Epidemiology of leishmaniases in Portugal

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Introduction

- **Zoonotic visceral leishmaniasis** caused by *Leishmania infantum*
- Endemic South America, Asia, Mediterranean basin and Portugal
- Dogs are major host and main reservoir for human infections
- Pathogen transmission occurs via the infective bite of **phlebotomine sand flies** (Diptera, Psychodidae)
In Portugal…

✓ Three main endemic regions:
  • Alto Douro
  • Metropolitan area of Lisbon (Lisbon and Setúbal)
  • Algarve
✓ Human and canine cases throughout the country
In Portugal...

- Compulsory notification for visceral leishmaniasis (VL) since the 50’s
- VL traditionally more prevalent in infantile age group than in adults
- Since the 90’s most of the cases in immunocompromised adults (HIV+)

VL cases diagnosed in IHMT

1982 to 1992 (n=138)

- 46 Paediatric cases
- 80 HIV-
- 12 HIV+

1993 to 2000 (n=137)

- 81 Paediatric cases
- 22 HIV-
- 10 HIV+
- 24 HIV+ (relapses)
Zymodemes ...

✓ Five *L. infantum* zymodemes

✓ 95% of isolates *L. infantum* MON-1

✓ *L. infantum/L. major* hybrids identified in Portuguese autochthonous human cases

⇒ Enhanced transmission potential by *L. infantum* and *L. major* competent vectors

Rapid communication

*Increased transmission potential of Leishmania major/Leishmania infantum* hybrids

Petr Volf a,*, Ivana Benkova a, Jitka Myskova a, Jovana Sadlova a, Lenea Campino b, Christophe Ravel c
In Portugal...

Five phlebotomine sand fly species:

- *Phlebotomus perniciosus*
- *P. ariasi*
- *P. sergenti*
- *P. papatasi*
- *Sergentomyia minuta*
VL cases diagnosed in hospitals from Continental Portugal (1999-2009)

N = 375

✓ Incidence: 0.294/100000 inhabitants
✓ 30.4% (0-10 years old)
✓ 48.8% HIV+/AIDS

Notified cases by Health authorities = 145 ⇒ 38% under-reporting!

(Serrada, 2010)
VL cases diagnosed in IHMT

2001 to 2014 (n=199)

HIV+ = 119

Paedriatic cases
HIV-
HIV+
HIV+ (relapses)
Cutaneous Leishmaniasis cases diagnosed in IHMT

2001 to 2014 (n=23)

Children: 12
Adults: 11
Portuguese National Leishmaniasis Observatory - ONLeish

Main objective

• To implement epidemiological surveillance on canine leishmaniosis (CanL)
“Leishmaniasis Week”  
(A Semana da Leishmaniose)

- First national free CanL survey made in Europe
- N=3974
- Continental Portugal (January 2009)

- Direct Agglutination Test
- Cut-off: ≥ 1:400
Overall seroprevalence 6.3%
⇒ Endemic country for CanL
**Leishmania** infection in cats – North and Centre of Portugal

- 9/316 (2.8%) antibodies to *L. infantum* (domestic cats)
- 1/320 (0.7%) domestic cats detection of *L. infantum* DNA
Leishmania infection in cats – South of Portugal

Importance of Cats in Zoonotic Leishmaniasis in Portugal

CARLA MAIA, MÓNICA NUNES, AND LENA CAMPINO

7/23 (30.4%) stray cats
detection of L. infantum DNA

28/138 (20.3%) domestic cats
detection of L. infantum DNA

64/649 (9.9%) cats (320 domestic and 329 stray)
L. infantum DNA
Leishmania infection in cats – South of Portugal

1/180 (0.6%) domestic cats antibodies to L. infantum

10/271 (3.7%) cats (86 domestic and 185 stray) antibodies to L. infantum
Leishmania infection in other vertebrate hosts

7/173 (4.0%) horses antibodies to *L. infantum*

✓ Seropositive
✓ *L. infantum* DNA (Skin)
Leishmania infection in other vertebrate hosts

Mus musculus
- 9/27 (33.3%) Leishmania DNA
- 8/27 (29.6%) positive histopathology (liver and/or spleen)

Rattus norvegicus
- 1/3 positive histopathology

1/78 (1.3%) foxes L. infantum DNA (peripheral blood + bone marrow)
Phlebotomine sand fly surveys

- Afonso et al. 2007
- Branco et al. 2011
- Afonso et al. 2005; Cortes et al. 2007; Maia et al. 2011-2012
- Pires et al. 2007
- Maia et al. 2010

Algarve (2006-2013)

- Touristic region
- Human immigration from Magreb
Algarve region- sand fly surveys

Objectives

✓ Detection of *Leishmania* DNA

✓ Identification of blood meal sources in wild caught female sand flies

Biotopos

✓ Between *May* and *October*

✓ CDC light traps

✓ Two-three consecutive days/month

✓ Domestic, peridomestic and sylvatic environments
**Hosts**: Humans, dogs, livestock, horses, pigs, rabbits and/or poultry
Leishmania infection (2006)

