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Note from the editors: Eurosurveillance special issue on leishmaniasis painting a picture of the situation in Europe

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After the publication of a special issue on Chagas disease in September 2011, this is the second time we focus on diseases caused by pathogens belonging to the family Trypanosomatidae. Even though *Trypanosoma cruzi* and *Leishmania spp*. infections have different impact on health, they are (still) neglected and we would like to raise awareness for aspects related to public health.

At the end of March 2012, we invited contributions for a special issue with the main aim to contribute to the existing body of evidence and to make available data that can help paint a better picture of the epidemiological situation and burden of autochthonous leishmaniasis in Europe [1]. The initial response was limited and we prolonged the deadline for submissions until the end of August 2012. The prolongation coincided with the allocation the first impact factor for *Eurosurveillance* [2] and, in addition to this, a leading European expert kindly supported the call and spread the word among his peers. We are not able to judge which element had most influence, however, by the end of the August deadline we had received 35 contributions from 16 countries for the special issue.

The evaluation of these manuscripts was a challenge for the editorial team and the supporting experts. We needed to apply stricter criteria and select only those papers which we deemed of highest interest for the readers of Eurosurveillance. This led to favouring papers focusing on human disease and in particular surveillance.

In the selection process we were forced to reject also manuscripts of good quality, and after peer review we agreed to publish 12 articles from 10 countries in Europe to ensure a good geographical representation. The coordination of the special issue took some time and we thank all contributors, peer reviewers and supporters, in particular Luigi Gradoni from the Istituto Superiore di Sanità in Rome, Italy, for their engagement and patience.

In this first part of the special issue we present surveillance data from five endemic countries in southern Europe: Bulgaria, Greece, Croatia, Italy and France, together with a rapid communication on an increase in leishmaniasis cases in northern Italy in 2012-13. The second part of the special issue will be published on 25 July. It will feature an editorial by Luigi Gradoni and two papers from Spain on a recent outbreak in Madrid with some unusual findings. Data from the Netherlands, a non-endemic country where a series of cases imported from within Europe were detected, will also be presented. Moreover, it will cover various relevant topics such as the role of indigenous phlebotomine sandflies and mammals in spreading the pathogen as well as aspects related to treatment with tumour necrosis factor-alpha antagonists and new diagnostic methods.

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Ongoing outbreak of visceral leishmaniasis in Bologna Province, Italy, November 2012 to May 2013

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An increased number of autochthonous visceral leishmaniasis (VL) cases has recently been reported in Bologna Province in northern Italy. Over six months from November 2012 to May 2013, 14 cases occurred, whereas the average number of cases per year was 2.6 (range: 0–8) in 2008 to 2012. VL was diagnosed in a median of 40 days (range: 15–120) from disease onset. This delay in diagnosis shows the need for heightened awareness of clinicians for autochthonous VL in Europe.

From November 2012 to May 2013, public health authorities, microbiologists and clinicians in Bologna Province, northern Italy, noted an upsurge in human cases of visceral leishmaniasis. During these six months, 14 cases were notified, an over five-fold increase compared with the annual average of 2.6 cases (range: 0–8) from 2008 to 2012. Here, we report preliminary epidemiological, microbiological and clinical findings.

Background

Visceral leishmaniasis (VL) is a severe disease primarily affecting the host's reticuloendothelial system and is caused by parasitic protozoans belonging to the *Leishmania donovani* complex. VL is endemic in Mediterranean Europe, where the disease is caused by *L. infantum*. Transmission is mainly zoonotic and occurs via the bite of a phlebotomine sandfly species of the genus Phlebotomus [1,2]. In Italy, the Tyrrhenian littoral, the southern peninsular regions and the islands have been considered classical endemic zones for VL, whereas in continental northern regions, VL has mainly affected human immunodeficiency virus (HIV)-positive patients. Since the early 1990s, however, a northward spread of the disease to previously non-endemic Italian regions has been observed. These regions also exhibited a progressive decrease in HIV co-infection rates [3]. A recent survey conducted in a continental area of north-western Italy, which was previously considered to be non-endemic, detected anti-leishmanial antibodies in 7.4% of healthy adults; for half of the seropositives, ongoing infection was confirmed by PCR analysis [4]. Currently, the occurrence of both asymptomatic and symptomatic leishmaniasis seems to be underestimated in Italy.

Outbreak description

In Italy, laboratory-confirmed cases of VL are reported by local public health departments to the regional authorities, which report cases to the Ministry of Health. The case definition for VL in Italy is based on the World Health Organization (WHO) case definition

Figure 1

Epidemic curve of human cases of visceral leishmaniasis, Bologna Province, northern Italy, November 2012–May 2013 (n=14)

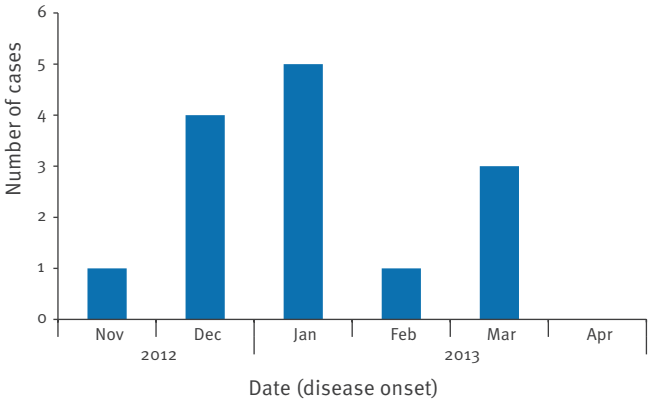
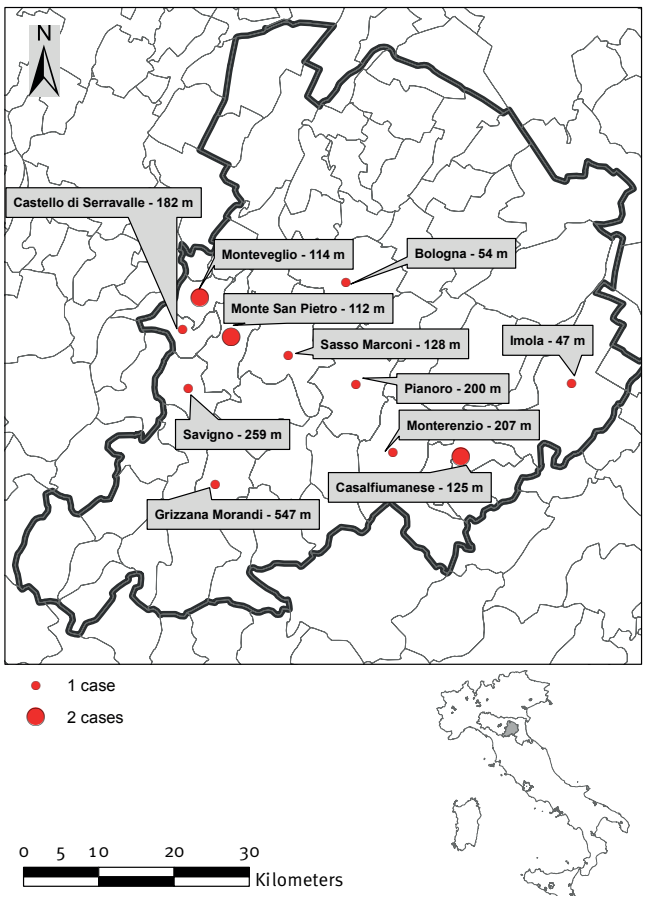


Figure 2

Geographical location of human cases of visceral leishmaniasis, Bologna Province, northern Italy, November 2012–May 2013 (n=14)



m: meters above sea level

and includes positive serology (indirect immunofluorescence antibody test (IFAT), ELISA, rK39-based immunochromatographic test (IC), direct agglutination test) and/or parasitology (microscopy, culture or PCR) for patients with suspected clinical symptoms [5].

