

Sand fly borne diseases in the region

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Sand Fly-Borne Diseases

- Leishmaniases
 - Human
 - Canine
- Bartonellosis
- SF Viruses (Phleboviruses)



Three important life cycle for Leishmania spp.

Enzootic

Maintained in Animals
(Canids or small mammals)



Host or Reservoir

Leishmania

Vector



Sand fly

Zoonotic

Anthroponotic

(VL caused by *L. donovani*
CL caused by *L. tropica*)

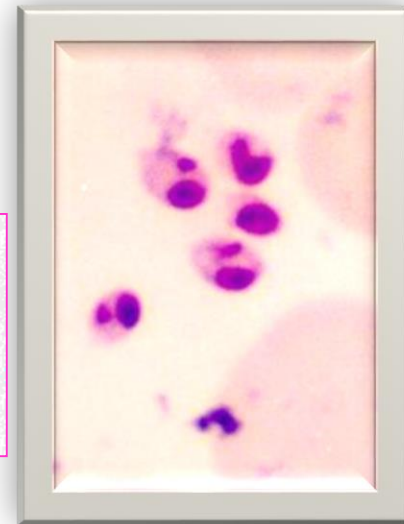
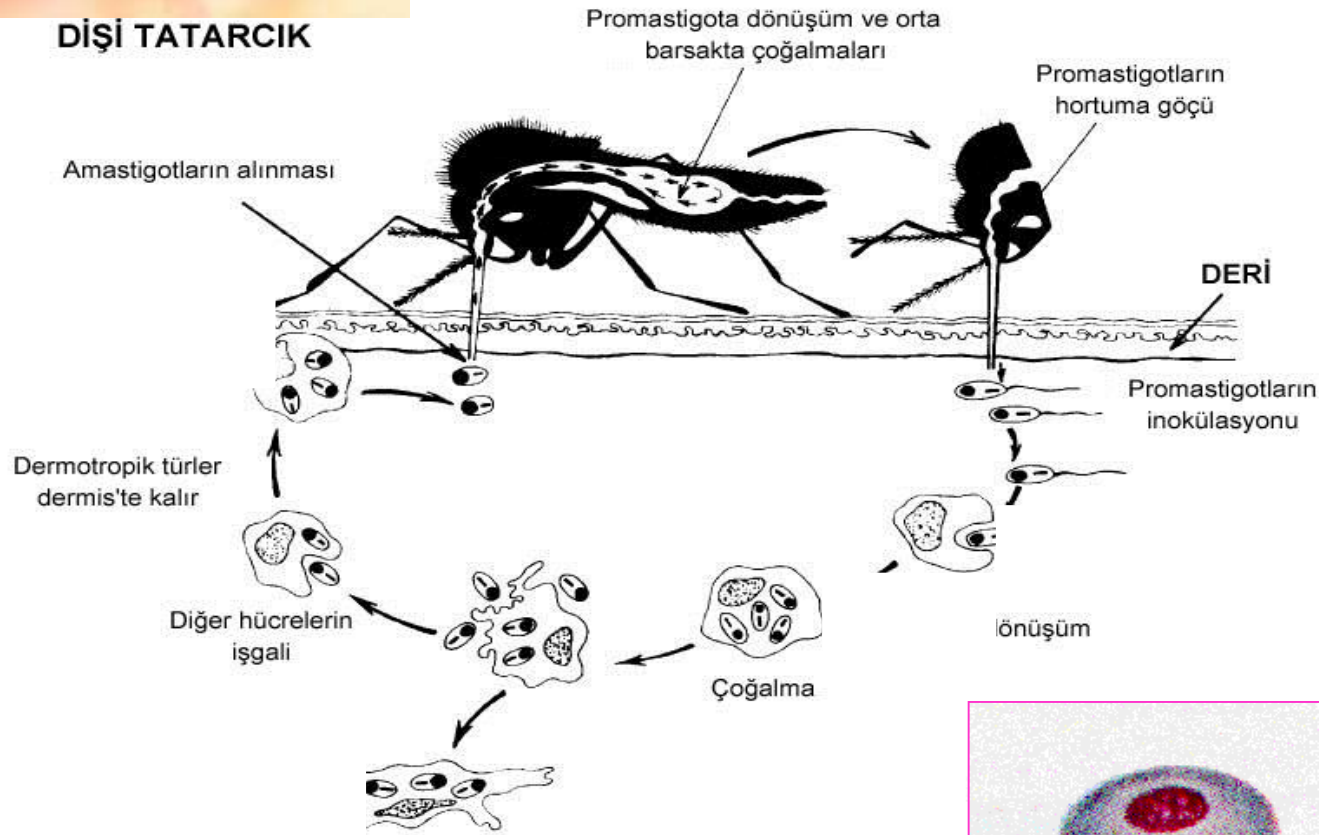




Female Sandfly



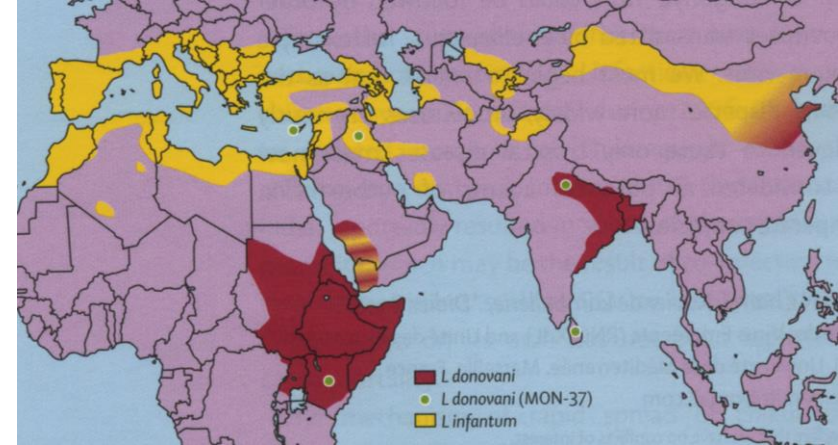
DİŞİ TATARCIK



Human, animal or reservoir host

Old World Leishmaniases

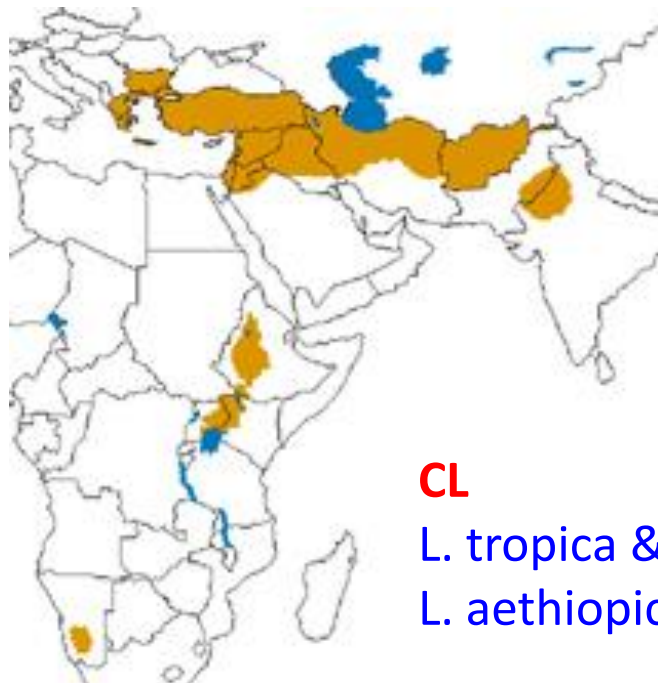
- It is prevalent in more than 98 countries or territories on 4 continents
- Each year; 1.5 million new cases of CL and 500.000 cases of VL
- Annually (2004-2008)
- VL: 42 in Greece, 29 in Turkey, 14 in Syria
- CL: 22.882 in Syria, 2.465 in Turkey, 3 in Greece



