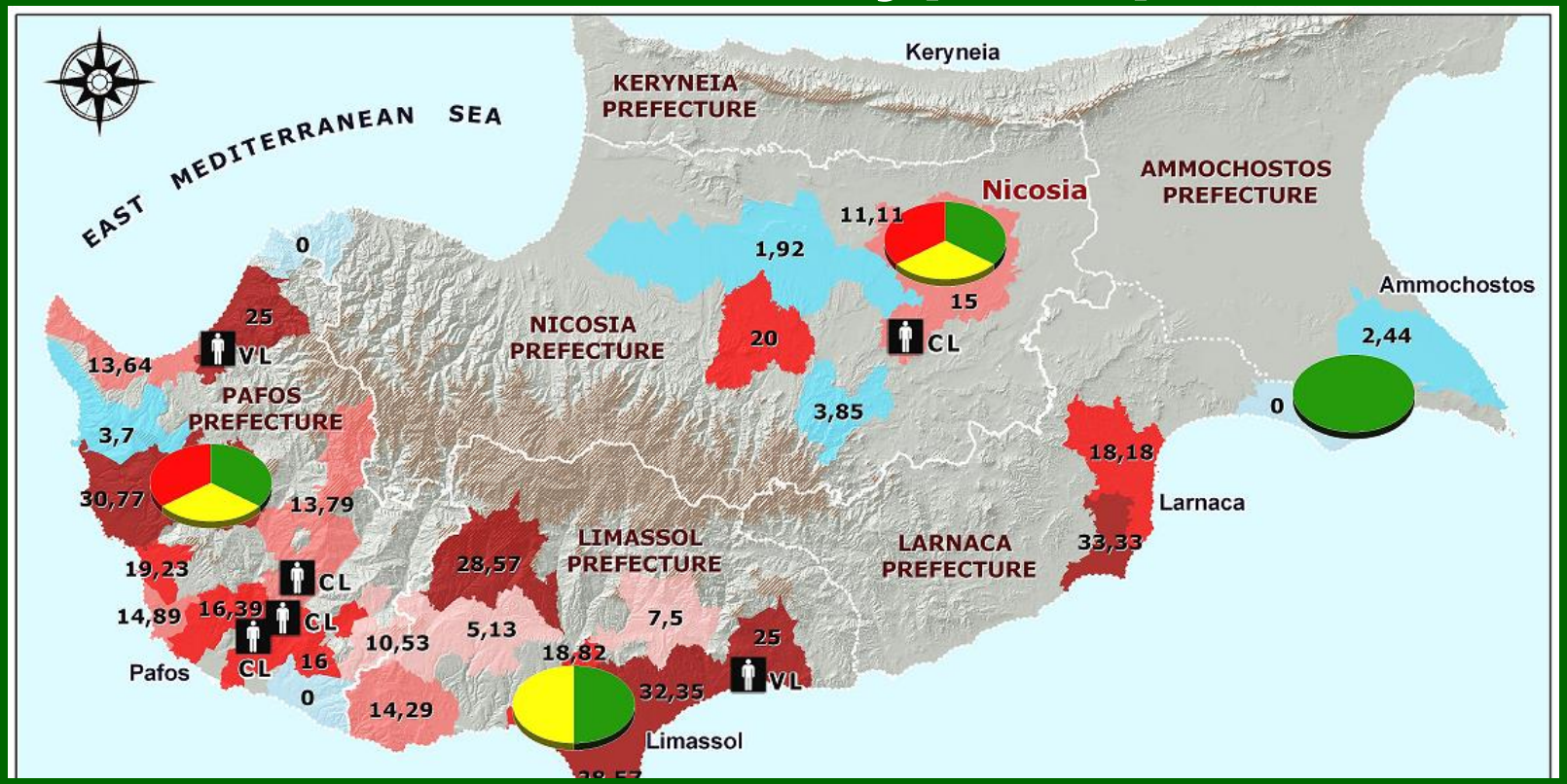
Sand flies and the Cyprus paradox



Maria Antoniou
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University of Crete Medical School,
Heraklion, Crete, Greece

Akrotiri, Cyprus, April 2018

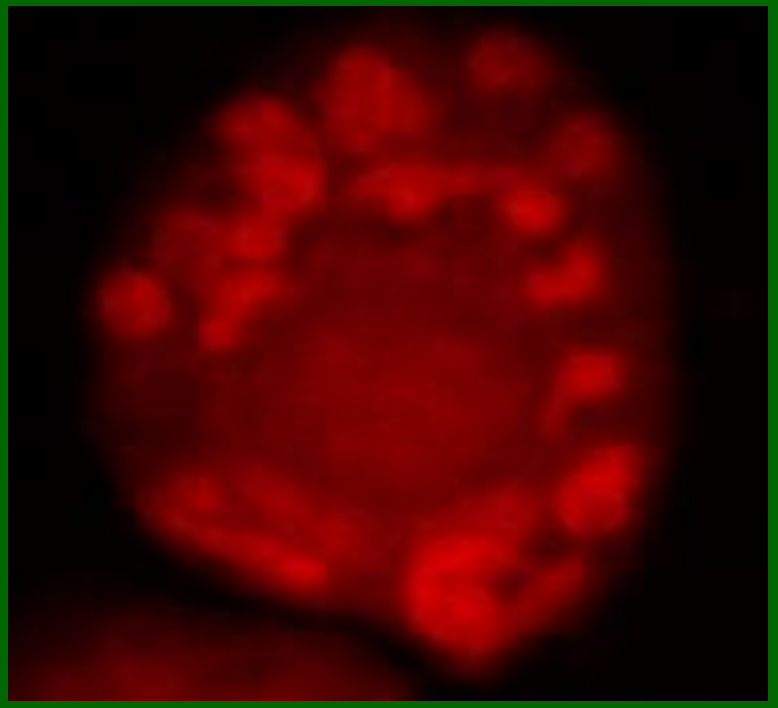
Points to discuss

- A short Introduction about the Parasite and the Disease
- The sand fly vectors
- Leishmaniasis in Cyprus: past and present
- *Leishmania infantum* cases
- *L. donovani* cases and *L. donovani* in Cyprus
- What factors may explain the 2 parallel epidemiological cycles?
- Drug resistant isolates in Cyprus
- New introductions and epidemiological “games”
- The Northern Cyprus situation?

Leishmania

Promastigote stage

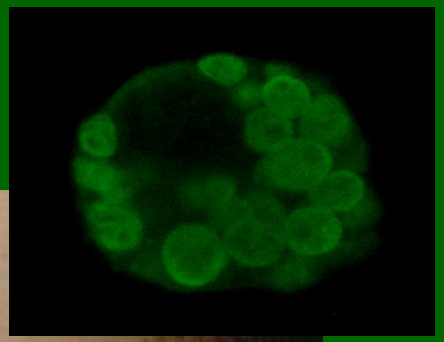
(in insect gut)



Amastigote stage

(in macrophages)