From November 2012 to May 2013, 14 new cases of VL occurred in Bologna Province in northern Italy (Figure 1). Most patients resided in hilly, rural areas. Municipalities and their altitudes are reported in Figure 2.

The age range of patients was five months to 83 years, four cases were aged 18 months or younger, five were between 48 and 60 years-old, and five were between 62 and 83 years-old. The majority, 11 patients, were male. One patient was HIV-positive. Overall, five patients had known risk factors for VL, i.e. being under two years-old, (n=4) or being HIV-positive (n=1). All patients had symptoms compatible with VL, including a fever of unknown origin (n=13), mild to moderate anaemia (n=12), leukopenia (n=12), thrombocytopenia (n=10), hepatomegaly (n=7), splenomegaly (n=14) and weight loss (n=7). Interestingly, four of 14 VL cases presented with haemophagocytic syndrome (HS), a systemic hyperinflammatory disorder with severe dysfunction of immune homeostasis that may be secondary to VL [6,7].

VL was diagnosed in a median time of 40 days from disease onset (range: 15–120) and was supported by parasitological and serological means.

Laboratory investigations

Bone marrow aspirate was performed in 12 cases, but smear was available for microscopic diagnosis only in 10 cases. The detection of amastigotes was positive in three of 10 cases investigated, and the culture was negative in all nine tested cases (Table), suggesting a low parasite load in the bone marrow [8]. As expected [9], molecular methods enhanced parasite detection in bone marrow samples. Leishmanial DNA was detected by nested PCR and/or real-time PCR in seven of 10 patients and in the peripheral blood of an additional patient who was HIV-positive; thus, molecular methods were positive in eight of 13 VL cases (not performed in one case).

Serology performed using an indirect haemagglutination assay (IHAT) was negative in all eight cases tested, whereas the rk39-based IC [10] and IFAT were positive in all tested patients (10 of 10 and 13 of 13, respectively, see Table). Thus, serodiagnosis by IFAT or IC in patients with suspected symptoms was fundamental.

Indeed, in four of five cases in whom a diagnosis of VL was posited based on only suggestive symptoms and serological tests, the response to anti-leishmanial treatment (liposomal amphotericin B, 10 mg/kg intravenous, single dose) was appropriate, suggesting that our diagnostic approach was correct. One case resolved symptoms without treatment and is currently under clinical and laboratory evaluation.

Discussion

The risk of the emergence or resurgence of several exotic vector-borne pathogens in Europe, including chikungunya and dengue virus, has become a hot topic over the past decade, whereas other infections, such as leishmaniasis, have been neglected [11]. In fact, clinicians and microbiologists are often ill informed on the prevalence and symptoms of, and detection methods for VL, which may lead to initial misdiagnosis and a delay in diagnosis and treatment.

According to the epidemic curve (Figure 1), most cases of the ongoing outbreak in Bologna Province had disease onset in the winter months, indicating that patients were probably infected during summer and autumn 2012. Furthermore, half of the cases were diagnosed more than 40 days from disease onset and one of these cases was diagnosed 120 days after initial symptoms. This indicates a frequent delay in identifying the

Table

Parasitological and serological findings in visceral leishmaniasis cases, Bologna Province, Italy, November 2012–May 2013 (n=14)

Case number	Time to diagnosis	Parasitological findings	Serological findings
1	78 days	BM: microscopy - , PCR+	IHAT-, IC+, IFAT+ (1:80)
2	27 days	BM: microscopy- , culture-, PCR+	IHAT-, IC+, IFAT+ (1:160)
3	15 days	BM: microscopy -, culture-, PCR-	IHAT-, IC+, IFAT+ (1:160)
4	42 days	BM: microscopy - , culture-,PCR-	IC+, IFAT+ (1:40)
5	40 days	BM: microscopy+, culture-, PCR+	IHAT-, IFAT+ (1:80)
6	15 days	BM: microscopy -culture-, PCR-	IC+, IFAT+ (1:1,280)
7	80 days	BM: microscopy -, culture-, PCR-	IHAT-, IC+, IFAT+ (1:5,120)
8	40 days	PB: PCR+	IHAT-, IC+, IFAT+ (1:10,240)
9	120 days	PB: culture-, PCR-	IHAT-, IC+, IFAT+ (1:5,120)
10	20 days	BM: PCR+	IHAT-
11	30 days	BM: microscopy +	IFAT+ (1:320)
12	25 days	BM: microscopy - , PCR+	IFAT+ (1:1,280)
13	60 days	BM: microscopy+, culture-, PCR+	IC+, IFAT+ (1:320)
14	50 days	BM: culture- , PCR+	IC+, IFAT+ (1:640)

BM: bone marrow; PB: peripheral blood; IFAT: indirect immunofluorescence antibody test; IHAT: indirect haemagglutination test; IC: rk39-based immunochromatographic test; -: negative; +: positive.

parasitic infection. One third of the identified cases were revealed as having HS, and one child was erroneously diagnosed as having familial haemophagocytic lymphohistiocytosis (FHL). Symptoms for FHL and HS due to VL may overlap, and the differential diagnosis can be difficult [7, 12]. Thus, awareness of the increasing incidence of VL in areas previously considered to be at low risk is fundamental to avoid misdiagnosis, especially in infants in whom HS might be confused with FHL.

L. infantum is considered to be autochthonous in Bologna Province and *P. perfiliewi* is the predominant vector in this area [13]. In 1971–72, an outbreak of VL occurred in Bologna Province that affected 60 individuals, with 13 deaths [14]. Afterwards, the area remained endemic, with a low prevalence and an annual mean of 2.6 reported cases (range: 0–8 years) for the years 2008 to 2012 (R. Cagarelli, personal communication, May 2013). The reasons for the recent upsurge of VL cases in Bologna Province are unknown. Theoretically, current global warming in the Mediterranean area [15] may enhance leishmaniasis distribution due to the effect of temperature on parasite development and vector spread. However, according to WHO, there is no clear evidence indicating that sandfly and VL distribution in Europe have changed in response to climate change [2].

In conclusion, the increase in human VL cases in an area of northern Italy raises important public health concerns. Firstly, there is an urgent need to expand the existing control measures for canine leishmaniasis [13]. Secondly, healthcare professionals need to be informed of the upsurge in VL in the area to proceed with appropriate parasitological and serological tests in suspected cases, to promptly identify and treat cases of VL. Finally, the public needs to be aware of the potential exposure to sandfly bites in areas in which the parasite circulates, and to be educated in the use of appropriate preventive measures, such as mechanical and chemical repellents.

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Conflict of interest

None declared.

Authors' contributions

Designed the study: SV and MPL. Wrote the first draft: SV. Collected, synthesised and analysed the data: RC, ACF, LA, CS, FM, MG, AS, TDM, RT, GAG, RR and RT. Interpreted the results critically and revised the article to ensure important intellectual content: PV, LG and MPL. All authors read and approved the final manuscript.

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Geographical distribution and epidemiological characteristics of visceral leishmaniasis in Bulgaria, 1988 to 2012

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Visceral leishmaniasis is a sporadic illness in Bulgaria. However, cases in humans are registered nearly every year. This study describes the geographical distribution of the disease in Bulgaria from 1988 to 2012, over a period of 25 years. Cases were analysed according to age, sex, and place of residence. A total of 122 cases were registered in 25 years, 118 of which were autochthonous and four of which were imported from endemic countries in southern Europe. The average annual incidence for the study period was 0.06 per 100,000 population, or an average of five cases per year (maximum 15 in 1989; no cases notified in 1991, 1995, 1996 and 2008). Cases of visceral leishmaniasis were recorded in 13 out of 28 regions in Bulgaria, mainly in the southern part of the country. The highest number of cases were registered in the regions of Blagoevgrad (n=36) and Stara Zagora (n=34). Data presented in this study show that there is ongoing transmission of visceral leishmaniasis in Bulgaria with a high mortality rate (1:7), affecting mostly children.

