. brasiliensis, L. guyanensis, L. panamensis, L. amazonensis*
- *Reservoir*: forest mammals
- less common forms of leishmaniasis
- 90% cases in the Bolivia, Brazil and Peru
- cause extensive damage and disfiguration
Mucocutaneous leishmaniasis

- leads to destruction of mucous membranes: nose, mouth and throat
- clinically evident within several years of the original cutaneous lesions
- parasite spread from the skin
- the initial manifestations: nasal symptoms: stuffiness or bleeding
- prevention: adequate treatment of the original cutaneous (skin) infection
- untreated disease progress perforation of the nasal septum

**Pharyngeal involvement**
Ulceration often extends to the pharynx & soft palate, and the first symptoms may be related to tissue destruction in this area here: destructive lesion of the hard palate
• Brazil
• Progress arrested by intensive chemotherapy
• Need for reconstructive surgery
Seroprevalence and asymptomatic carriage of *Leishmania* spp. in Austria, a non-endemic European country

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- explorative cross-sectional serological study
- ELISA
- 1048 healthy Austrians
- 47 individuals (4.5%) tested positive
- after 12 months 18 were persistently positive
- screened using a commercial PCR test to detect parasite DNA
- 4 were PCR positive (Leishmania donovani/infantum complex & Leishmania (Viannia) guyanensis)
- significant risk of exposure to *Leishmania* spp. was found for travel to the New World (Caribbean)

**Conclusion**

*Leishmania* spp. seroprevalence in non-endemic countries has been considerably underestimated
Thank you for your attention