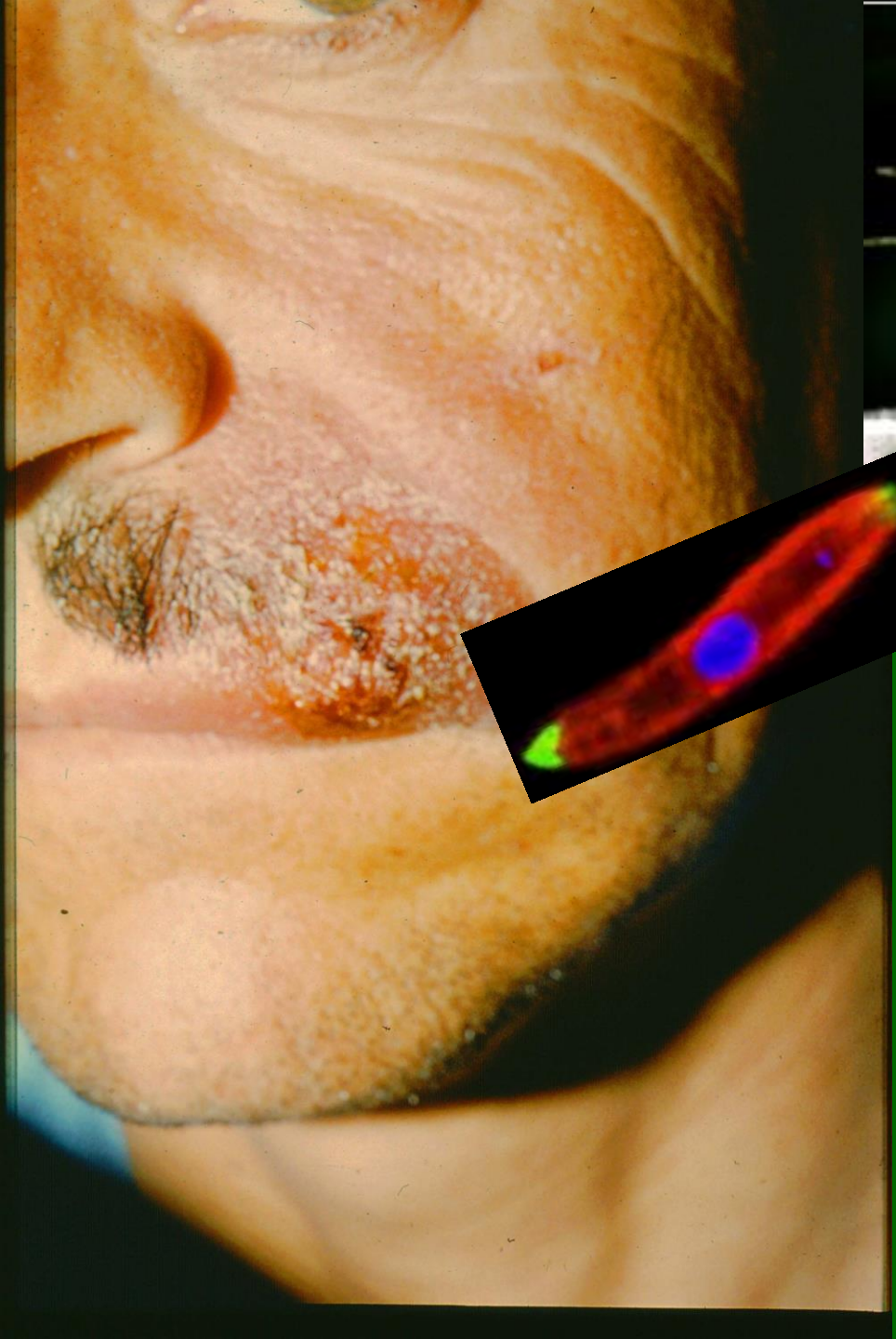
The leishmaniases



Visceral leishmaniasis by *L. infantum*

Zoonosis

Sandflies active
in Mediterranean basin
May-October

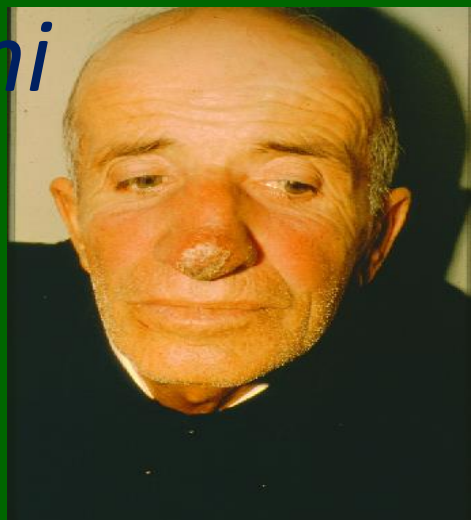


Cutaneous leishmaniasis

L. donovani

L. tropica

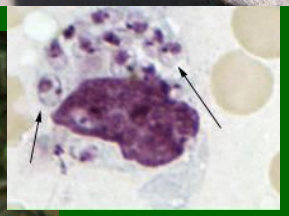
anthroponosis

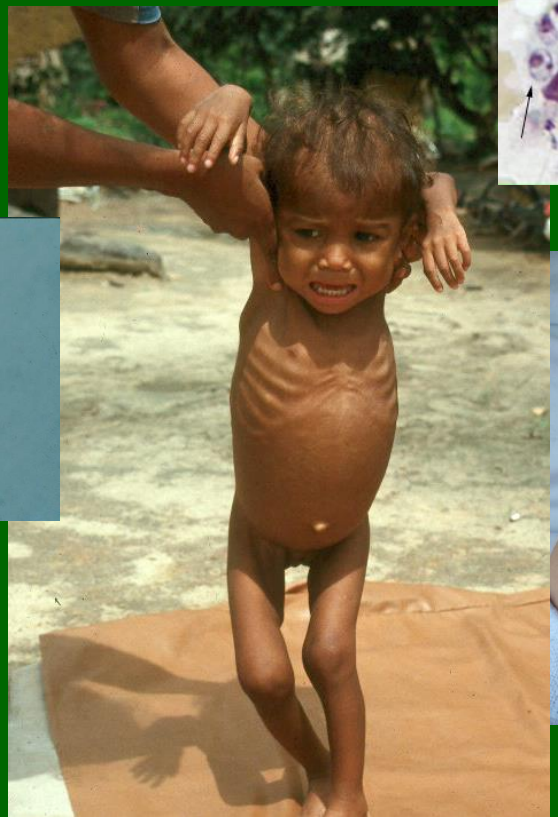


CanL and VL caused by *L. infantum* coexist

Leishmaniasis in Cyprus

L. infantum





L. donovani

The variety and distribution of sandfly vectors of *L. infantum* in Europe.

Paul Ready Natural History Museum London UK

P.ariasi *P.perniciosus* *P.neglectus,* *P.tobbi* *P.major* *P.kandelakii* *P. perfiliewi*

Also vectors of the bacterium Bartonella bacilliformis and arthropodborne viruses (phleboviruses and vesiculoviruses) causing human diseases.

Sicilian virus

Geographic distribution of neutralising antibodies against Toscana virus (panel A), Sand fly fever Sicilian virus (panel B), Arbia virus (panel C), and Adana virus (panel D) in Cyprus. **Alwassouf et al., 2016**

Leishmania vectors in the Mediterranean Basin

L. infantum (VL)

Phlebotomus ariasi
Phlebotomus kandelakii
Phlebotomus langeroni
Phlebotomus neglectus
Phlebotomus perfiliewi
Phlebotomus perniciosus
Phlebotomus tobbi
Phlebotomus balcanicus

L. donovani (VL+CL)

Phlebotomus tobbi (Cyprus) – Suspected

L. tropica (CL)

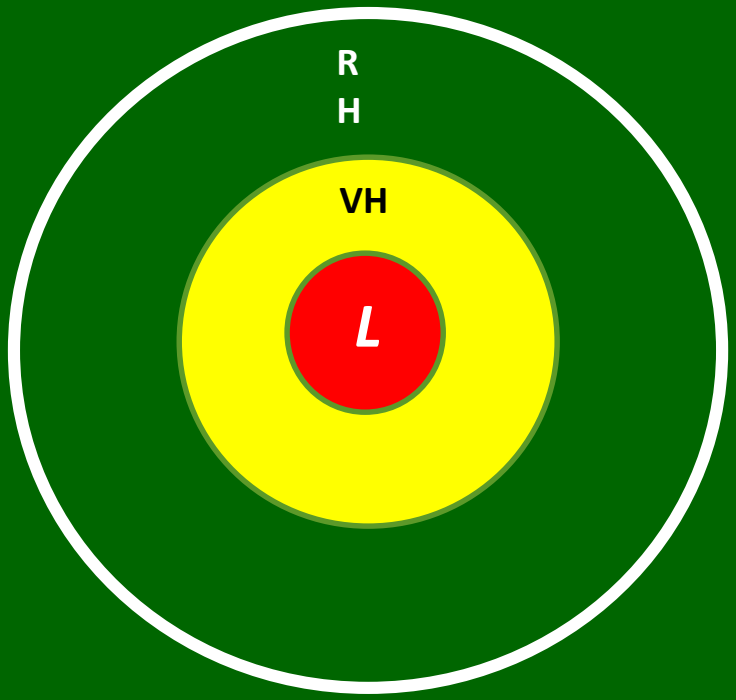
Phlebotomus sergenti
Phlebotomus similis (Greece) - Suspected
P. arabicus

L. major (CL)

Phlebotomus papatasi

Parasite + Vector co-evolution:
- Killick-Kendrick, 1985
- Ready & Pesson, 1999

WHO 2010, Gradoni 2017



RH: *Reservoir host*
VH: *Vector host*
L: *Leishmania*

Killick-Kendrick, 1985
Ready & Pesson 1999


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Image © 2006 NASA
Image © 2006 TerraMetrics

The emerging and re-emerging of *leishmaniases* in Cyprus

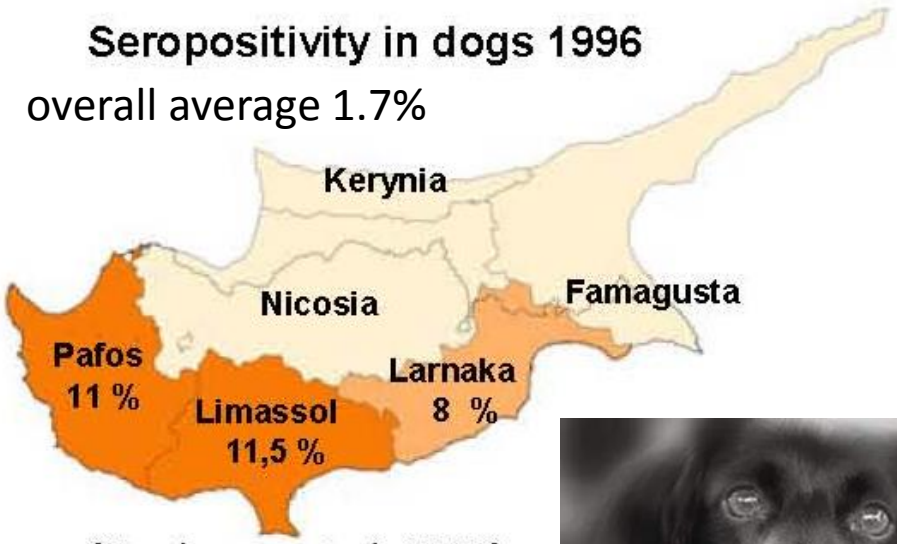
- In Cyprus, leishmaniasis was prevalent before 1945.
- Was nearly eradicated by 1996 as a consequence of the destruction of reservoir hosts and vectors.
  - The **malaria-eradication** campaign, 1940–1950
  - The **anti-echinococcosis** campaign, 1970–1975

    (dog numbers fell from 46,000 to 6,000)

  Stayed clear of the disease for over 20 years.