Introduction

Visceral leishmaniasis (VL) is a protozoan, vector-borne disease characterised by chronic course, remittent fever, hepatosplenomegaly, and anaemia to complete pancytopenia and secondary immunosuppression. *Leishmania infantum* is the causing agent of VL in the Mediterranean region. In areas endemic for VL, the disease tends to have a chronic course and children are especially affected [1]. The average incubation period of the disease varies from a few weeks to six months. Until recently, children aged between one and four years were the group most affected by endemic VL caused by *L. infantum* in southern Europe, North Africa, west and central Asia [2]. According to some authors, the ratio between children and adults with leishmaniasis in the Mediterranean region is 7:3 and the average age of the affected children is under four years [3]. However, in recent years, about a half of leishmaniasis cases in Europe have occurred in adults, following the appearance of the human immunodeficiency virus (HIV) infection and the increased number of patients receiving immunosuppressing treatments due to transplantation, malignancies or other underlying conditions [4].

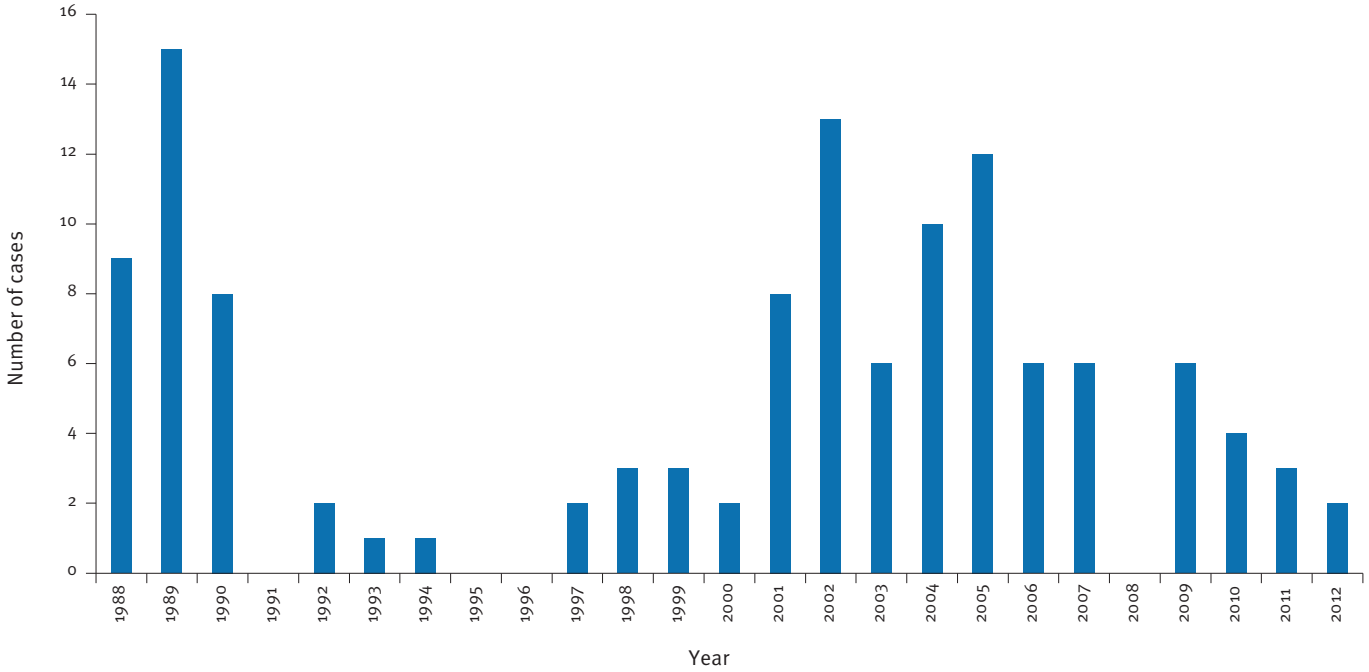
The first cases of VL in Bulgaria were reported by Mollov in 1921, who then described two clinical cases imported from Greece, and in 1937, the same author described the first autochthonous VL case in the country [5]. In the following 16 years, until 1953, a total of 57 autochthonous cases of VL were reported in Bulgaria, 50 of which were children. At that time, the disease occurred sporadically, mostly in the southern part of the country [6,7]. Between 1953 and 1988, only sporadic cases of VL were registered in the country. This was attributed to the mass use of dichlorodiphenyltrichloroethane, an organochlorine insecticide used during the eradication of malaria in Bulgaria, which led to the decrease in number and density of mosquitoes and phlebotomine sandflies that serve as vectors for leishmaniasis [8].

Studies on VL cases in Bulgaria were mainly focused on clinical aspects, diagnosis and treatment [9-13]. Over the last 15 to 20 years, the incidence of VL in Bulgaria increased significantly and the disease is now present in most parts of the country. Since 1988, autochthonous VL cases are registered almost annually.

Dogs are the principal reservoir hosts of *L. infantum* under domestic and peridomestic conditions [2]. In recent years, several studies on canine VL in Bulgaria were conducted. In 2004, a seroepidemiological screening for leishmaniasis among dogs was performed in 11 regions in Bulgaria: five regions in southern Bulgaria (Plovdiv, Stara Zagora, Yambol, Burgas, Blagoevgrad) and six regions in northern Bulgaria (Varna, Silistra, Ruse, Veliko Tarnovo, Pleven, Montana) (Figure 3) [14]. Sera from 220 dogs were tested by immunofluorescent assay, but none was seropositive. In 2006, clinical manifestations of leishmaniasis were observed and described in two domestic dogs in Petrich in southwestern Bulgaria [15]. In 2007, a seroepidemiological

Figure 1

Notified cases of visceral leishmaniasis, Bulgaria, 1988–2012 (n=122)



survey on the seroprevalence of *L. infantum* among dogs was carried out in two municipalities – Svilengrad and Petrich. Ten new cases of canine VL with clinical manifestations were diagnosed between November 2006 and November 2007 in these two municipalities [16].

There are no recent data on species composition of the phlebotomine sandfly fauna in Bulgaria. Few publications on the subject exist from the beginning of the 20th century and a single more recent study from 2011. Five species of the genus *Phlebotomus* were identified in the country so far: *Phlebotomus papatasi, Ph. sergenti, Ph. perniciosus* [17], *Ph. balcanicus* [18], *and Ph. tobbi* [19]. *Ph. perniciosus* is considered as a main vector, and *Ph. balcanicus* and *Ph. tobbi* are considered as potential vectors of VL [20].

The aim of the present study is to describe and summarise data from all registered cases of VL in Bulgaria from 1988 to 2012, by demographic information and geographic distribution and to compare the results with those published for other endemic European countries.

Methods

According to the Bulgarian legislation, VL is a mandatorily notifiable disease since 1978 [21]. Notification and registration of all cases of VL are regulated in two ordinances [22, 23]. All cases of VL are reported to the regional health inspectorate (RHI) by the diagnosing physician. Each case must be confirmed by additional tests at the Department of Parasitology and Tropical Medicine (DPTM) at the National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia. According to the legislation [23] cases of infectious diseases are classified into the following categories: possible, probable, and confirmed. Only confirmed cases of VL are subject to mandatory notification. RHI submits a summary of all recorded cases to the National Health Information Centre on a daily basis. The Centre processes the data collected from RHIs and sends a monthly summary of the situation to the Ministry of Health, the NCIPD, and the RHIs. Every year, the DPTM at NCIPD analyses the parasitic morbidity in the country. Based on this information, the Ministry of Health takes measures aimed at increasing the effectiveness of the surveillance system. Surveillance for VL comprises a set of activities including an epidemiological investigation of the cases on site and filling in a registration card. After treatment, the patients are subject to follow-up for one year.

For this study we used (i) data from registration cards of VL cases, (ii) data from the annual analyses of the parasitic morbidity in the country released by NCIPD each year and (iii) data from the clinical exams of patients checked at the DPTM at NCIPD.

The registration cards contain personal information, medical history, clinical and laboratory data, epidemiological and treatment information and data from parasitological laboratory tests.