VL

Antoniou et al. 2008

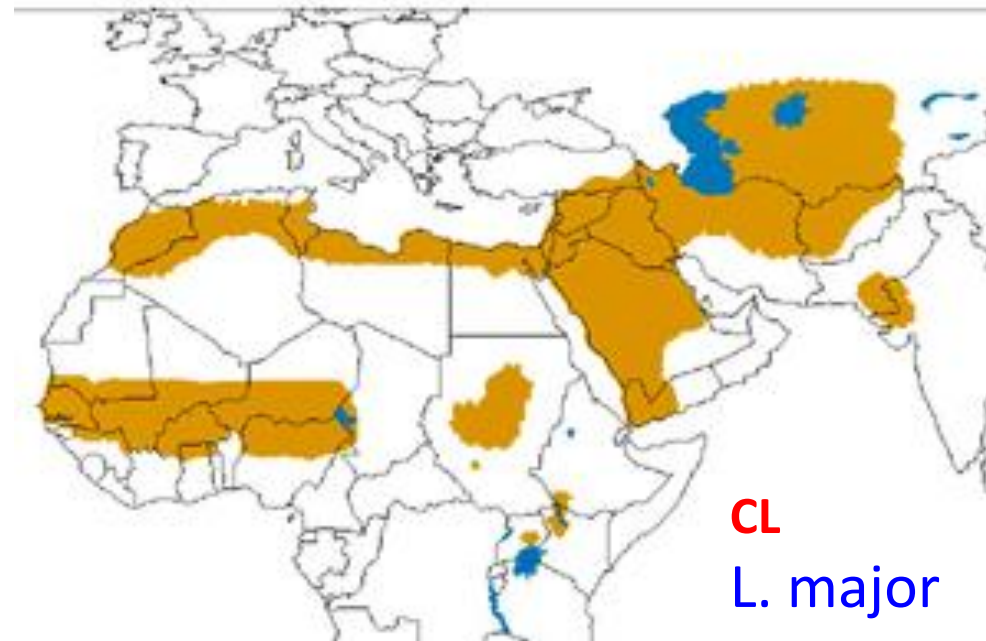
L. donovani; L. infantum



CL

L. tropica & L. aethiopica

Alvar J. et al. PLoS ONE, 7(5), e35671, 2012



CL

L. major

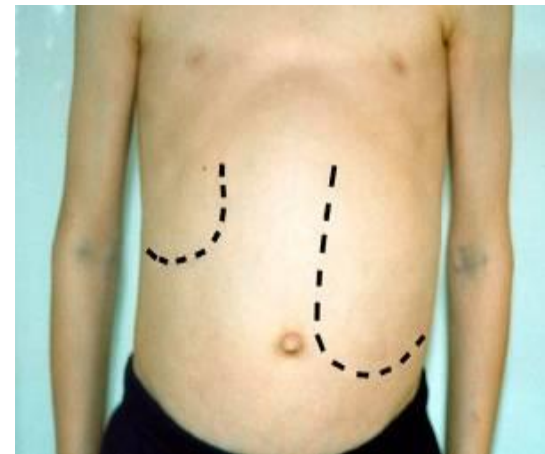
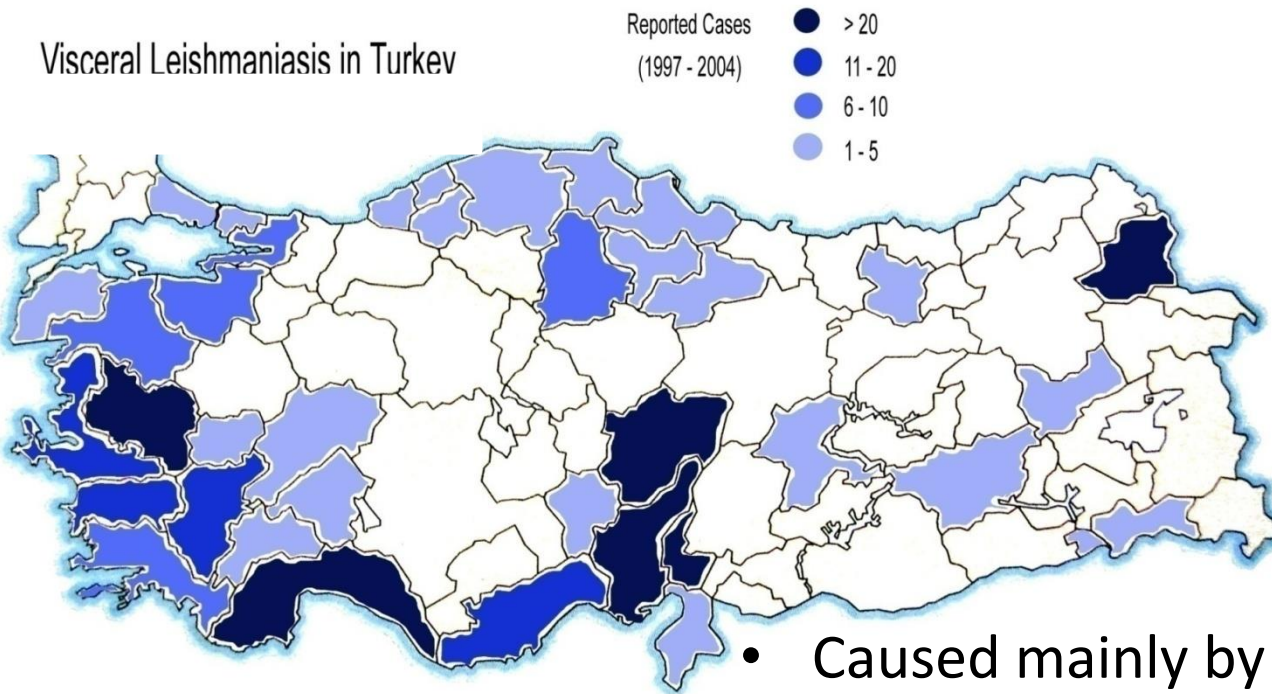
WHO Expert Report, 2010

Two clinical forms of leishmaniasis are present as human disease in Turkey and Cyprus

- Visceral leishmaniasis (VL, Kala-Azar)
- Cutaneous leishmaniasis (CL)
 - Different types of lesions are seen including mucozal involvement
- Canine Leishmaniasis
 - Important reservoir for VL
 - An important life threatening disease for the dog population

Visceral Leishmaniasis in Turkey

Visceral Leishmaniasis in Turkey



Annually 25-30 cases

- Caused mainly by *L.infantum*
- And sporadically by *L.tropica* and *L.donovani*
- Notifiable disease and reported in 38 provinces out of 81
- Antimonials/Liposomal amphotericine B first line drugs



Cutaneous leishmaniasis in Turkey

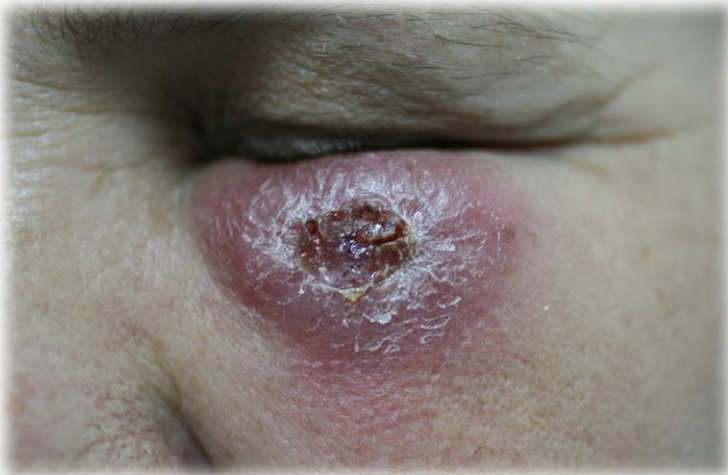


- The causative agent:
 - *L. tropica*: In Southeastern, Central and western regions
 - *L. infantum* : In East Mediterranean region
 - *L. major* ve *L. donovani*: Rarely
- Notifiable and reported: In 41 provinces since 1994
- Mostly reported: In 6 provinces in southeast Turkey
- Diagnosis: Mainly clinical and parasitological
- First line treatment: Antimonials



What is CL?

- Generally seen in children
- Mainly located in uncovered parts of the body: face, arms, legs
- Single or more than one
- Leaving scar without treatment, potentially causing functional loss in lesions located in face, nose and lips