  After which, sand-fly populations increased and the number of dogs recovered to an estimated 100,000.

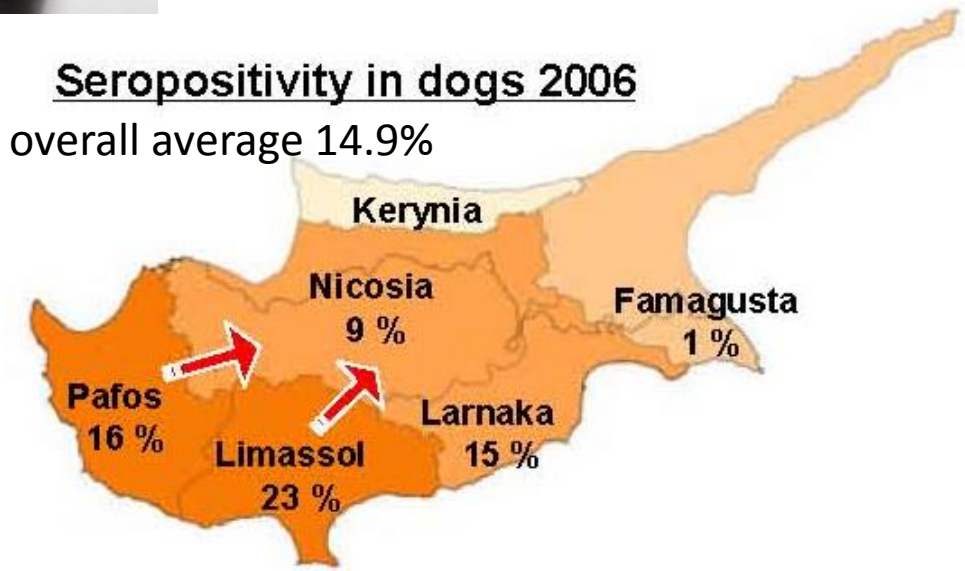
(Mazeris et al., 2010)



- **Overall, a nine-fold increase in dog seroprevalence** from 1.7% to 14.9%
- **Dog cases spread in all areas**
  - *L. infantum* responsible
- Only two human cases (infantile) described since 1935 **due to ??**
- The assumption: No anthropophilic vectors on the island.

Phlebotomus tobbi

Leishmaniasis in humans and dogs is notifiable



Leishmaniasis in Cyprus

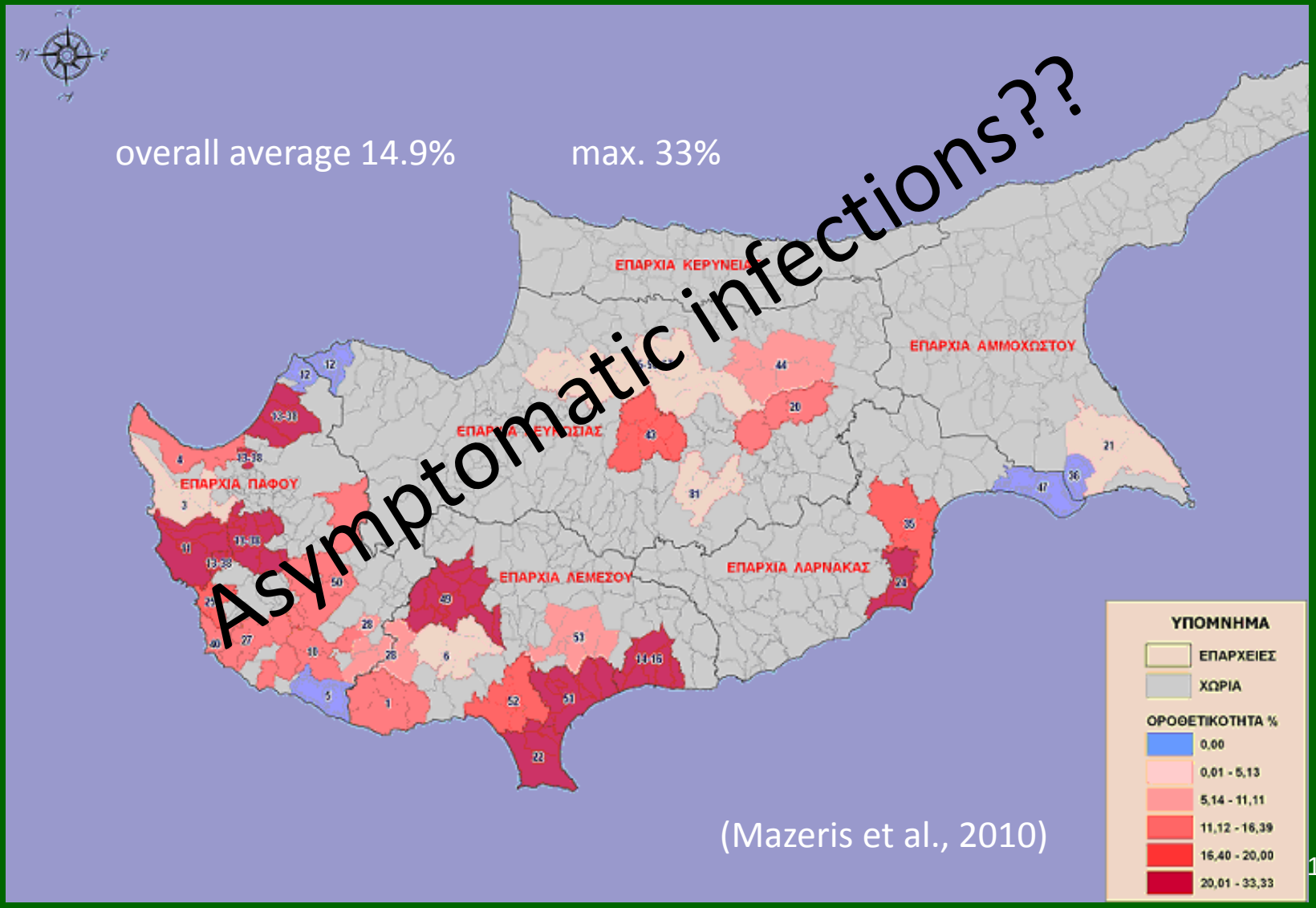
"not of recent origin since no dogs were imported from endemic centers for at least 30 years" (Adler 1945)

The situation in Cyprus is **Unusual**

since typically, the greater the number of infected dogs in an area, the greater the possibility of human infection