We conducted a retrospective analysis of the medical records of the confirmed VL cases (both autochthonous and imported) in Bulgaria. The incidence per 100,000 population was calculated on the basis of

the information available from the National Statistical Institute about the number of the Bulgarian population per years [24, 25].

With the existing surveillance system in Bulgaria, underreporting of diagnosed VL cases is unlikely. Based on this, we consider the limitations or bias in this study as minimal. An approval from the ethical commission was not necessary for this study.

Results

During the study period, 122 cases of VL were registered (Figure 1). Of these, 118 were autochthonous with patients from 51 settlements (urban and rural) in 13 of the 28 regions in Bulgaria. Four imported cases were recorded in Bulgarian citizens who had visited European countries in the Mediterranean region [9-11,26].

Cases of VL were registered in all years between 1988 and 2012, except for 1991, 1995, 1996 and 2008. The peaks of incidence were in 1989 (n=15 cases), 2002 (n=13), 2005 (n=12), 2001 (n=10) and 2004 (n=10).

Throughout the years we could observe that the incidence of VL was fluctuating. In different periods as in 1988–1990, 2001–2007 and 2009 the incidence was higher compared to the established average annual incidence of 0.06 per 100,000 population for the whole period (Figure 2).

Age and sex distribution

Sixty-eight (56%) of the 122 patients were children and teenagers up to 18 years of age, and 54 (44%) adults. When analysing the age among children we found that the most affected group were children between one and two years of age (n=18), followed by children under one year (n=15). The group of children between zero and five years of age prevails among all persons with VL (n=48; 71% of cases among children and 39% of the total number of cases). The average age of the affected children in Bulgaria is 4.7 years. Ninety-one (75%) of the cases were male.

HIV co-infection

Among the 54 adults diagnosed with VL, only one person was co-infected with human immunodeficiency virus (HIV).

Mortality

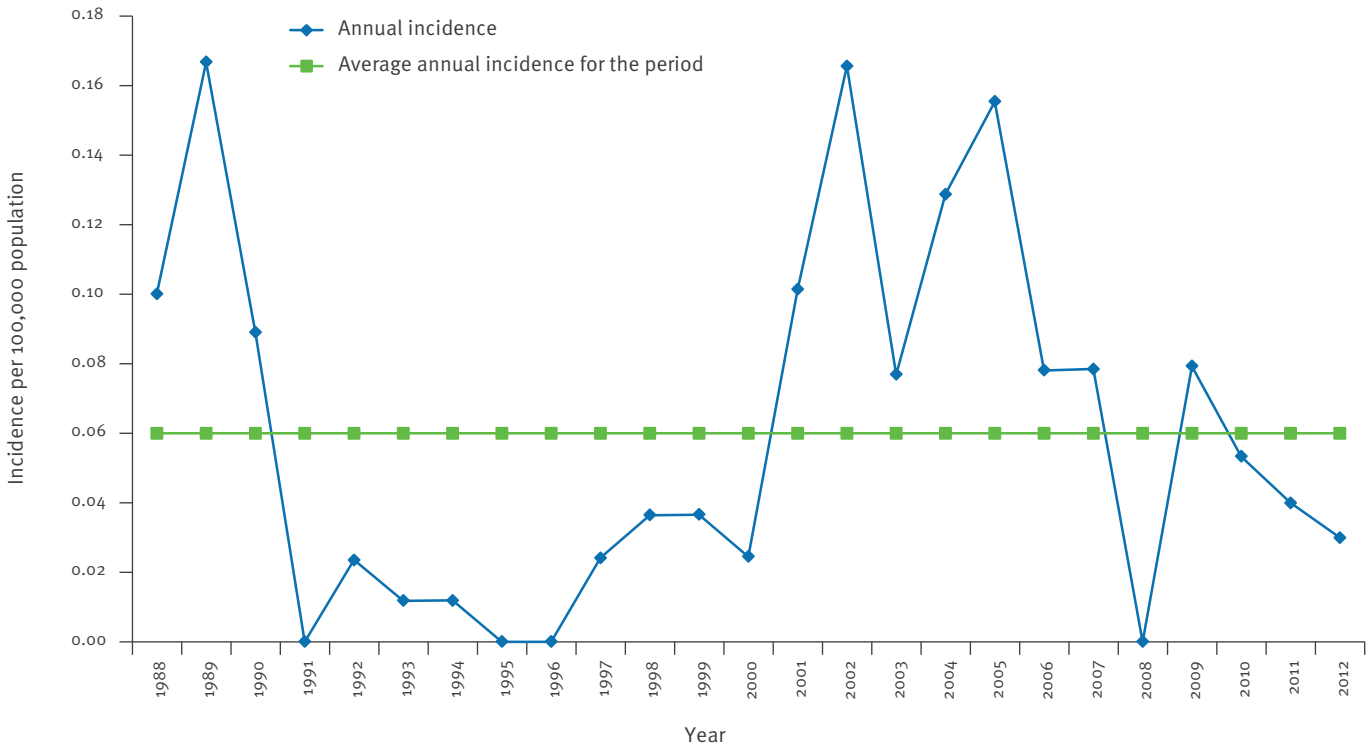
Seventeen people (13.9%) died from VL during the study period. The highest number of deaths (n=9) was registered in 1989. Average annual mortality for the whole period 1988 to 2012 was 0.01 per 100,000 population.

Seasonality

Cases of VL were recorded in all months of the year. In more than a half of the cases (61 of the 118 autochthonous cases) the first clinical symptoms were noticed during the months from October to January.

Figure 2

Incidence of notified cases of visceral leishmaniasis per 100,000 population, Bulgaria, 1988–2012 (n=122)



Table

Visceral leishmaniasis cases by age, sex, residence, and place of infection, Bulgaria, 1988–2012 (n=122)

Characteristic	Age		Sex		Residence[a]		Place of infection	
	Children	Adults	Men	Women	City	Village	Autochthonous	Imported
Number of cases	68	54	91	31	82	36	118	4
Percentage%	56	44	75	25	69	31	97	3

[a] For autochthonous cases only (n=118).

Figure 3

Autochthonous human cases of leishmaniasis (n=118) and results of seroepidemiological screening of dogs by region, Bulgaria, 1988–2012

FYROM: Former Yugoslav Republic of Macedonia.
Sources of data on dogs: [14,16].

Autochthonous and imported cases

The majority of cases (n=118; 97%) were autochthonous. In 2006 and 2007 four (3%) imported VL cases were registered in Bulgarian citizens, two of whom had travelled to Italy and two to Spain.

Residence and geographical distribution of cases

In terms of the type of residence in which the cases lived, 82 (69%) of them lived in urban and 36 (31%) in rural settlements (Table).

Cases of VL were recorded mainly in southern Bulgaria (Upper Thracian Plain and the Valley of Struma River), but cases of VL occurred also in northern Bulgaria indicating that the whole territory of the country is potentially endemic.

Figure 3 shows the notified autochthonous VL cases by region of residence. The highest number of autochthonous cases (n=96; 81%) was recorded in urban and rural settlements located at altitudes below 300 meters and typically with hilly landscape.

Discussion

In this paper we studied the distribution of VL in Bulgaria over a 25-year period from 1988 until 2012. The vast majority of patients were autochthonous cases and this is convincing evidence for the presence of local transmission of VL in rural and urban areas. Although Bulgaria is not situated in the Mediterranean, there are favourable environmental conditions for local transmission of the disease. Our results showed that most cases were probably infected during the warmer months of the year (June-October) when phlebotomine sandflies are active.

The average age of the affected children in Bulgaria (4.7 years) is similar to the Mediterranean countries. Most of the patients lived in urban areas and a possible explanation for this finding could be that some of the sylvatic foci are located close to the cities with recorded cases. Most people living in cities in Bulgaria are closely linked to the rural settlements because they have relatives living there and visit them often (farming, hunting, recreational activities). The fact that they visit the villages often for various activities means that they are more frequently in contact with these foci.