CL



L. donovani - CL



L. major - CL



L. infantum - CL



L. tropica - CL

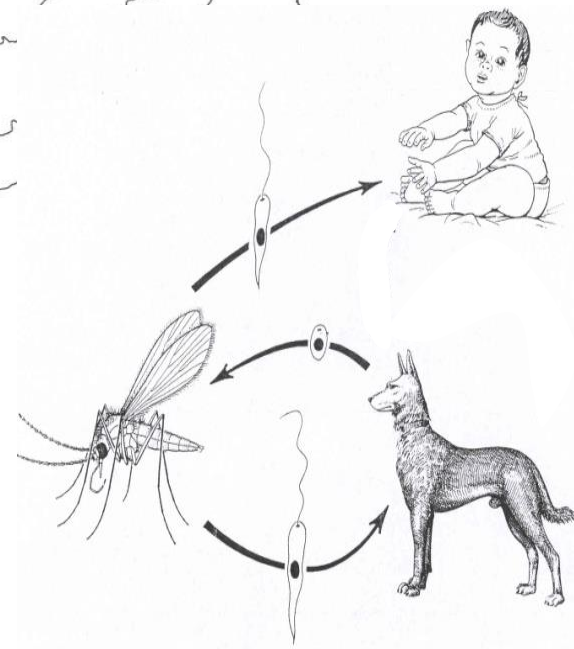
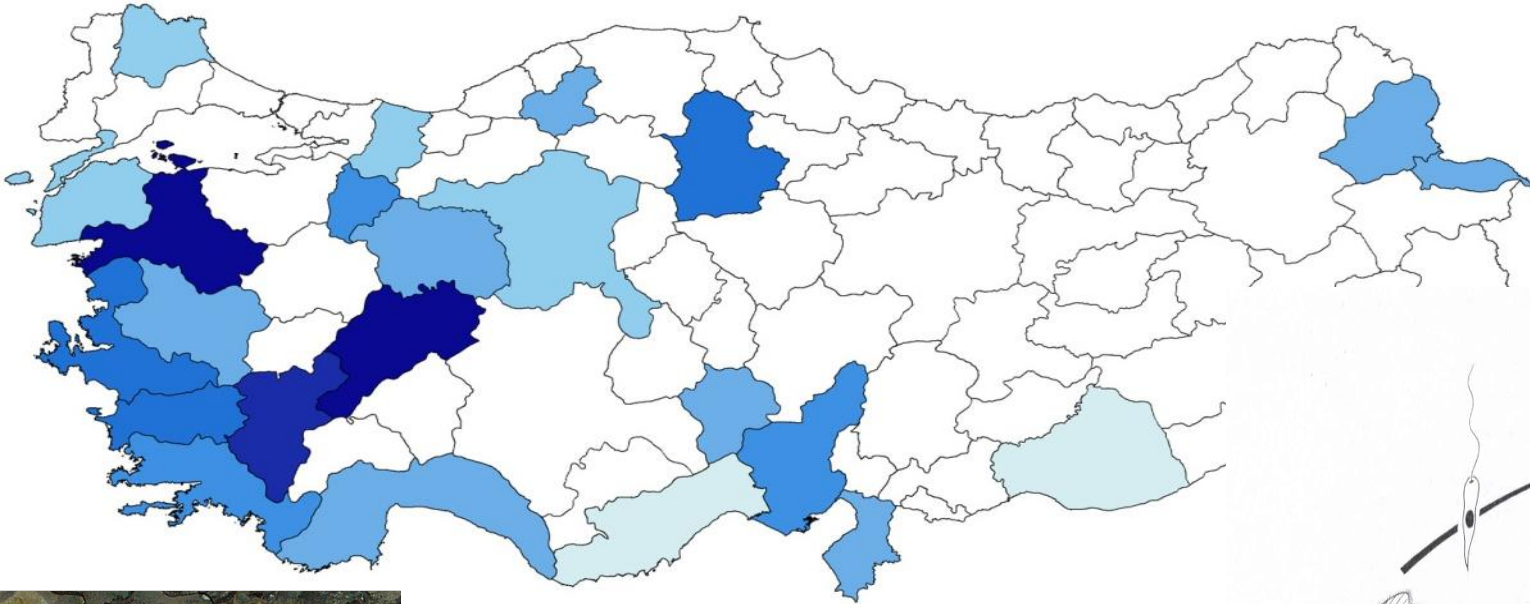


Before treatment

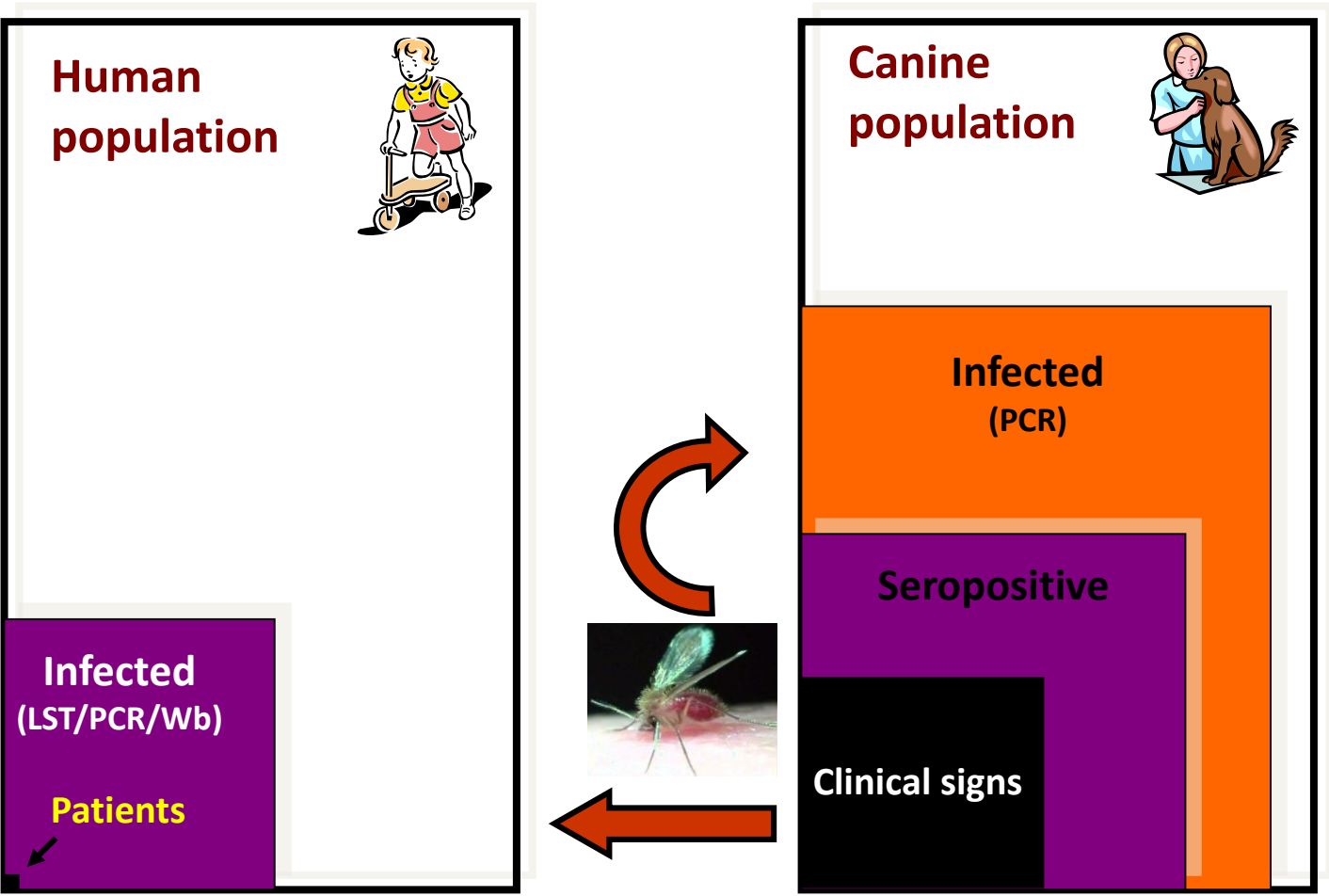
After treatment

Canine Leishmaniasis

- CanL is important veterinary and public health problem with much higher prevalence than human infection in all regions (%11.32)
- Agents: *L. infantum* ve rarely *L. tropica*

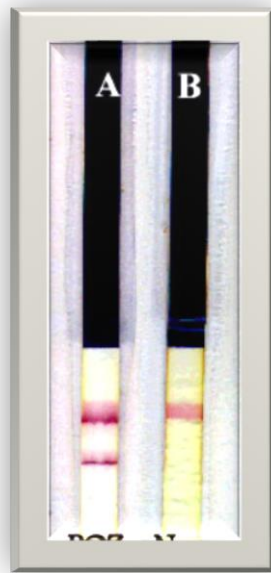


Relationship between human and canine leishmaniasis cases in endemic regions



Diagnostic Methods

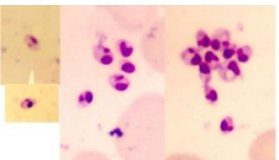
- Direct
 - Microscopy (VL: bonemarrow, CL: lesion smear, CanL: lymphnode)
 - Molecular (PCR)
- Indirect (serology)
 - IFAT, ELISA, IHA, DAT, Western Blot, dipstick



rK39 Dipstick



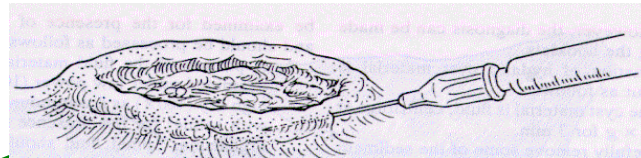
Giemsa
Staining



Amastigote



NNN Culture Medium

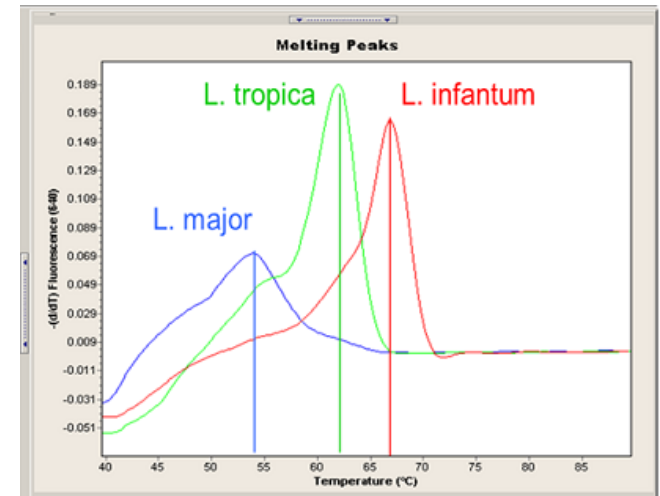


6-21 days



Promastigote

DNA amplification (PCR)