✓ One *P. perniciosus* female found infected with *L. infantum*
✓ County: Silves
✓ Date: July
✓ Sand fly overall infection rate: 0.47% (1/213)

![Map of Algarve Region, Portugal with emphasis on Silves]

Molecular detection of *Leishmania infantum* in naturally infected *Phlebotomus perniciosus* from Algarve Region, Portugal

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\textsuperscript{a} C. Maia
**Leishmania** infection (2007) & Blood meal identification

- One *P. perniciosus* female was found infected with *L. infantum*
- County: Olhão
- Date: September
- *P. perniciosus* infection rate: 0.13% (1/773)

**Blood meal identification**

<table>
<thead>
<tr>
<th>Species</th>
<th>Fed females</th>
<th>Blood meal identification</th>
<th>Blood meal source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. perniciosus</em></td>
<td>52</td>
<td>30</td>
<td>chicken</td>
</tr>
<tr>
<td><em>P. ariasi</em></td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><em>P. sergenti</em></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>S. minuta</em></td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>59</strong></td>
<td><strong>36</strong></td>
<td>6</td>
</tr>
</tbody>
</table>
Leishmania infection (2007)

- One *S. minuta* female was found infected with *L. major*
- County: Albufeira
- Date: July
- *S. minuta* infection rate: 1.21% (1/82)
Leishmania infection (2010)

✓ 2008-2009: No *Leishmania* DNA was detected

✓ 2010:

➢ Two *P. perniciosus* females were found infected with *L. infantum*

➢ Date: June

➢ Sand fly overall infection rate: 0.47% (2/426)
Leishmania DNA detection (2011-2013)

✓ *Leishmania* DNA was detected in three unfed females (0.13%)

   *P. perniciosus* (n = 1) and *S. minuta* (n = 2)

✓ Positive females were collected in peridomestic biotopes:

   - *P. perniciosus* (September 2011)
   - *S. minuta* (August 2012 and October 2013)
Leishmania DNA – P. perniciosus

✓ BLASTn ITS-1 sequence from *P. perniciosus* revealed >99% identity with *L. infantum*, *L. chagasi* or *L. donovani* sequences deposited at GenBank

✓ Phylogenetic analyses ⇒ segregation in a monophyletic cluster that included *Leishmania* species from *L. donovani* complex
Leishmania DNA – S. minuta

✓ BLASTn revealed ITS-1 sequences from both S. minuta revealed >93% identity with Leishmania sequences of Chinese origin referred to as Leishmania sp.

✓ Phylogenetic analyses ⇒ segregation in an assemblage of multiple Leishmania sp. sequences from human and canine origin from China (Yang et al. 2010)
Vertebrate DNA detection in fed females

✓ **Identification** vertebrate host source of blood meal of 30 out of 78 engorged females

<table>
<thead>
<tr>
<th>Sand fly host</th>
<th>P. ariasi</th>
<th>P. perniciosus</th>
<th>S. minuta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse (<em>Equus caballus</em>)</td>
<td>0</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Chicken (<em>Gallus gallus</em>)</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Human (<em>Homo sapiens</em>)</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Rabbit (<em>Oryctolagus cuniculus</em>)</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Pig (<em>Sus scrofa</em>)</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Cattle (<em>Bos taurus</em>)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Sheep (<em>Ovis aries</em>)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Lizard (<em>Tarentola mauritanica</em>)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1</strong></td>
<td><strong>24</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>
Conclusions

✓ Portugal continues an endemic country for leishmaniasis

✓ High prevalence of canine and feline infections → increase and spread of *L. infantum*

✓ Number of *Leishmania/HIV* co-infection cases has not diminished as expected after HAART introduction

✓ *L. infantum/L. major* hybrids ⇒ Epidemiological and medical implications?
Conclusions

✓ No dog or human blood was detected in engorged *P. perniciosus/P. ariasi*

   - presence of other larger sized vertebrates (e.g. horses)
   - presence of other vertebrates in greater numbers (i.e. chicken, rabbits, rodents)

✓ Detection of *L. infantum* in *P. ariasi* and *P. perniciosus* → reinforces the maintenance of both species as vectors in Portugal
Conclusions

- The **apparent anthropophilic behaviour** of *S. minuta*
- Detection of *L. major* and *Leishmania sp.* DNA phylogenetically related to those responsible for **human** and **canine leishmaniosis** in China
  → need to determine the **role played by this sand fly species** in the transmission of **pathogenic Leishmania** to **humans** and other vertebrates
Conclusions

✓ **Official notification** of **visceral** and **cutaneous** human clinical cases

✓ **On-going surveillance** on *Leishmania* **vectors** and **reservoirs crucial** ⇒ increased migration and travelling flow ⇒ ↑ risk of **introduction** and **spread** of infections by *Leishmania species* which are **non-endemic**

Acknowledgments

✓ The inhabitants that allowed the placement of the CDC and sticky traps in their properties

✓ Veterinarians, dogs and cats ownes

Thank you very much for your attention!

C. Maia