Only one case of HIV/VL co-infection was recorded. Although HIV-testing was not performed in all patients, we consider this number correct because in the follow-up observation for at least one year, no cases of relapse have occurred and all adult patients were definitely cured. Furthermore, this assumption may be supported to some extent by the low number of HIV-positive people in Bulgaria. According to the National Program for Prevention and Control of HIV/acquired immunodeficiency syndrome (AIDS) at the Bulgarian Ministry of Health, by the end of 2012, the total number of officially registered persons living with HIV/AIDS in the country was 1,647 [27]. This is different from other countries in southern Europe where in the past up to 70% of the cases of VL in adults were associated with HIV infection, and HIV/VL co-infection was distributed mostly among adults where 77.3% of the recorded cases with co-infection affected the age group between 31-50 years old [4,28]. The incidence of new VL cases in HIV-positive patients dropped about 50 to 65% in the Mediterranean area after highly active antiretroviral therapy (HAART) was introduced [29].

Even though cases occurred in nearly all parts of the country, most VL cases during the study period (110 of the 118 autochthonous cases) occurred among residents of the southern part of the country and the highest number of cases was found in south-western Bulgaria (Blagoevgrad region) and central-southern Bulgaria (Stara Zagora). The presence of single cases of VL in the northern part of the country is probably due to the very similar climatic, geographic and faunistic features of the separate geographical zones in Bulgaria which define the epizootology and epidemiology of the disease. Another potential explanation could be the fact that people living in the northern part of the country have acquired the infection while travelling in the rural regions of the south.

Still considered by some as a tropical disease, zoonotic VL is endemic in a number of southern European countries. Cases of VL have been recorded in all countries neighbouring Bulgaria: Turkey, Greece, Romania, Serbia and the former Yugoslav Republic of Macedonia [30,31]. In southern Europe the incidence is relatively low: 0.02–0.49 per 100,000 population (estimated at around 700 new cases per year) [32]. Our data are in line with these estimates: the average incidence of VL from 1988 to 2012 is 0.06 per 100,000 population and new cases of the disease are recorded locally almost every year.

Although the incidence of VL in Bulgaria is relatively low, its severe course and the possibility of a lethal outcome is a reason to regard leishmaniasis as an illness with high impact on public health.

Conflict of interest

None declared.

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Epidemiology of human leishmaniasis in Greece, 1981-2011

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Leishmaniasis is endemic and mandatorily notifiable in Greece. Epidemiological surveillance data for leishmaniasis in Greece between 1981 and 2011 are presented. In 1998, the notification system began distinguishing between visceral and cutaneous leishmaniasis. The mean annual incidence of reported leishmaniasis cases between 1998 and 2011 was 0.36 per 100,000 population. Of a total 563 leishmaniasis cases reported after 1998, 523 (93%) were visceral leishmaniasis cases. Incidence of reported visceral leishmaniasis cases fluctuated during this period, generally decreasing after 2007, with a small re-increase in 2011. The mean annual incidence rate of reported visceral leishmaniasis cases was significantly higher in less than four year-olds (p <0.001). Leishmaniasis cases occurred both in the country mainland and islands. Between 1998 and 2011, Attica concentrated almost half of the reported visceral leishmaniasis cases, with incidence rates in western Attica and western Athens above 12.00 per 100,000 population. Compared to visceral leishmaniasis, cutaneous leishmaniasis had a rather sporadic distribution, with many prefectures appearing free of cases. From 2004, the notification also included risk factors and of 287 cases with known immune status, 44 (15%) were immunocompromised. Moreover having a dog at home was reported by 209 of 312 leishmaniasis cases (67%), whereas 229 of 307 cases (75%) reported the presence of stray dogs near their residence. Linking clinical surveillance data with laboratory data and improving collaboration with the veterinary public health sector are some of the future challenges for leishmaniasis surveillance in Greece.

Introduction

Leishmaniasis is a vector-borne disease, caused by parasitic protozoans of the genus *Leishmania* and the disease is transmitted by phlebotomine sandflies [1]. Less common ways of infection include infected blood transfusion, congenital infection and parenteral transmission [2]. The most common forms of the disease in humans are the visceral and the cutaneous form. Visceral leishmaniasis causes a systemic disease characterised by fever, hepatosplenomegaly, anaemia and lymph node enlargement, and may be fatal without appropriate treatment, while cutaneous leishmaniasis mainly causes skin ulcers and is considered a less severe form of the disease [3].

Greece is considered to be an endemic country for both forms of the disease, with visceral leishmaniasis being the predominant form, endemic in nearly all geographical areas of the country and cutaneous leishmaniasis occurring sporadically [4,5]. *L. infantum* is the responsible species for the clinical manifestations of visceral leishmaniasis (and some cases of cutaneous leishmaniasis), while the vector species that transfer this type of parasite are *Phlebotomus neglectus, P. tobbi and P. perfiliewi* [6-9]. Anthroponotic cutaneous leishmaniasis is also present in Greece, caused by *L. tropica*, which is transmitted by P. sergenti [6]. Sporadic cases caused by *L. tropica* have been diagnosed both in the Greek mainland and in Greek islands [5,10,11].

The objective of this article is to present epidemiological surveillance data for human leishmaniasis in Greece, collected the last 30 years (1981–2011).