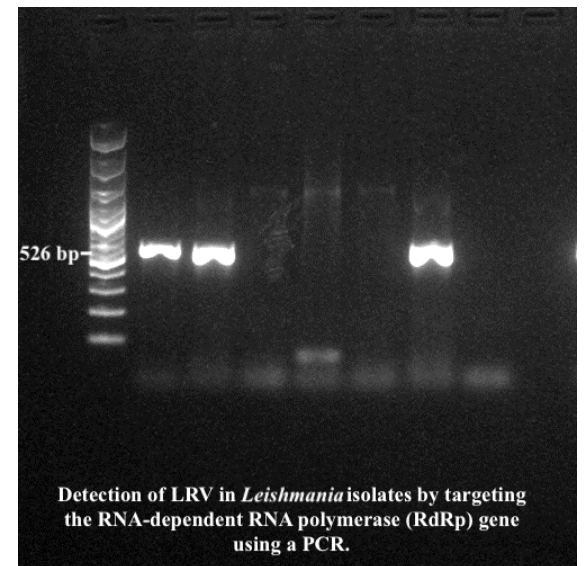
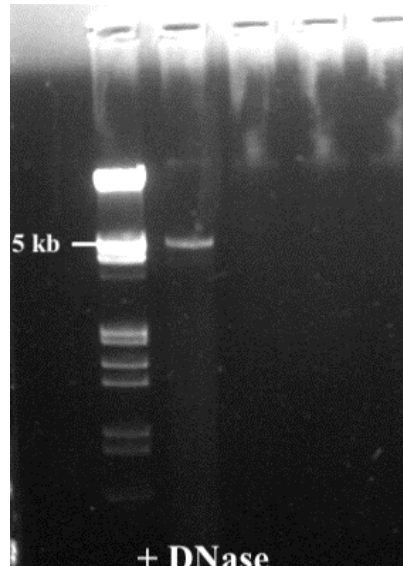
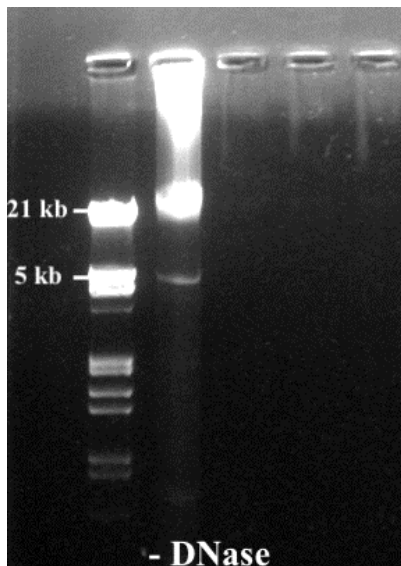
Treatment

- VL treatment in Turkey;
 - Pentavalent Antimonials: free of charge
 - Liposomal Amphotericine B: patients with poor health situation
- CL treatment;
 - Intralesional injection of Pentavalent Antimonials: one or limited number of lesions
 - Sistemic treatment with Pentavalent Antimonials: multipl lesions or lesions in risky locations
- Miltefosin (oral drug)
 - Drug resistance began in Asia and India
 - It is not used in humans yet in Europe and Turkey
- Prognosis
 - VL: 90% death if left untreated
 - CL: 95% healing with scar even left untreated

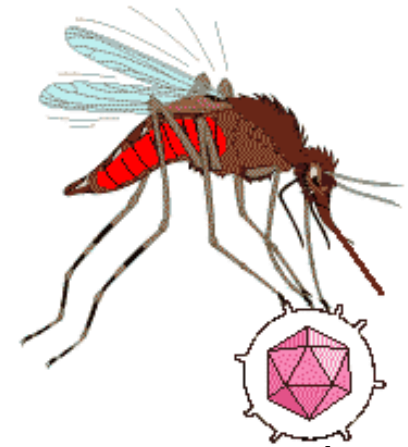


Leishmania virus (LRV) positivity in Old World *Leishmania* Species

- LRV2 was found in *L. major*, *L. infantum* and *L. aethiopica* in Old World countries like Turkmenistan, Portugal, Iran and Ethiopia
- We found LRV2 positivity in *L. major* and *L. tropica* isolates (MSc thesis of Muhammed Nalçacı)
- This needs to be evaluated as a virulence factor for the region

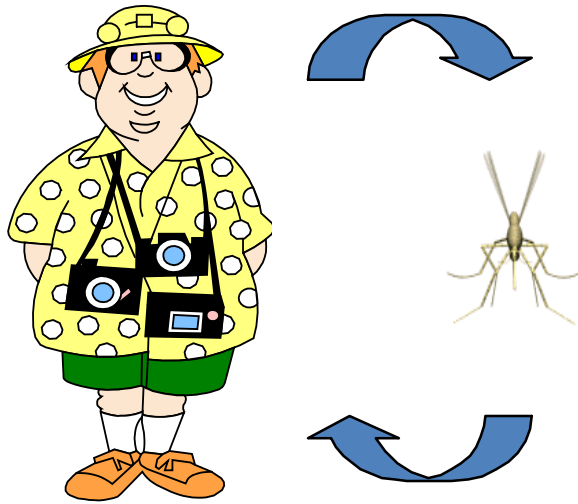


Arthropod-borne virus

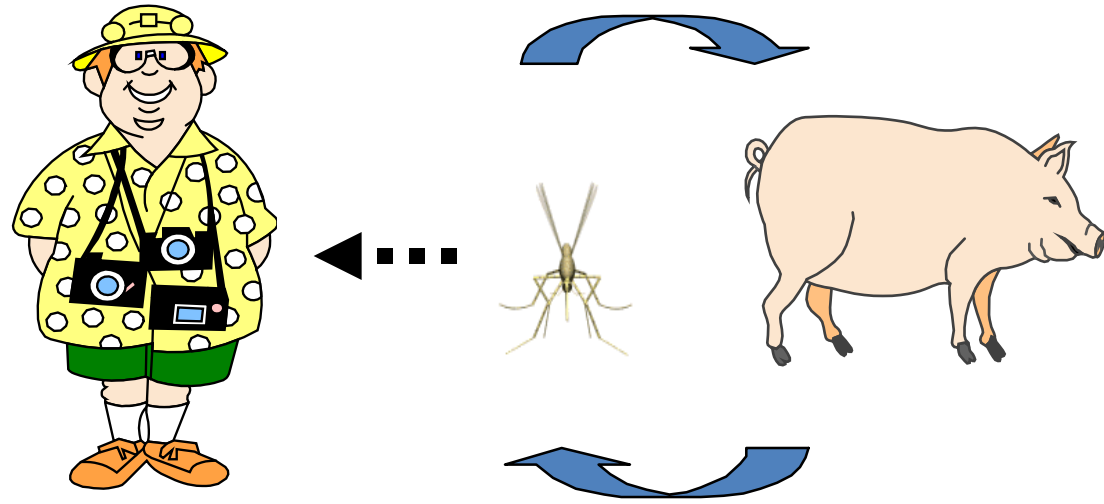


- Arthropod-borne pathogens are transmitted among vertebrate hosts by hematophagous arthropods
 - Mosquitoes, ticks, other biting flies, sandflies
- Widespread in tropical and subtropical regions
- 500 arbovirus species
- Mosquitoes will be infected only biting the patient
 - 12 hours before → Sunset of illness → 72 hours after
- They can infect humans → after 8-14 days and be a vector during 3 months

Arbovirus Infection Routes



Human - Arthropod - Human



Animal – Arthropod - Human

RESEARCH



Open Access

Phlebovirus and *Leishmania* detection in sandflies from eastern Thrace and northern Cyprus

Koray Ergunay^{1*}, Ozge Erisoz Kasap², Serra Orsten¹, Kerem Oter³, Filiz Gunay², Ayse Zeynep Akkutay Yoldar⁴, Ender Dincer⁵, Bulent Alten² and Aykut Ozkul⁴

- Phleboviruses are arthropod-borne viruses
- Enveloped viruses with single-stranded RNA genome in three segments encoding viral polymerase and proteins
- Classified as a genus in the Bunyaviridae family
- Phleboviruses are not all transmitted by phlebotomine flies
 - Some are transmitted by mosquitoes or ticks depending on the virus
- Some phleboviruses are pathogenic for humans causing:
 - a febrile disease usually as fever of unknown origin (FUO); having different names as sandfly fever/papatasi fever/three-day fever
 - or neuroinvasive diseases of CNS such as meningitis, encephalitis, myelitis