Dog seropositivity in 30 randomly selected areas

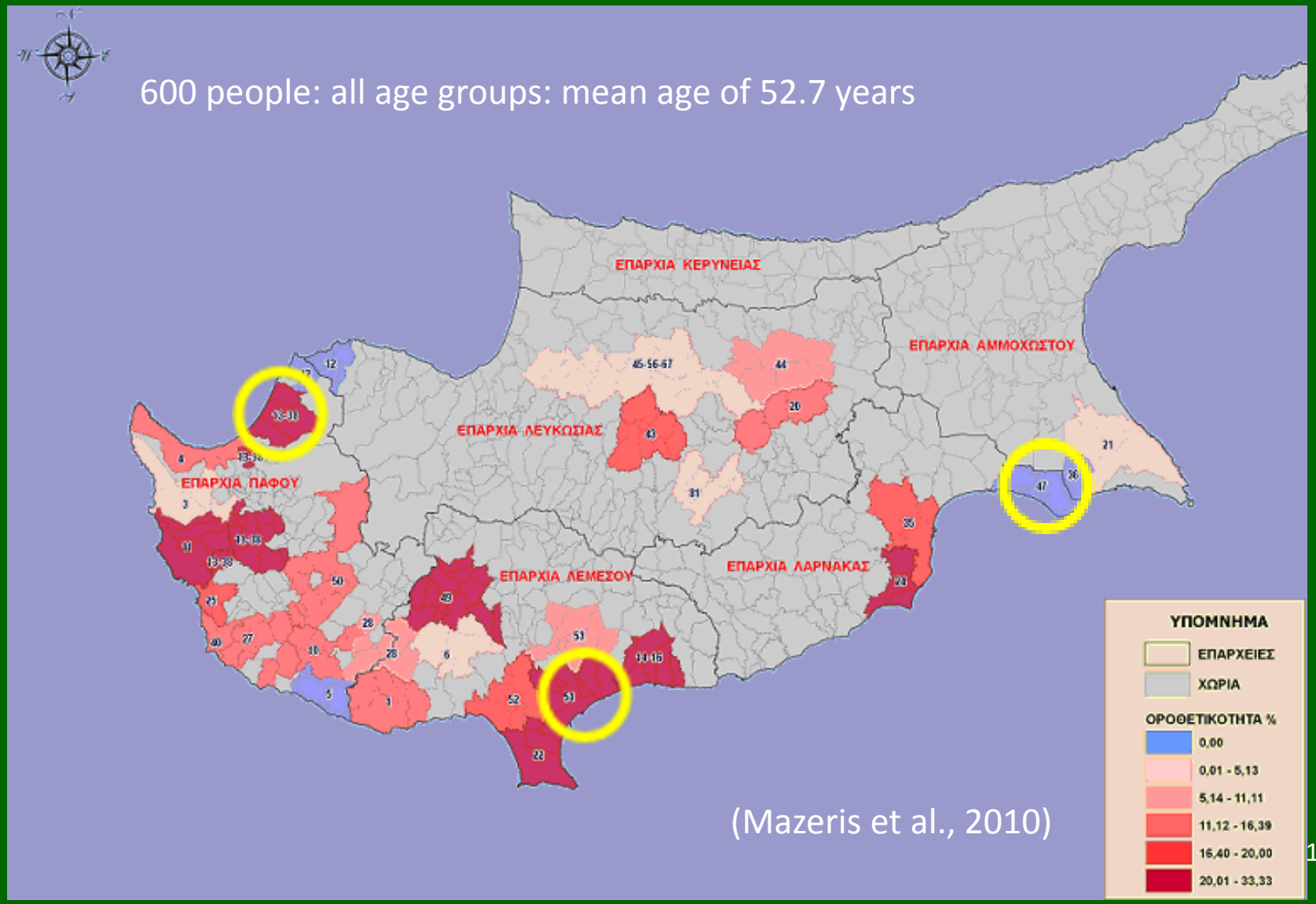
All cases *L. infantum* (2005)



(Mazeris et al., 2010)

Dog seropositivity in 30 randomly selected areas

1st Human seroepidemiological study in 2006



(Mazeris et al., 2010)

Cyprus

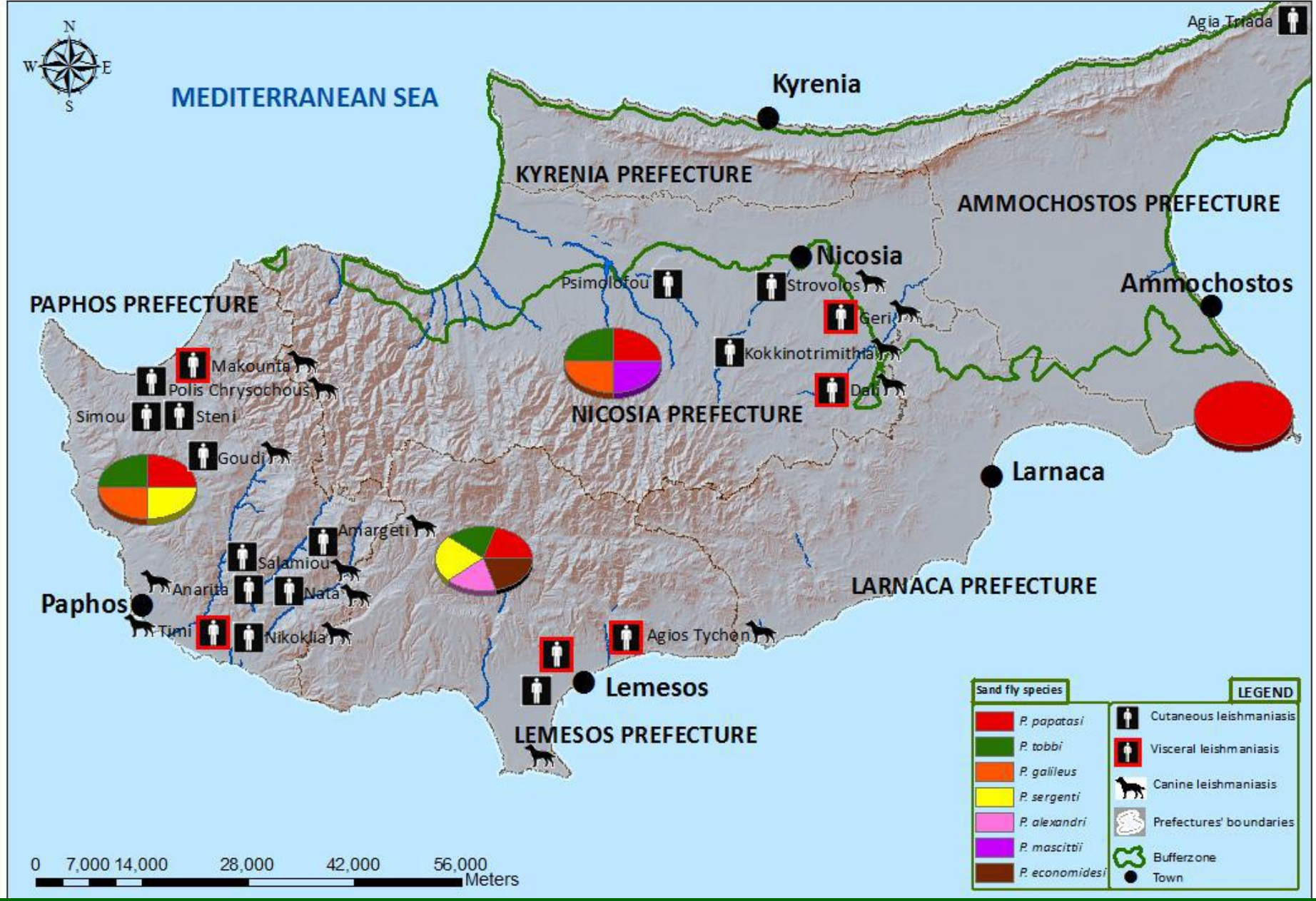
- NO seropositive human cases
- Does *L. infantum* not infect humans in Cyprus?
- Is there a different situation in the island?

Human leishmaniasis in Cyprus

In **2006**, the first human cases were found; both cutaneous (CL) and visceral (VL), with new sporadic cases occurring annually.

- All human cases, due to the anthroponotic ***L. donovani***
- One dog/20 carrying both species.
- Therefore, indigenous competent vectors are present on the island.

(Antoniou et al., 2008; Mazeris et al., 2010)



P. tobbi **(proven vector of *L. infantum* and *L. donovani* in countries in the Middle East: in Cyprus?),**
P. galilaeus **and *P. papatasi* were the predominant species captured**

Reported up to date:
26 human leishamniasis cases

22 *L. donovani*
CL: 13/20 G.C.
VL: 1/6 G.C.

4. *L. tropica*

The human isolates

Typing of the isolates by:

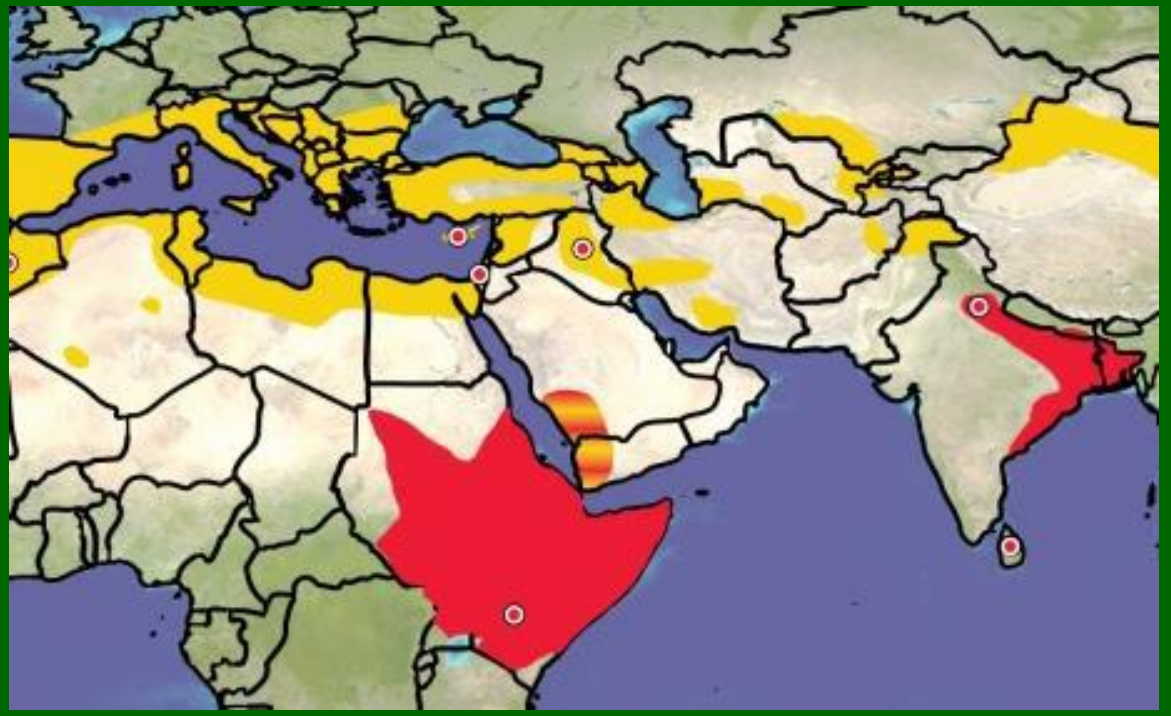
1. ***K26*-PCR** assay (discriminates species/subspecies of the *L donovani* complex, based on the amplicon size. (Haralambous et al., 2008)
2. Multilocus enzyme classified it as **MON-37 (zymodeme profile)**

A dog was co-infected with both parasite species

(Analysis with nine microsatellite polymorphic markers confirmed). Antoniou et al., 2008; 2009

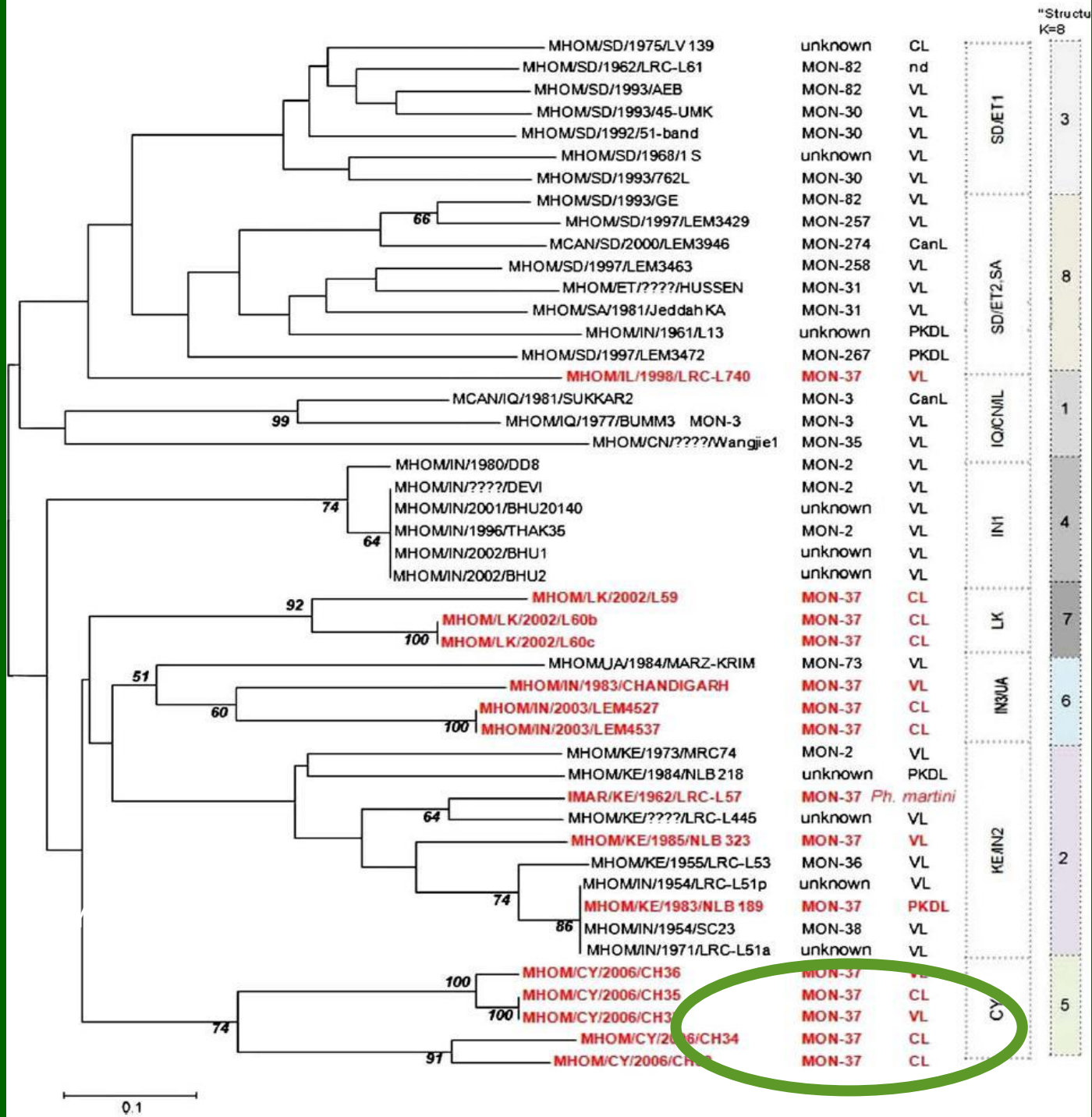
L. donovani MON-37

Multilocus microsatellite typing, showed that the *L. donovani* MON-37, Isolated from human cases in Cyprus, bear substantial **genetic differences** to *L. donovani* MON-37 isolates from : India, Israel, Sri Lanka and Kenya. (Antoniou et al., 2008)



They revealed **genetic similarities to isolates from Turkey** suggesting that the Cypriot isolates may have originated from Turkey. (Gouzelou et l., 2012)

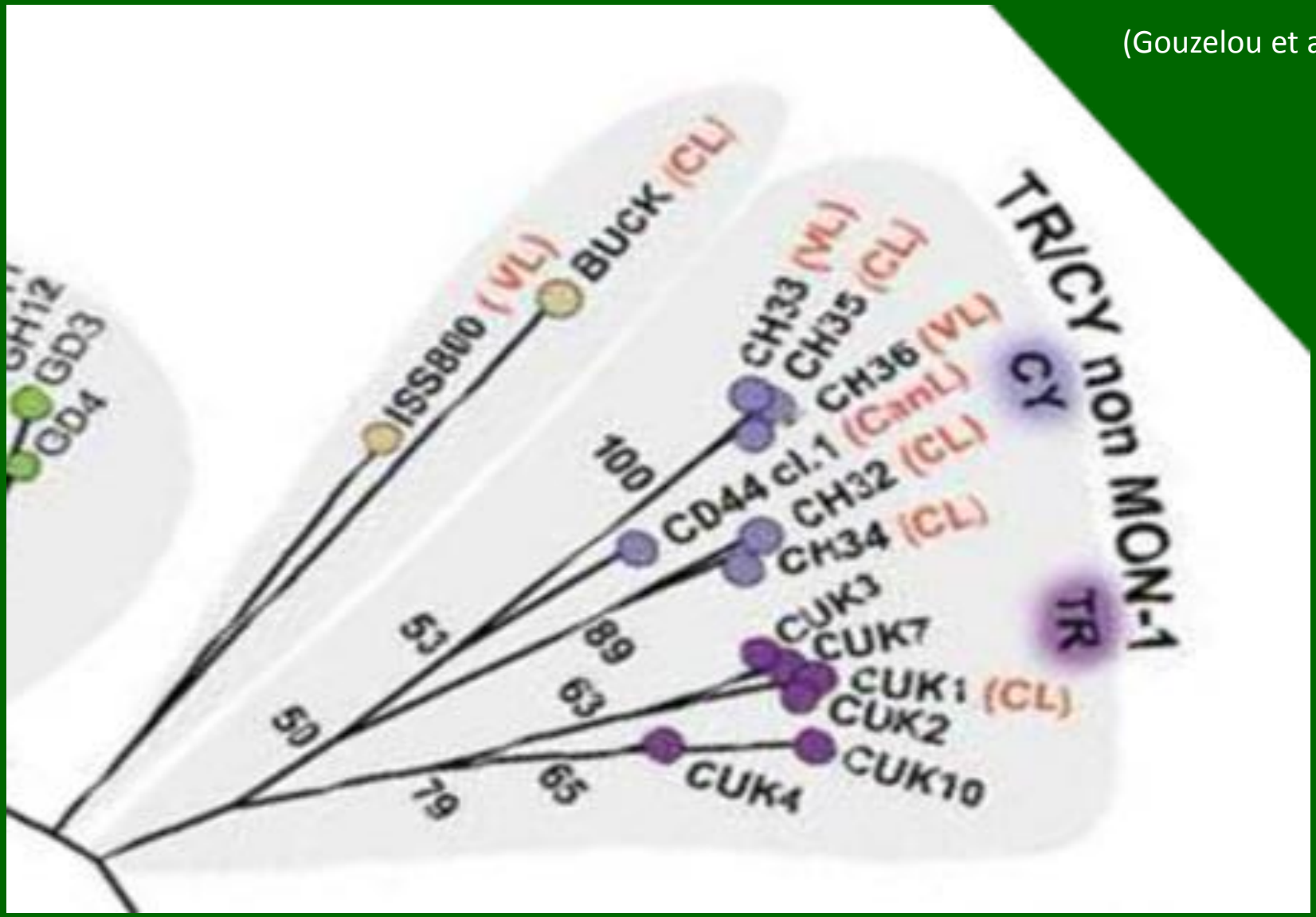
The Cypriot MON-37 forms a distinct sub-group within the Leishmania donovani complex