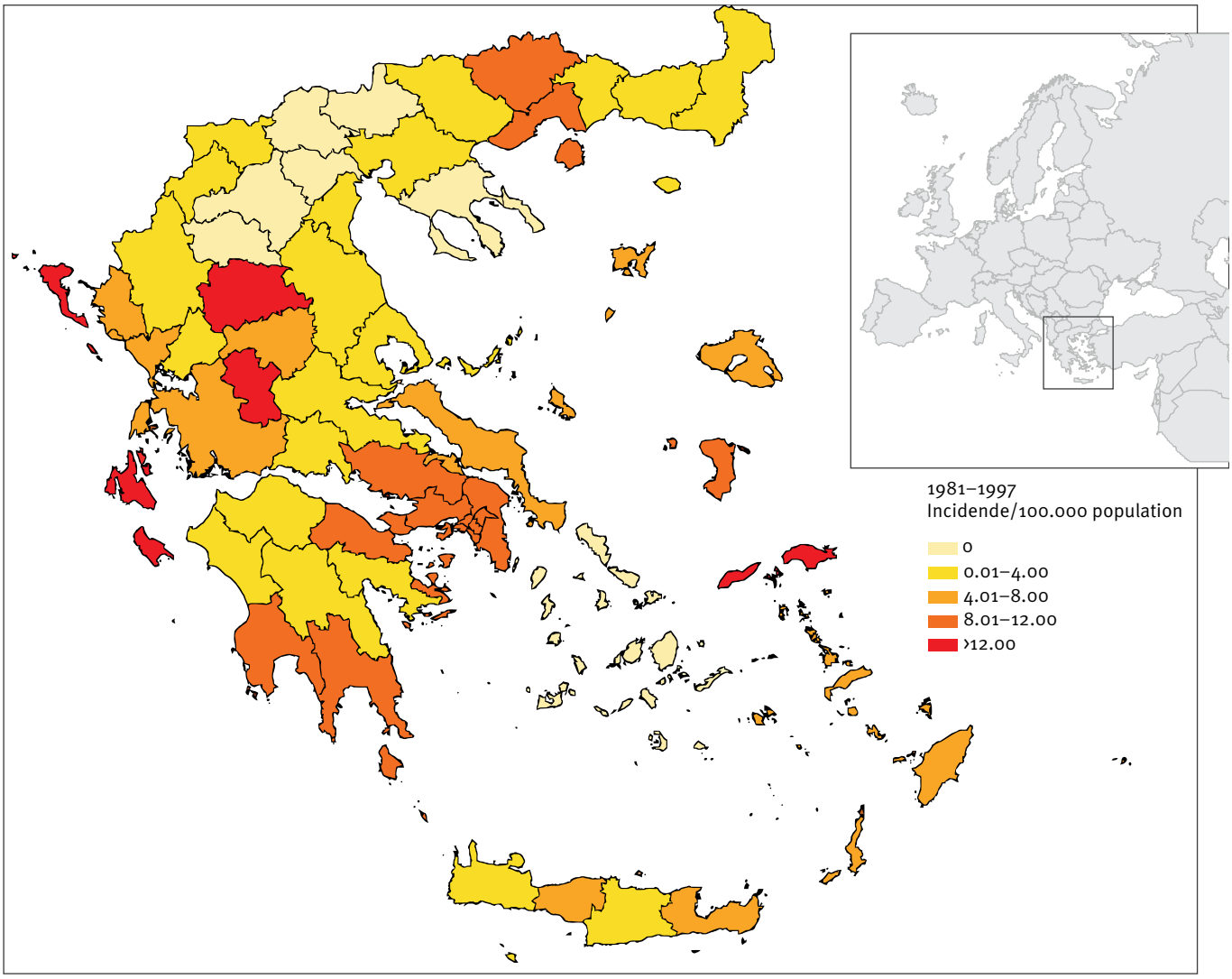
Methods

Leishmaniasis is a mandatory notifiable disease in Greece. The national mandatory notification system is operating since 1998 under the auspices of the Hellenic Center for Disease Control and Prevention, which is responsible for the collection, processing and analysis of epidemiological data on communicable diseases in the country. Prior to 1998, aggregated leishmaniasis data were notified directly to the Hellenic Ministry of Health via the prefectures' public health directorates of the country.

In 2003, the mandatory notification system was redesigned both in the context of harmonising the national surveillance system with the European Union (EU)

Figure 1

Laboratory-confirmed leishmaniasis cumulative incidence rate per 100,000 population by prefecture of cases' residence, Greece, 1981–1997 (n=688)



surveillance framework and in the context of preparations for hosting the 2004 Olympic Games. The consequent changes regarding leishmaniasis surveillance were mainly the alteration of the notification time frame from a monthly to a weekly basis and the use of a redesigned notification form that included risk factors for infection, as well as clinical manifestations and laboratory findings.

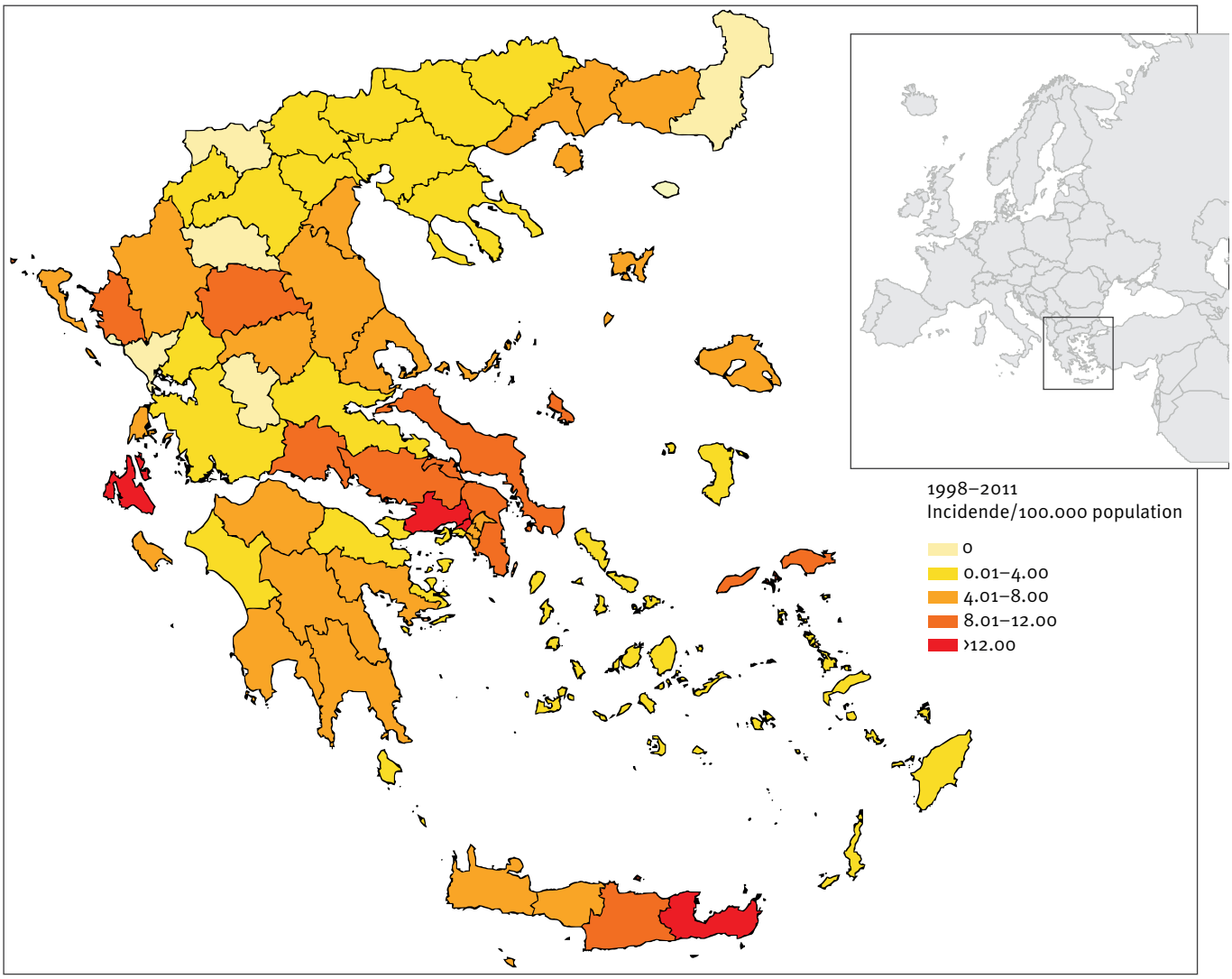
According to the Hellenic mandatory notification system, a confirmed visceral leishmaniasis case is an individual with clinical manifestations compatible with visceral leishmaniasis (persistent fever, splenomegaly, substantial weight loss, anaemia, lymph node enlargement) and laboratory confirmation via serology and/or detection of the pathogen on clinical samples. A confirmed cutaneous leishmaniasis case is an individual with clinical manifestations compatible with cutaneous leishmaniasis (appearance of skin lesions – nodular or ulcerative – usually on exposed areas of the body, which can be followed in some cases by the appearance of mucosal lesions) and laboratory confirmation via detection of the pathogen on clinical samples (in case of presence of mucosal lesions only, laboratory confirmation is performed via serology).

From 1981 to 1997, aggregated data on laboratory-confirmed leishmaniasis cases were derived from the Hellenic Ministry of Health records, while data on laboratory-confirmed leishmaniasis cases from 1998 to 2011 were derived from the national mandatory notification system. The distinction between visceral and cutaneous leishmaniasis cases was introduced in the notification process in 1998.

For a period limited to between 2004 and 2009, leishmaniasis cases were also reported from the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and

Figure 2

Laboratory-confirmed visceral leishmaniasis cumulative incidence rate per 100,000 population by prefecture of cases' residence, Greece, 1998–2011 (n=558)



Of a total 614 visceral leishmaniasis cases reported to the surveillance system in Greece between 1998 and 2011, the figure shows the 558 with available information on prefecture of residence.

Geographical Medicine of the Medical School of the University of Crete and from the Reference Laboratory for Opportunistic Infections of the Department of Parasitology, Entomology and Tropical Diseases of the National School of Public Health. Data on age, sex and risk factors were not available for these cases.

Data about leishmaniasis cases were compiled from 1998 through 2011 with respect to age, sex, Greek citizenship and hospitalisation. In addition, for the period from 2004 to 2011, during which the reformed mandatory notification system was operational, data were compiled regarding risk factors for the disease (owning a dog, presence of sandflies in the area of residence, presence of stray dogs in the proximity of the patients' residence, being immunocompromised), clinical manifestations and laboratory findings.