Virus-Sand fly Interaction

- Phlebotomus is biologic vector for phleboviruses
- Phleboviruses are transmitted transovarially and can be also found in male sandflies
 - Timing is important; If eggs developed before virus multiplication in sandfly, virus can not be reached to the egg,
- Virus infection rate is gradually decreased in subsequent generations
- This suggested that phleboviruses cannot be maintained in the vector population by transovarial transmission alone
 - Virus stays in larvae during its hibernation in winter
- Female Phlebotomine will be infected by biting the patient during;
 - 2 days before  sunset of illness  1 day after
- Phlebotomine can infect human 6-10 days after receiving virus

Old World Phleboviruses

- There are app. 60 phlebovirus serotypes
- Sandfly fever agents
 - Sandfly fever Sicilian virus (SFSV)
 - Sandfly fever Naples virus (SFNV)
 - Sandfly fever Cyprus virus (SFCV)
 - Sandfly fever Turkey virus (SFTV)
- Toscana virus (TOSV), Granada virus and Adria virus are reported to be associated with phlebovirus-induced febrile conditions as well as sporadic seasonal meningitis/meningoencephalitis
- TOSV, SFSV, SFTV and Chios virus have sporadically been reported to cause neuroinvasive diseases

Case Definition

- A self limited febrile disease
- Neuroinvasive disease with CNS symptoms

AND

- For Mediterranean region: occurring between May and October

AND

- Settlement or recently travel to Mediterranean Countries

AND

- Confirmation of diagnosis using one of the determined laboratory tests

Symptoms of Sandfly Fever

- Flu like mild symptoms
 - Generally in summer months
 - 3-6 days after bite: acute malaise, abdominal pain, nausea/vomiting
 - 1st day: chilling, than for 1-2 days: 39-40 °C fever, tachycardia, severe headache, myalgia, arthralgia: reddish face without eruption
- Fever will become normal in 2-4 days (3 day fever, rarely 1-9 day)
 - After fever period, malaise, bradycardia and generally hypotension
 - Occasionally konjunktivitis, eye pain
- Healing in a few days or few weeks
- Symptoms were gone spontaneous
- Treatment is symptomatic and immunity is acquired

Toscana (TOS) Virus

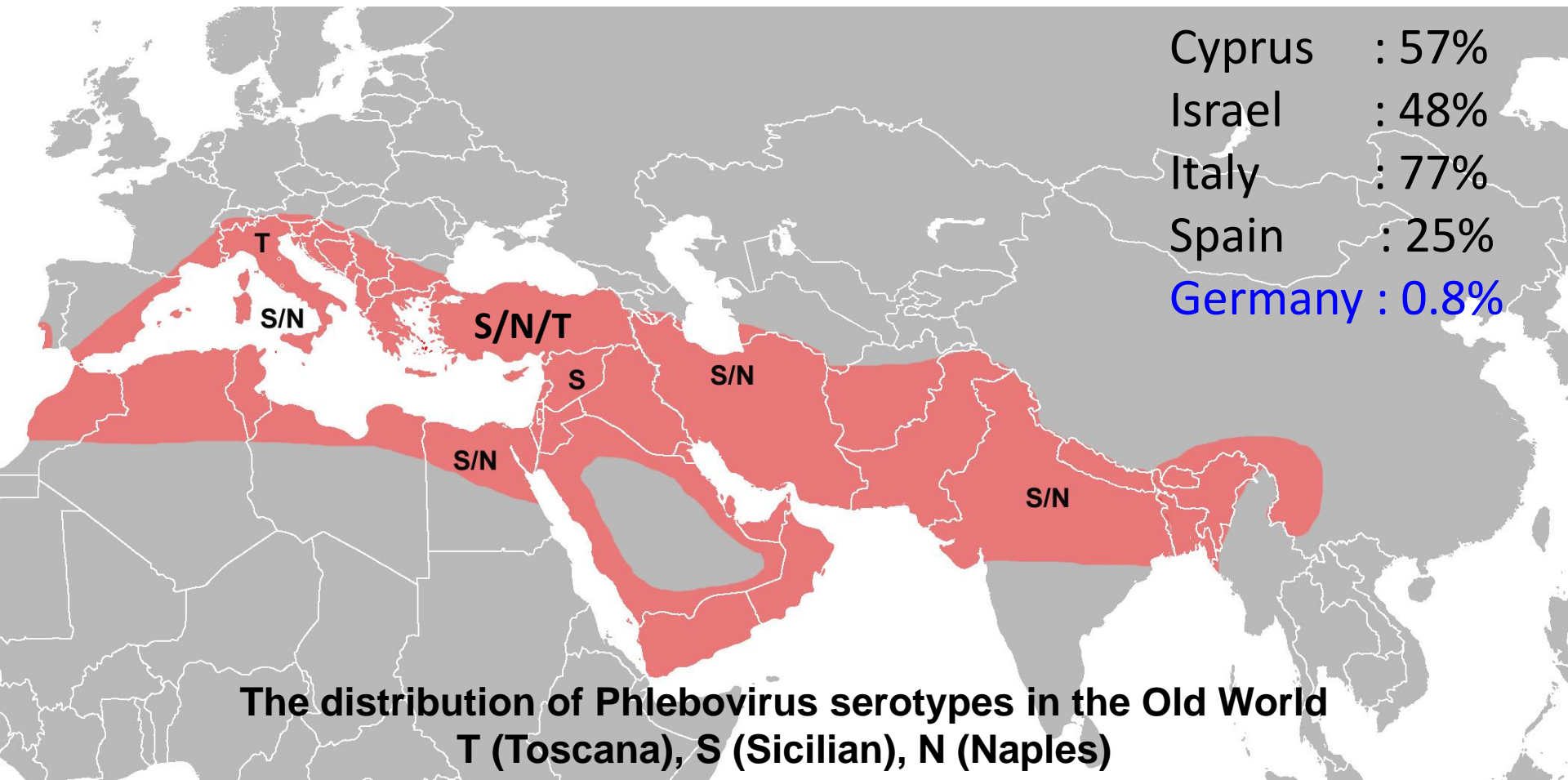
- Causing agent of aseptic meningitis or encephalitis in humans
 - Neuroinvasive TOSV infections can also result in severe/fatal CNS involvement, peripheral neurological symptoms and sequelae such as paresis, persistent speech disorders and hearing loss
- Isolated from *P. perniciosus* in 1971, in Tuscany
- Subsequent isolations from *P. perniciosus* and *P. perfliewi*
- Toscana virus is closely related but antigenically distinct to Naples virus
- Toscana virus cause 80% of summer meningitis especially in children in central Italy
- Human cases were reported from Spain, Portugal, France, Greece and Cyprus

Toscana Virus

- Spain 1988 & 2003: serologic and molecular evidence
 - 15 strains isolated from patients,
 - 26% seroprevalence rate in Granada (Mendoza-Montero et al 1998 Clin Infect Dis)
- France 2004: serologic and molecular evidence (*Hemmersbach-Miller et al 2004 Eur J Intern Med, Peyrefitte et al 2005 Emerg Infect Dis*)
 - seroprevalence 12% in healthy blood donors southeastern France
 - Toscana virus is in the top-3 of causes of meningitis in France and Spain
- Turkey 2009: serologic and molecular evidence (Ergunay et al Clin Microbiol Infect 2010).
- Croatia 2010