(Alam et al., 2009)

Genetically-distinct monophyletic group of L. donovani complex strains from Cyprus and Turkey

(Gouzelou et al., 2012)



In the Cyprus Rebuplic

- Two transmission cycles run in parallel :

  A) in dogs with ***L. infantum*** (zoonotic visceral leishmaniasis; dogs the main reservoir hosts).

- B) in humans with ***L. donovani*** (anthroponotic, causing VL, CL).

Phlebotomus tobbi was incriminated as the vector of *L. infantum*.

Vector of *L. donovani?*

L. donovani MON-37 CL / VL in Cyprus

Parasites of same genetic subgroup exhibit different clinical pathology in humans.

Is this due to differences in the parasite/the host/vector?

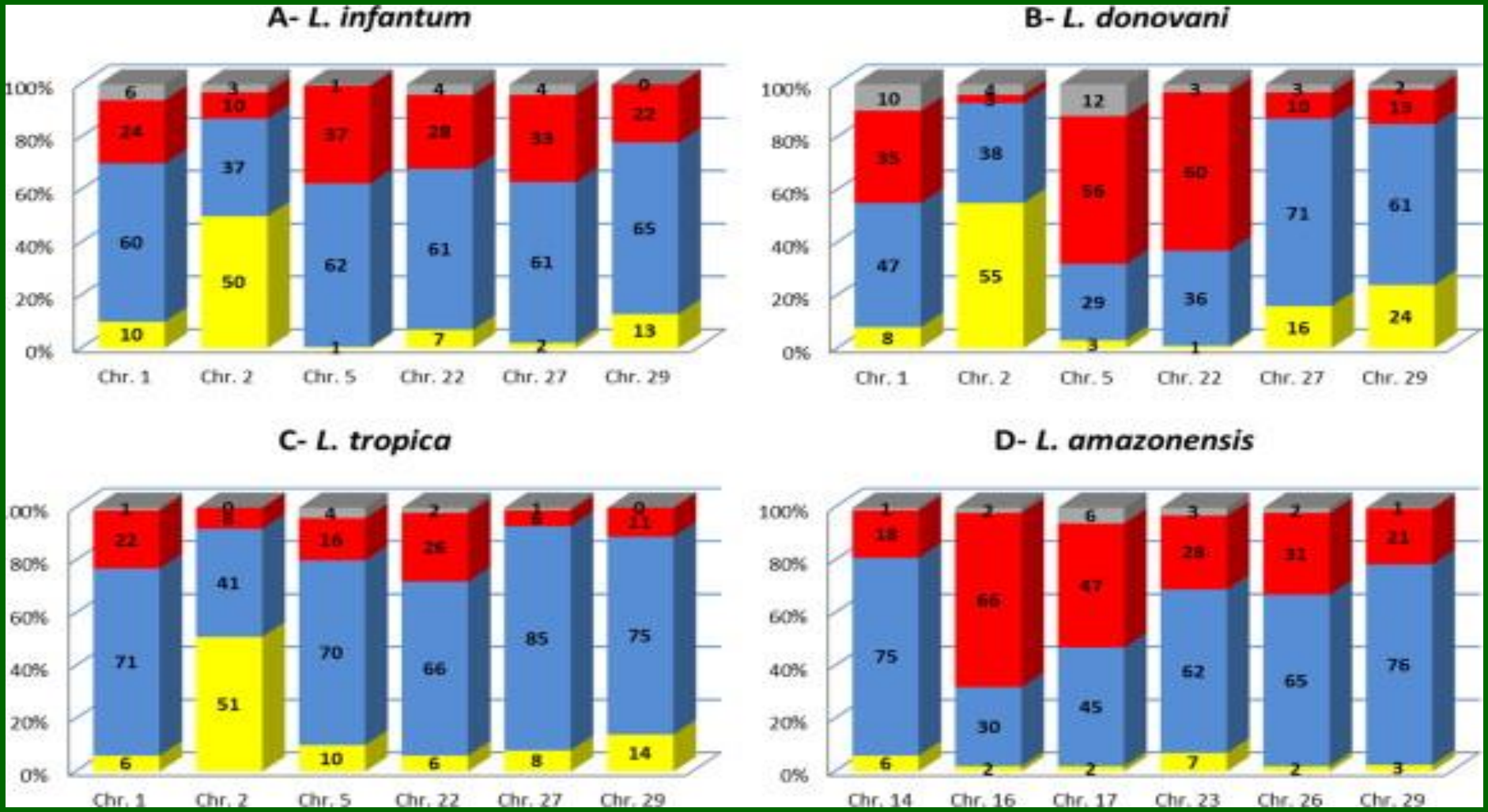
Animal models

- L. donovani MON-37 in Cyprus, in humans capable to cause both CL and VL.

- In hamsters and Balb/C mice display differences in virulence levels which appear to correlate with the different tropism phenotype in humans.

(Gouzelou et al., 2017)

Mosaic aneuploidy in Leishmania (fluorescence in situ hybridization (FISH))



Mosaic aneuploidy in four Leishmania species of the old and new world. Histograms show the proportions (percentages in ordinate) of mono-(yellow), di-(blue), tri-(red) and tetrasomic (gray) chromosomes in the different Leishmania species studied (Laurence et al., 2014)

Are Pathogen and/or host genetic factors involved?

There is evidence showing that genetic factors are involved in human susceptibility to parasitic diseases (schistosomiasis, malaria).

Studies have shown that the Nramp1 and H-2 genes are implicated in the control of *Leishmania donovani* infection in mice.

L. donovani in humans ?

Studied: 37 families with at least two affected sibs living in a village in eastern Sudan, where an outbreak of visceral leishmaniasis had occurred.

Concluded that genetic variations of the NRAMP1 promoter affects susceptibility to visceral leishmaniasis in this population.

(Bucheton et al., 2003)

The situation in Crete, similar to Cyprus

Thirty years ago, leishmaniasis was not considered a health problem in Crete.

Before the 1940s, VL and CL were widespread.

In Crete, VL re-emerged in 1907 with high incidence in the dog and the human populations

(Adler et al. 1983; Antoniou et al., 2009; Christodoulou et al., 2012)

The number of human cases increases each year reaching 17 VL and 3 CL cases/ year.

Why Cretans get infected by *L. infantum* and Greek Cypriots not?

Susceptibility to infectious disease is influenced by multiple host genes

- **They are difficult to map in humans.**

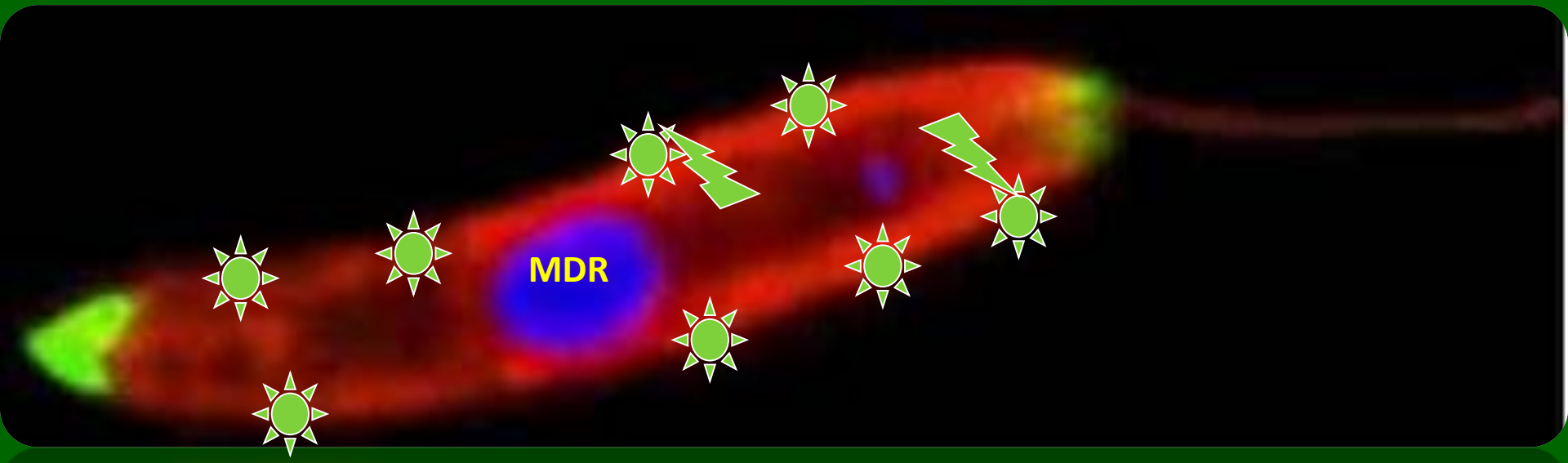
- Studies of animal models and epidemiological studies in humans have shown that many apparently non-hereditary diseases, including infectious diseases , develop predominantly in genetically predisposed individuals.