To assess temporal variation, annual incidences per 100,000 population were calculated for the period from 1998 to 2011, using data from the mandatory notification system and population data from the 2001 census population. Cumulative incidence per 100,000 population was calculated by prefecture of cases' residence, for the period from 1981 to 1997 (1991 census population), based on aggregated data from the records of the Hellenic Ministry of Health. On the other hand, cumulative incidence per 100,000 population was calculated by prefecture of cases' residence for the period between 1998 and 2011, based on data derived from the national mandatory notification system, and also including the cases reported from the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine of the Medical School of the University of Crete and the Reference Laboratory for Opportunistic Infections of the Department of

Parasitology, Entomology and Tropical Diseases of the National School of Public Health.

Incidence rate ratios were tested for significance using the chi-squared test. A p value ‹0.05 was considered significant. Data were analysed with Stata v 12.1., and area maps were created using Epi Map (EpiInfo v 3.4.3).

Results

Cumulative incidence rates of leishmaniasis per prefecture from 1981 to 1997

From 1981 through 1997, a total of 688 aggregated laboratory-confirmed cases of leishmaniasis were reported. The respective period's cumulative leishmaniasis incidence rate by prefecture of cases' residence is depicted in Figure 1.

From 1981 to 1997, in the mainland, the prefectures with cumulative leishmaniasis incidence rate of reported cases above eight per 100,000 population are located mainly in central Greece, Thessaly, southern Peloponnese, and eastern Macedonia. In the islands, cumulative incidence rates of reported cases above 12.00 per 100,000 population are observed for the islands of Corfu, Kefallonia and Zakynthos in the Ionian Sea, and in Chios and the island complex of Samos in the Aegean Sea.

Cumulative incidence rates of leishmaniasis per prefecture from 1998 to 2011

From 1998 to 2011, 563 laboratory-confirmed leishmaniasis cases were reported through the national mandatory notification system. An additional 101 cases were reported from the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine of the Medical School of the University of Crete and the Reference Laboratory for Opportunistic Infections of the Department of Parasitology, Entomology and Tropical Diseases of the National School of Public Health, for the years 2004 to 2009 (an additional 39% to the 260 cases reported via the mandatory notification system in the same period). From the total of 664 cases, 614 were visceral leishmaniasis cases and 50 were cutaneous leishmaniasis cases.

Figure 2 presents the 1998 to 2011 cumulative visceral leishmaniasis incidence rate by cases' prefecture of residence for a total of 558 cases for which residence was known.

During the years between 1998 and 2011, in the mainland, the prefectures with cumulative incidence rate of visceral leishmaniasis reported cases above eight per 100,000 population are located mainly in central Greece, with the Attica region, concentrating almost half of the reported visceral leishmaniasis cases (253 cases). In the islands, cumulative incidence rates of reported cases above eight per 100,000 population are observed mainly for the island of Kefallonia in the Ionian Sea (7 cases), for the Samos island complex of the Aegean Sea (5 cases), and for the island of Crete (Heraklion prefecture: 24 cases, Lasithi prefecture: 12 cases).

Of a total 50 cutaneous leishmaniasis cases reported to the surveillance system in Greece between 1998 and 2011, information on prefecture of residence was available for 47. In the mainland, the prefectures reporting cutaneous leishmaniasis cases were located in Peloponnese (Achaia, Arkadia, Ilia, Argolis, Lakonia), in central Greece (Aitoloakarnania, Phthiotis, Attiki, Evia), Thessaly (Trikala) and Macedonia (Thessaloniki and Serres). In the islands, cutaneous leishmaniasis cases were reported in Crete (Heraklion, Lasithi) and in Chios and Samos in the Aegean Sea. All of these prefectures had a cumulative incidence rate of cutaneous leishmaniasis reported cases below four per 100,000 population, with the exception of the Lakonia prefecture, which exceeded this value (6 reported cases in total).

Annual incidence rates of leishmaniasis

For the period from 1998 to 2011, the mean annual incidence rate of laboratory-confirmed leishmaniasis reported cases, based on data from the national mandatory notification system, was 0.36 per 100,000 population. Of the 563 laboratory-confirmed cases reported through the mandatory notification system, 523 cases (93%) were visceral leishmaniasis cases. The annual incidence rates of reported laboratory-confirmed cases of visceral leishmaniasis for the years between 1998 and 2011 (n=523) is depicted in Figure 3.

Visceral leishmaniasis annual incidence rate of reported cases presents fluctuations (mean annual incidence rate: 0.34, range: 0.17–0.46) and the lowest values are recorded in 1998 and 2003 (0.21 and 0.17 per 100,000 population, respectively). The low incidence in 1998 coincides with the beginning of reporting of leishmaniasis via the national mandatory notification system, while in 2003, the low incidence coincides with a reform of this system, whereby notification forms requiring information on risk factors for the disease, clinical manifestations and laboratory findings were introduced. From 2007, a decrease in the incidence rate below 0.36 per 100,000 population was observed, with a small re-increase in 2011.

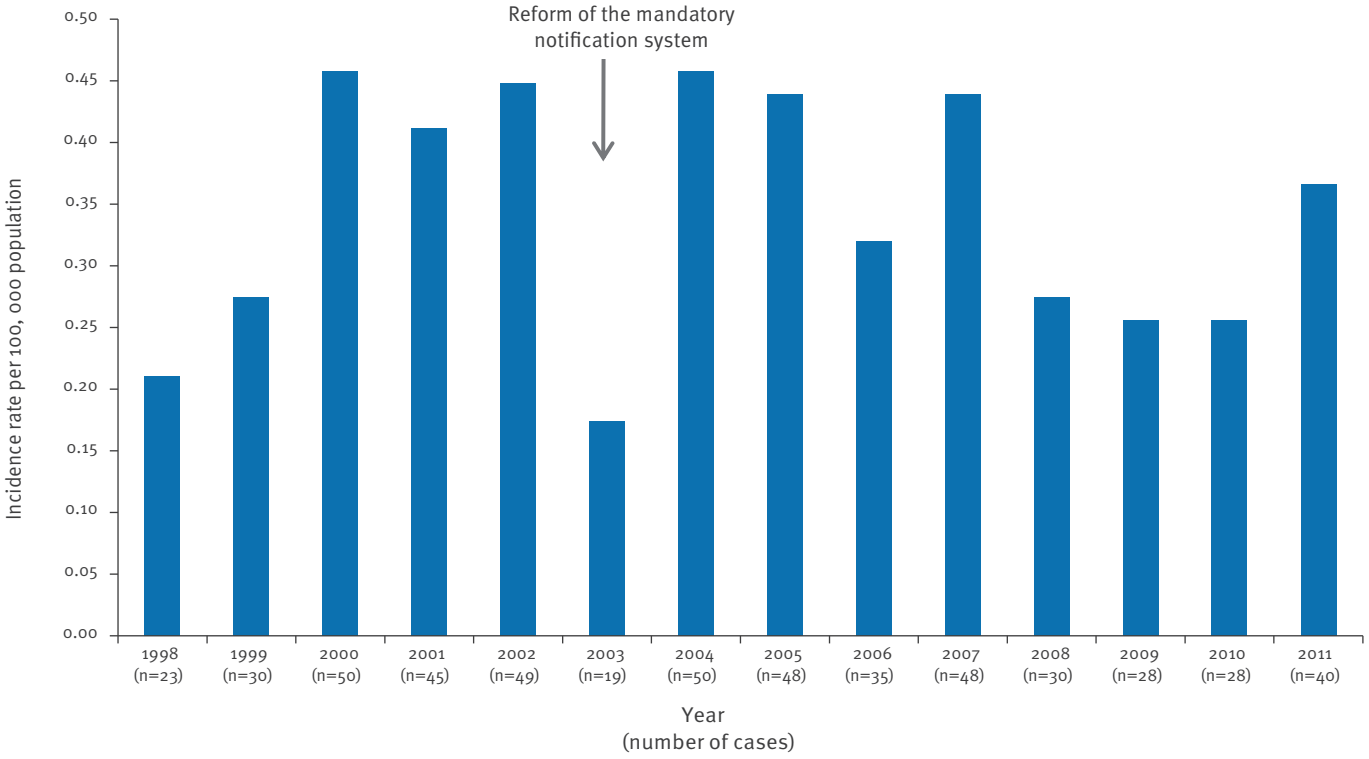
Age and sex distribution and origin of leishmaniasis cases

Information on sex and age was available for 500 (96%) of the visceral leishmaniasis cases, among which 330 (66%) were male. Distribution by sex and age is presented in Figure 4.