Sand fly fever viruses in the region

- Widespread in Mediterranean and Middle East Countries



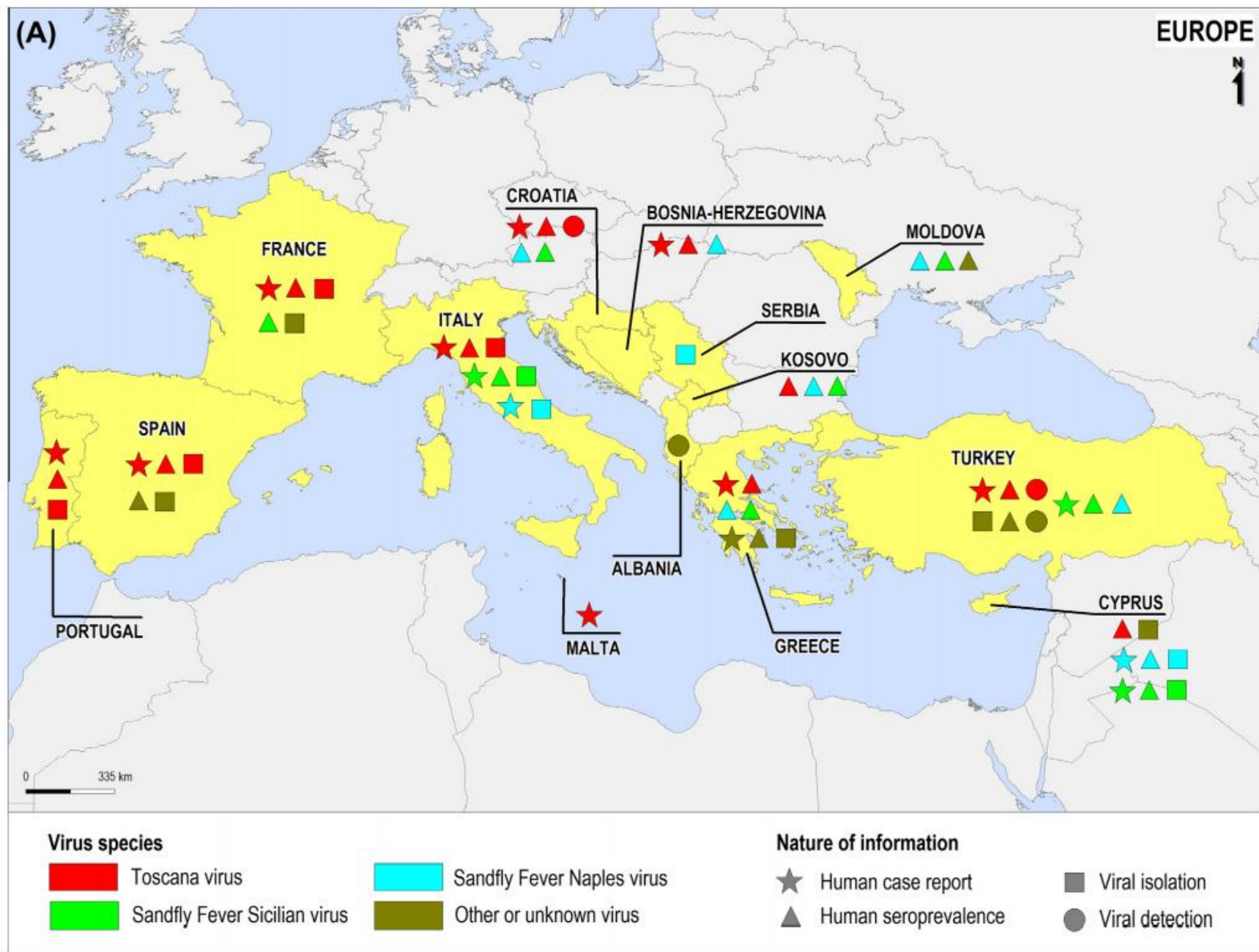
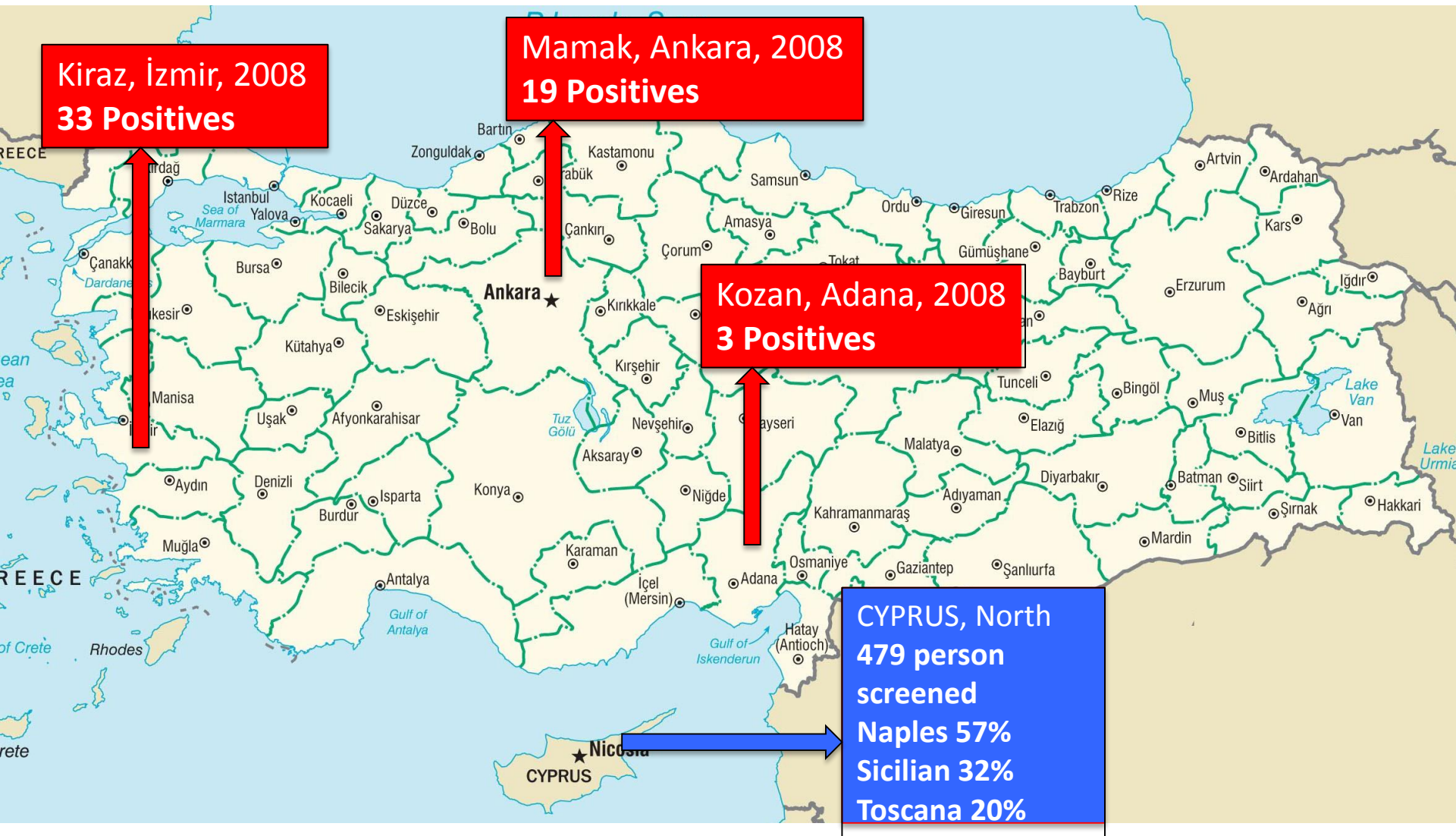


Fig. 4. Distribution of the sandfly-borne phleboviruses in the Old World. (A) Europe, (B) the Middle East, (C) Africa.

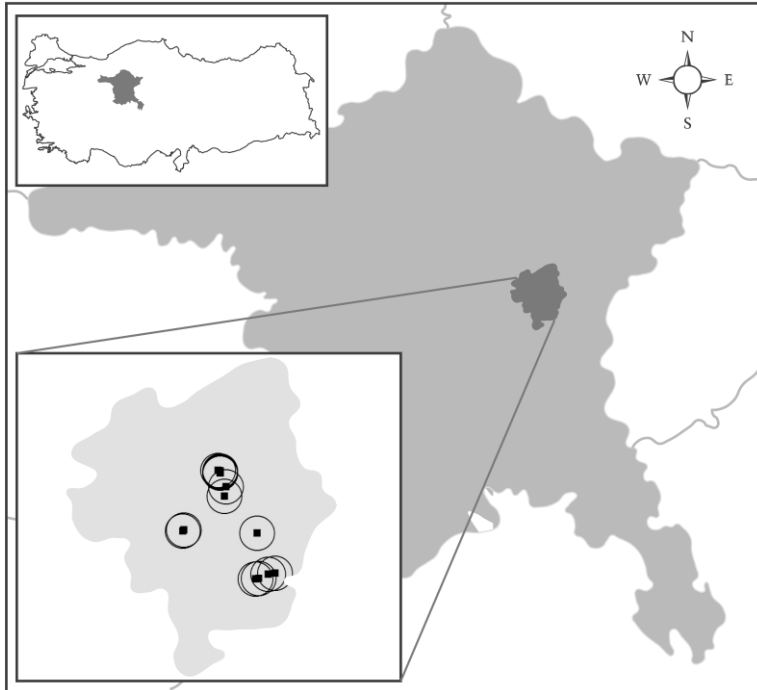
Phlebovirus outbreaks in Turkey



New virus is detected (Sand fly Fever Turkey Virus (SFTV))