- **This predisposition is caused by multiple genes.**

(Bucheton et al., 2003)

Consider that the symptoms of leishmaniasis vary so greatly from individual to individual infected by the same strain in the same area!

MDR phenotype



Pgp 170 (130 to 200 kDa) acts as a transmembrane efflux pump for a diverse group of lipophilic compounds, including many chemically diverse drugs and fluorescent dyes as well as calcium channel blockers.

The result is a reduced drug accumulation inside the cells and the survival of the parasites or the MDR neoplastic cells.

Leishmania drug resistance

The Pgp 170 activity, expressed as slope ‘α’ in **275 isolates** (0.01 to 6)

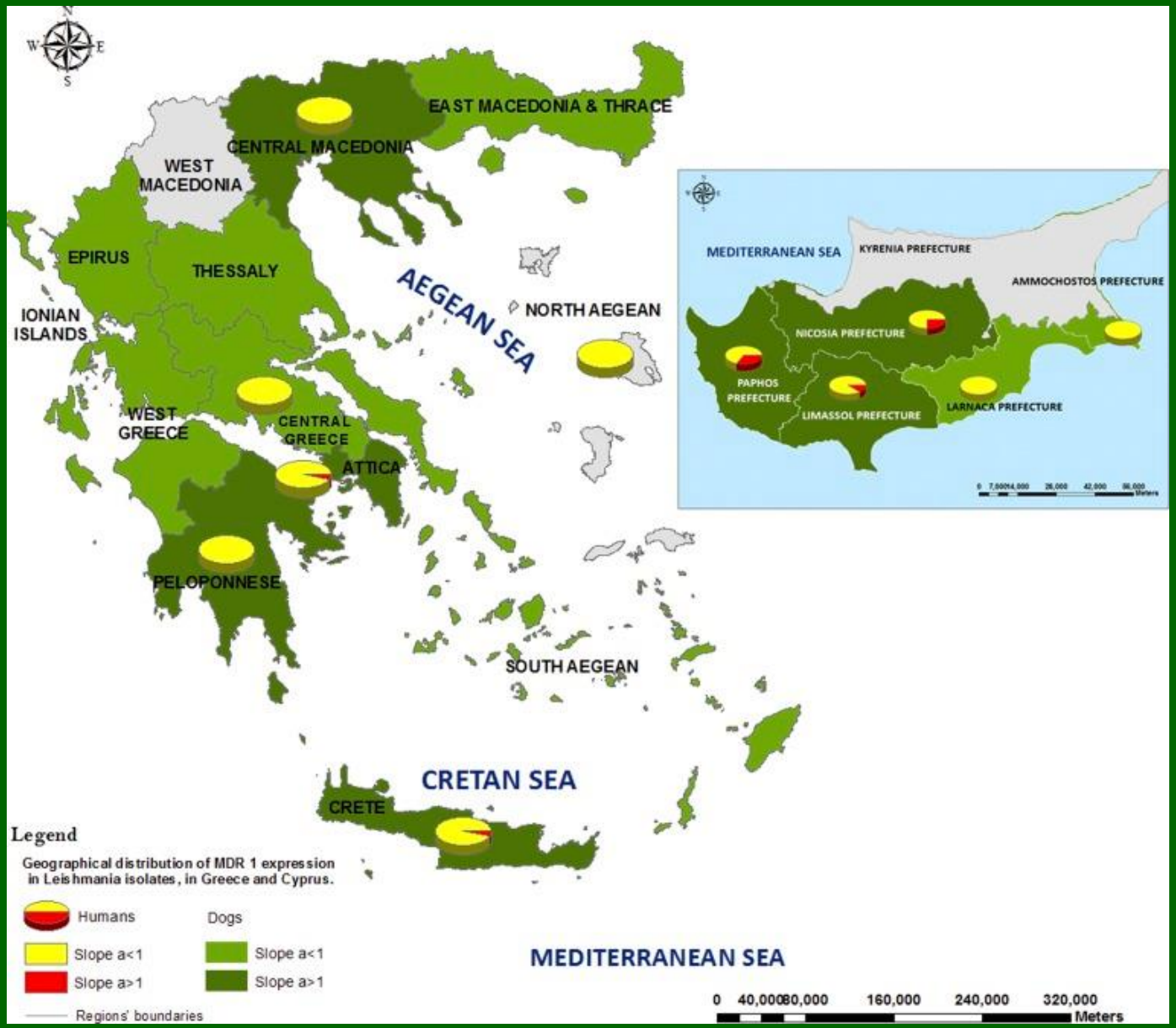
L. donovani isolates presentied a greater slope ‘α’ than *L. infantum*

80% of the *L. donovani* isolates tested were found to overexpress the MDR phenotype.

The highest efflux rate was presented by two isolates from Paphos prefecture: an *L. donovani* strain isolated from a VL patient and an *L. infantum* strain isolated from a dog (slope α 6 and 4.26, respectively).

Therefore, the emergence and spread of potentially drug resistant parasites in Cyprus signals a substantial public health threat.

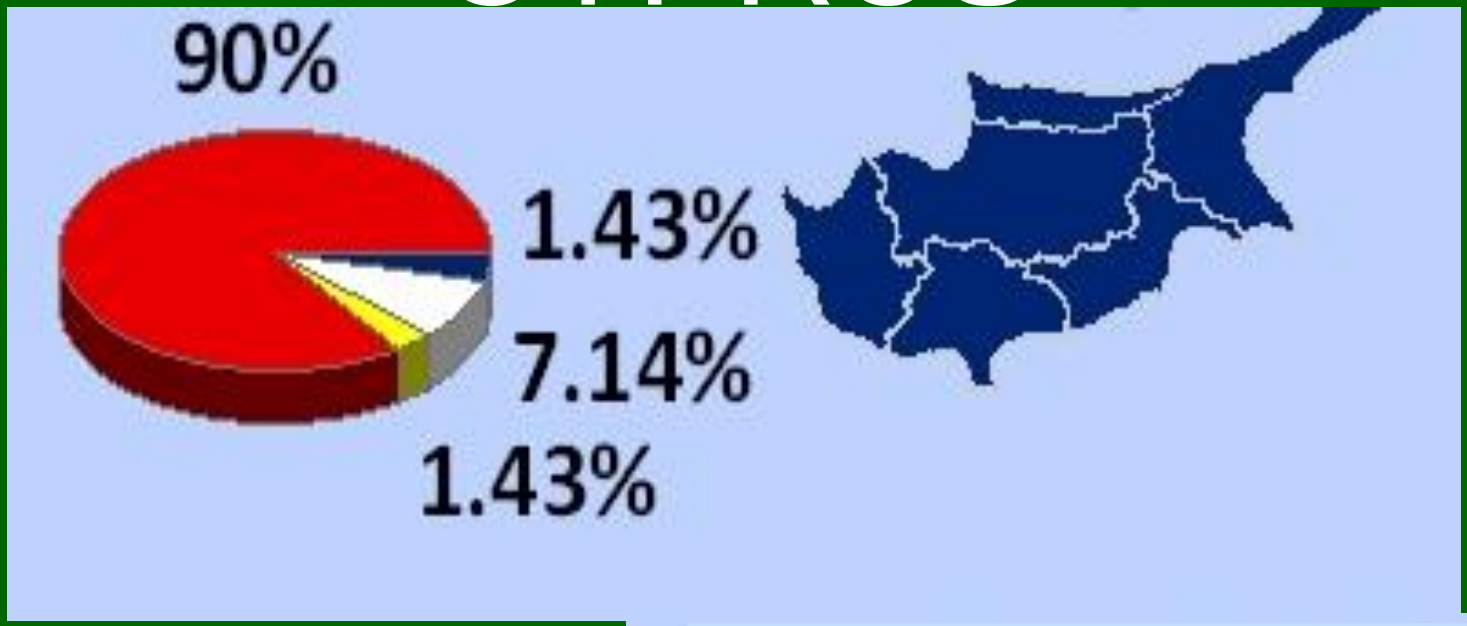
Messaritakis et al., 2013



The geographical distribution of multidrug resistance (MDR1) gene expression in 275 Leishmania strains, isolated from patients and dogs from Greece and Cyprus. The isolates were characterized as of high or low efflux potential (slope 'α' > 1, or low slope 'α' < 1, respectively).