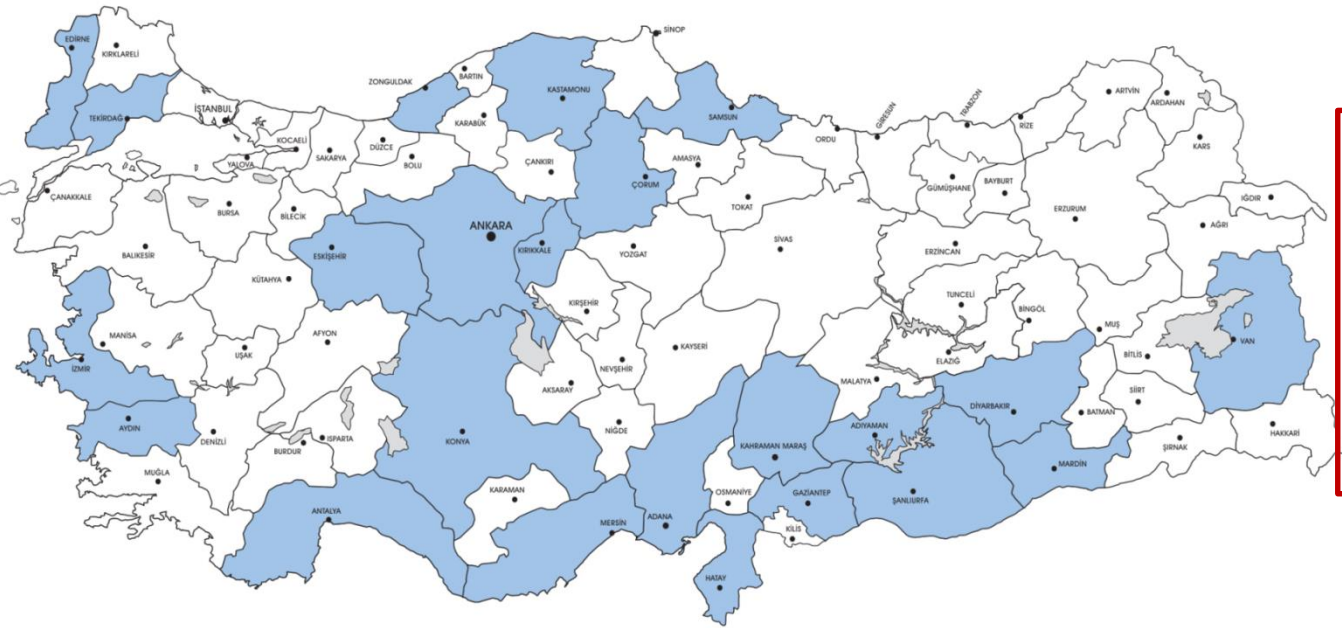
Phlebovirus in Sandfly

In 2012

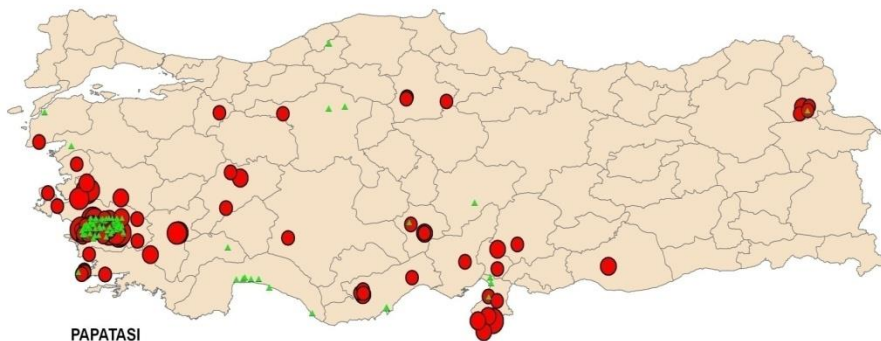


TOSCANA Virus detected in
Phlebotomus major s.l.
ANKARA

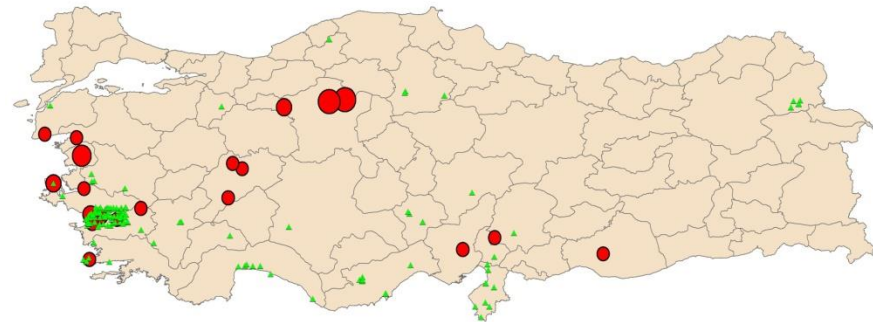
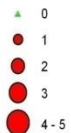
Phlebovirus Map of Turkey



3 new virus were detected
in ADANA
“Damyeri virus”
“Zerdali” virus
“Toros virus”



PAPATASI



PERFILEWİ

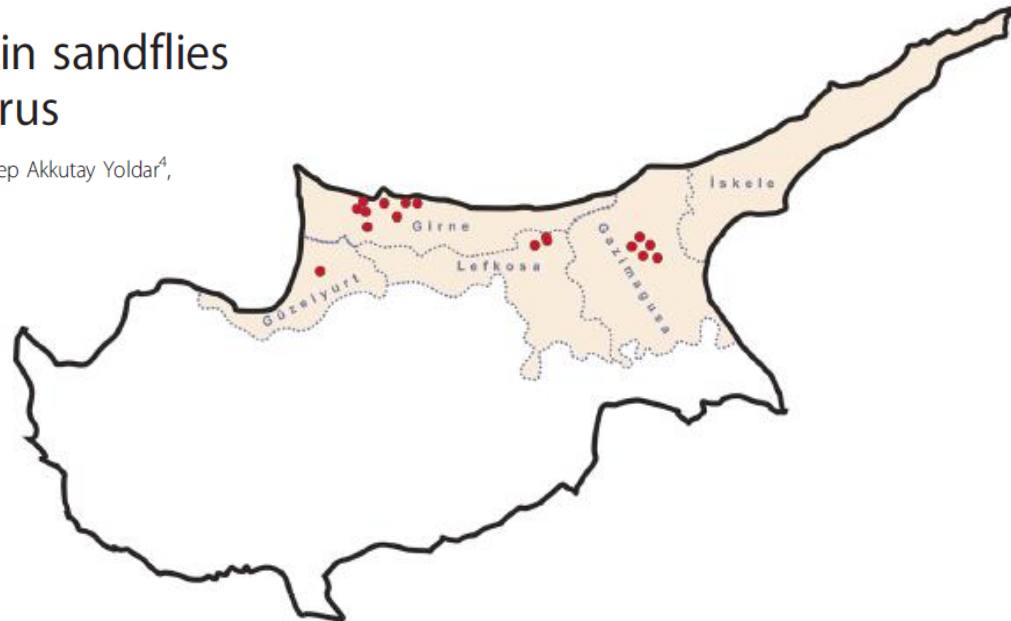


RESEARCH

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Phlebovirus and *Leishmania* detection in sandflies from eastern Thrace and northern Cyprus

Koray Ergunay^{1*}, Ozge Erisoz Kasap², Serra Orsten¹, Kerem Oter³, Filiz Gunay², Ayse Zeynep Akkutay Yoldar⁴, Ender Dincer⁵, Bulent Alten² and Aykut Ozkul⁴



- *L. infantum* DNA was demonstrated in *P. tobiasi* pools collected in Cyprus
- TOSV genotype A sequences were identified in *P. perfiliewi* s.l. and *P. tobiasi* pools from Cyprus
- According to the sequences two novel phleboviruses were also identified and named as Girne 1 and Girne 2

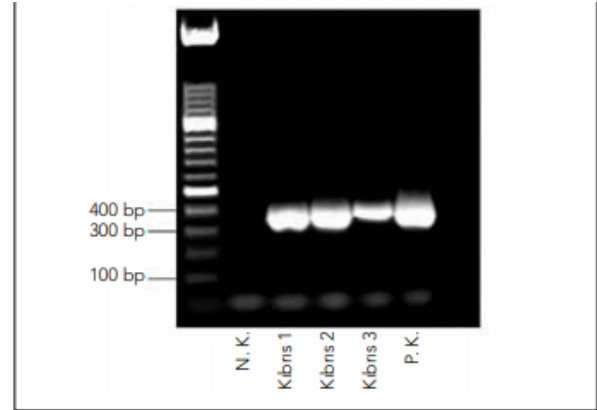
Kuzey Kıbrıs'ta Kanin Leishmaniasis ve Kum Sineklerinin Epidemiyolojisi

An Epidemiological Study on Canine Leishmaniasis (CanL) and Sand flies in Northern Cyprus

Seray Özensoy Töz¹, Hatice Ertabaklar², Bayram Göçmen³, Samiye Demir³, Mehmet Karakuş³, Suha Kenan Arserim³, İ. Cüneyt Balcıoğlu⁴, Tayfun Çanakçı⁵, Yusuf Özbel¹



A CanL case from North Cyprus



Şekil 2. 2012 yılında yapılan nested PCR sonuçlarının %1,2'lik jel elektroforezde gözlenmesi

N. K.- Negatif Kontrol, P. K.- Pozitif Kontrol

Tablo 1. Diseksiyon yapılan dişi kum sineklerinin türleri ve yüzde dağılımları

Kum Sineği Türleri	Sayısı	%
<i>P. tobbi</i>	92	31,08
<i>P. papatasi</i>	33	11,15
<i>P. galilaeus</i>	15	5,07
<i>P. alexandri</i>	8	2,70
<i>P. kyrenia</i>	1	0,34
<i>P. sergenti</i>	1	0,34
<i>S. azizi</i>	91	30,74
<i>S. minuta</i>	37	12,50
<i>S. fallax</i>	111	6,08

Pediatric Visceral Leishmaniasis Caused by *Leishmania infantum* in Northern Cyprus.

Sayili A¹, Ozkan AT^{2,3}, Schallig HD⁴.

⊕ Author information

Abstract

Visceral leishmaniasis (VL) is a vector-borne disease widespread in the Mediterranean basin, including Cyprus. During the last decades no cases were notified from northern Cyprus, but herein three cases of VL (female: 2, male: 1, median age: 24.6 months) diagnosed during their hospital admission between January 2011 and December 2012 are reported. Diagnosis was based on clinical findings; $1 \geq 1/64$ titer positivity of immunofluorescence antibodies, *Leishmania* amastigotes in Giemsa-stained slides of bone marrow, as well as molecular identification confirmed that in all three the infecting pathogen was *Leishmania infantum*. Fever, splenomegaly, and hepatomegaly were the typical clinical findings. First-line treatment with liposomal amphotericin B (AmBisome®; intravenous, 3 mg/kg) on days 1-5, followed by the same on days 10 and 21 yielded a successful outcome with no relapse in all cases. These confirmed VL cases found within 2 years demonstrate the presence of VL on the island.

J Vector Borne Dis. 2017 Oct-Dec;54(4):358-365. doi: 10.4103/0972-9062.225842.

Leishmaniasis in northern Cyprus: Human cases and their association with risk factors.

Ruh E¹, Bostanci A¹, Kunter V², Tosun O³, Imir T¹, Schallig H⁴, Taylan-Ozkan A⁵.

⊕ Author information

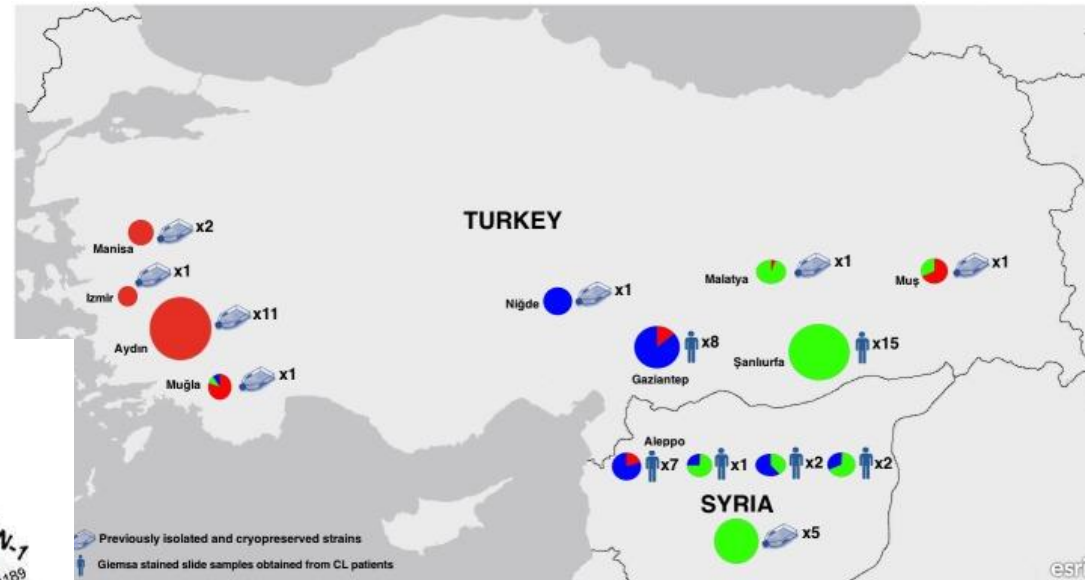
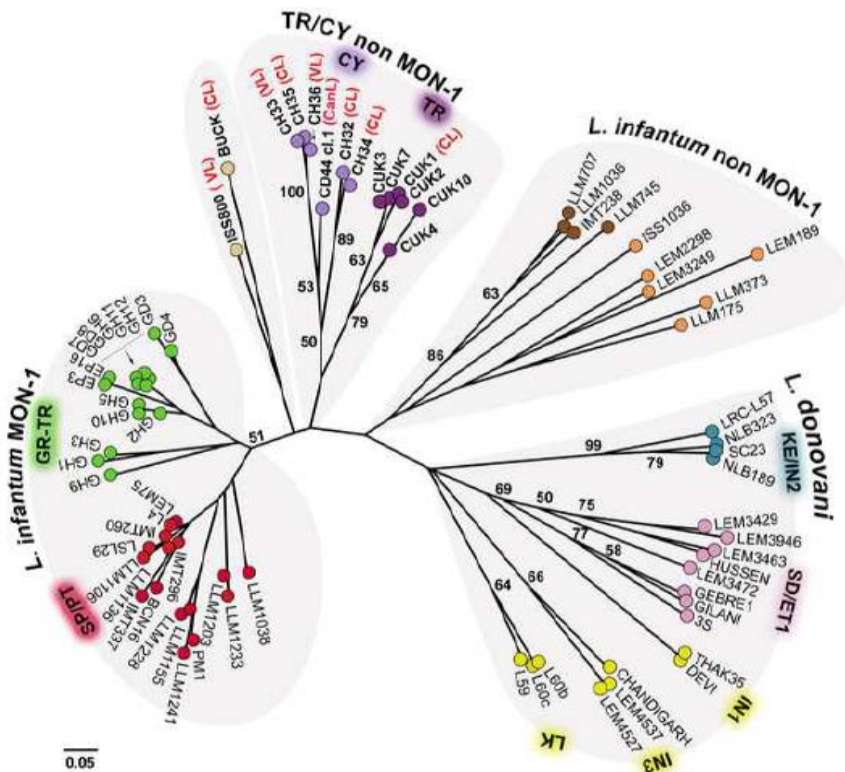
Abstract

BACKGROUND & OBJECTIVES: Cyprus is located in the eastern part of the Mediterranean Region where leishmaniasis is endemic. The primary objective of this study was to investigate human visceral leishmaniasis (VL) in the northern region of Cyprus where presence of canine leishmaniasis (CanL) and sandflies has been documented in earlier studies. The secondary objective was to assess the association of leishmaniasis with demographic and epidemiological variables.

METHODS: Intravenous blood samples were collected from 249 volunteers in Kyrenia district (located in the northern coastal region of Cyprus). Whole blood samples were tested for DNA of *Leishmania* spp by polymerase chain reaction (PCR), while serum samples were analyzed using direct agglutination test (DAT) and rK39 test. For evaluation of possible risk factors, a questionnaire was applied to the participants.

RESULTS: Only three (1.2%) of 249 participants were found seropositive by DAT (n = 2) or rK39 test (n = 1). The remaining samples were negative in serology, and no PCR positivity was detected in any of the 249 participants. Seven individuals, including the seropositive cases, had a history of cutaneous leishmaniasis (CL). Seropositivity and CL were not significantly related with gender (M/F: 40.2/59.8%), age [Mean: 42.85 ± 17.45, Median: 40 (7.86)], occupation (Indoor/Outdoor: 84.7/12.9%), dog ownership (52.6%), and CanL history (5.3%). However, a

Leishmania infantum and *Leishmania tropica* Geographical Distribution by Microsatellite Analysis



- Gouzelou et al. *PLoS Negl Trop Dis*. 2012;6(2):e1507.
- Karakuş et al. *PLoS Negl Trop Dis*. 2017 Apr 12;11(4):e0005538.

Multidisciplinary Diagnostic and Epidemiological Approach for Leishmaniasis

- Conventional diagnostic technique: Parasite positive or negative
- Serological and molecular techniques could help to understand the prognosis
 - When the parasite acquired
 - Is the patient asymptomatic or symptomatic
 - Drug resistance
- New diagnostic approaches
 - Optimisation and validation of the diagnostic tests
 - Compatibility of the test with the parasite species/subspecies in the geographical region
 - Preparation of diagnostic algorithm

Multidisciplinary Approach for Treatment

- We published CL guideline with dermatologists in Int J Dermatol recently
 - Active case detection and treatment for decreasing the morbidity
- We prepared CanL guideline with veterinarians in Turkey last year
- Treatment of CanL needs to be taken into account at multidisciplinary level
 - Antimonial and Milteforane usage for CanL treatment
 - Dog collar usage for controlling the disease

For Collaborative Studies

Establishing a common database for cases containing demographical, geographical, diagnostic technique, treatment/resistance, species info and prognosis

THANK YOU