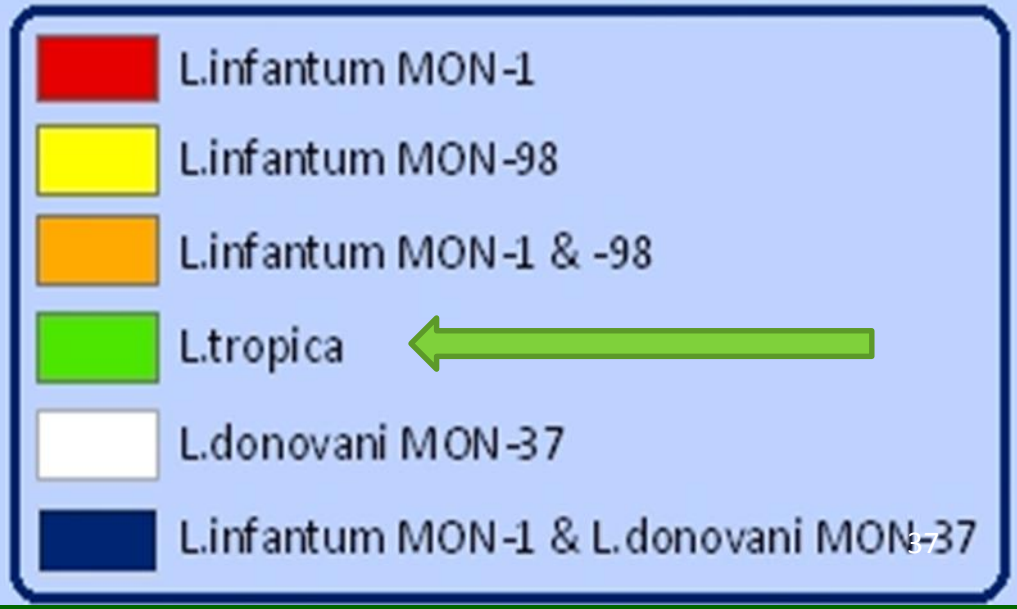
Messaritakis et al., 2013

CYPRUS



1. Mainly

2. new

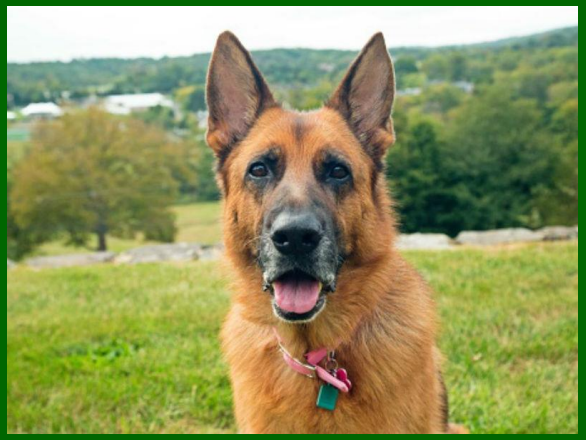


(Christodoulou, 2010)

Therefore, in Cyprus

Therefore, in Cyprus

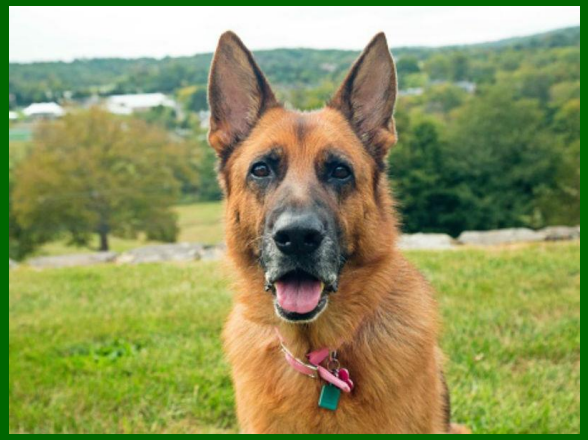
L. infantum



Therefore, in Cyprus

L. infantum

L. donovani

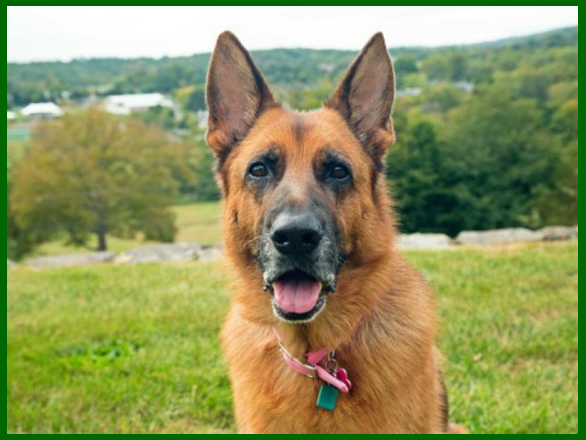


Therefore, in Cyprus

L. infantum

P. tobbi

L. donovani



Therefore, in Cyprus

L. infantum

P. tobbi

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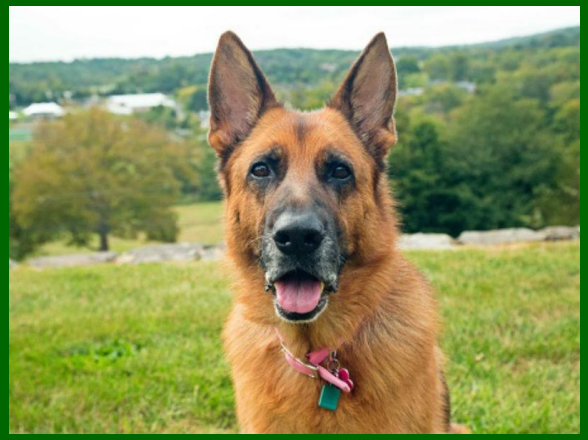

1/20

Hybrids?

Conclussions

- **The Republic of Cyprus presents an unusual situation in which two distinct leishmaniasis transmission cycles run in parallel:**
  - **Zoonotic ONLY in dogs with *L. infantum* (MON-1 and MON-98),**
  - **Anthroponotic in humans with the newly introduced *L. donovani* MON-37.**
- **The *Leishmania donovani* of Cyprus is close to the Turkish *Leishmania donovani***
- **What is the situation in N. Cyprus regarding *L. infantum*?**
- **High drug resistant *Leishmania* isolates are found in Cyprus compared to Greece.**
- **Are genetic factors involved in the parasite? The host? The vector? Explaining the unique situation in Cyprus?**
- **Although it is clear that in Cyprus, there are competent vectors for the transmission of *Leishmania* to dogs and humans the question of why there are no VL or CL cases caused by *L. infantum* remains unanswered.**
- **+ L. tropica ?**

The lessons we learned from our study in Cyprus

Epidemiology of leishmaniasis
Number of cases increases due to:

- Decrease in insecticide use against Malaria
- New endemic foci emerge due to the movement, importation of infected hosts
- People not “immunized” enter endemic areas
- Changes in the ecology of hosts/vectors
- Development of new diagnostic techniques
- Better monitoring of the disease

Can we stop this advancing enemy?

- Monitor high risk areas
- Reduce sand fly populations
- Isolate and treat sick dogs
- Isolate and treat HIV-leishmaniasis co-infected patients
- Prophylactic measures: dog collars etc, bed nets, window nets
- Legislation (dog movement, notify dog/human cases)

and

- Dogs must be tested for leishmaniasis serologically at Spring and Autumn
- Treatment in dogs should be given only if the animal will be followed closely by PCR every 6 months

  (resistance to drugs has emerged in *Leishmania*)
- Monitor for hybrids

Human leishmaniasis in Northern Cyprus???

Does the presence of *P. neglectus* affect the situation in a different way?

Now P. neglectus found in the Republic

Thank you for your attention

Toll-like receptor 9 polymorphism in Cretan patients with *Leishmania infantum* infection

- **Toll-Like Receptor 9 (TLR9; located intracellularly on the lysosomes of antigen-presenting cells) recognizes microbial structures and triggers inflammatory and adaptive responses (Tuon FF 2008).**
- **We investigated the association between the single nucleotide polymorphism (SNP) in TLR9 gene and the risk of endemic *L. infantum* infection.**
- **The presence of the C/C genotype and the C allele of the TLR9 gene polymorphism may represent an increased risk for *L. infantum* infection in the Cretan people, a population sharing a common genetic and cultural background.**

(Messaritakis et al., 2014)

Comparison of Cretan – Cypriot people