The disease was present in all age groups. The age group comprising individuals below four years-old had a statistically significantly higher mean annual incidence rate compared to every other age group (p value‹0.001 in all comparisons). The majority of cases in all age groups were of male sex.

Figure 3

Annual incidence rate of laboratory-confirmed reported cases of visceral leishmaniasis per 100,000 population, Greece, 1998–2011 (n=523)



The total 523 visceral leishmaniasis cases represented in the Figure are those reported by the mandatory notification system in Greece from 1998 to 2011.

Four hundred and forty seven cases of the 523 reported visceral leishmaniasis cases had Greek citizenship and 70 were of foreign origin (for 6 cases the relevant information was unknown). Among the 482 visceral leishmaniasis cases (92%) for which information about hospitalisation was known, 461 were hospitalised (96%). The number of reported visceral leishmaniasis cases showed no apparent seasonal trend. Cases were almost equally distributed by month of reporting (median number of reported cases by month: 43.6, range: 33–54).

A total of 40 of 563 cases reported by the mandatory notification system were cutaneous leishmaniasis cases. Information on sex and age was available for 38 cases, among which 22 were male. The age group comprising five to 14 year-olds had the highest mean annual incidence rate (0.044 per 100,000 population), followed by the age group with 15 to 24 year-olds, with a mean annual incidence rate of 0.032 per 100,000 population. Twenty four cases were Greek citizens and 16 were of foreign origin. Twenty one cases were hospitalised.

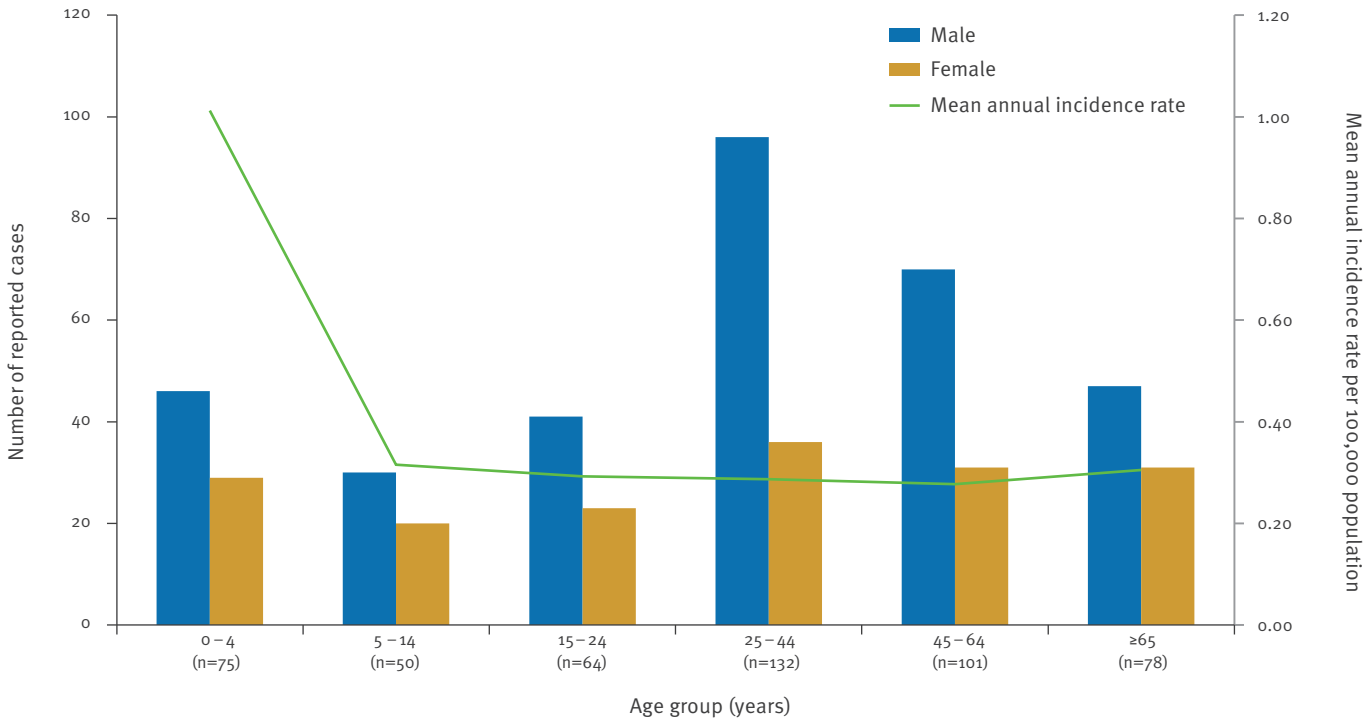
Clinical manifestations, laboratory findings and risk factors for leishmaniasis

From 2004 to 2011, a total of 330 leishmaniasis cases were reported via the reformed mandatory notification system. Information on immune status was available for 287 cases (87%), of which 44 (15%) were reported as immunocompromised. Information on the risk factor 'owning a dog' was available for 312 cases (94%), with a total of 209 (67%) cases reporting having a dog at home. Presence of sandflies in the area of residence was reported for 216 of the 298 cases for which information was available (72%). The respective percentage for the presence of stray dogs in the proximity of the patients' residence was 75% (229 of 307 cases, for which information was available).

Among the 330 cases reported from 2004 through 2011, a total of 307 (93%) were cases of visceral leishmaniasis. Two hundred and twelve (69%) of the latter were confirmed via serological testing and 121 (39%) via detection of the pathogen on clinical samples. Regarding clinical manifestations, 253 (82%) cases were reported with persistent fever, 260 (85%) with hepatomegaly or splenomegaly, 53 (17%) with

Figure 4

Age and sex distribution of reported visceral leishmaniasis cases, Greece, 1998–2011 (n=500)



The mean annual incidence is the mean annual number of leishmaniasis cases per 100,000 of the age group under consideration.
The 500 leishmaniasis cases represented in the Figure are those from a total of 523 reported by the mandatory notification system in Greece for which information on sex was available.

lymphadenopathy, six (2%) with cutaneous nodular lesions, two (1%) with cutaneous ulcerative lesions and five (2%) with mucosal lesions.

Among the 23 cases of cutaneous leishmaniasis reported from 2004 through 2011, five (22%) were confirmed via serological testing and 21 (91%) via detection of the pathogen on clinical samples. Information on clinical manifestations was available for all 23 cases. Of these 11 (48%) were reported with cutaneous nodular lesions, 14 (61%) with cutaneous ulcerative lesions and three (13%) with mucosal lesions.

Discussion

This report aims to provide epidemiological information for leishmaniasis in Greece during the last 30 years (1981–2011), by analysing national epidemiological surveillance data.

During this period, there were two important alterations in the way the disease is reported in the country; one in 1998 (which involved change from aggregated data collection to case by case data collection) and one in 2003 (which consisted in a reform of the national mandatory notification system, whereby disease specific notification forms were introduced, that included information on risk factors, as well as clinical manifestations of the disease and laboratory findings). Both alterations aimed to improve the disease's surveillance via a more thorough collection of information. We believe that once the introduced changes were incorporated in the system and assimilated by the reporting physicians, they contributed to a better description of the disease's epidemiological features.

The mean annual incidence of reported leishmaniasis cases per 100,000 population for the years 1998 to 2011 in Greece was 0.36. According to data from the Centralized Information System for Infectious Diseases/World Health Organization (CISID/WHO), the 1998 to 2010 mean annual incidence of reported leishmaniasis cases per 100,000 population for Italy and Spain was 0.23, whereas the respective number for France, for the years 2003 to 2010 was 0.24 [12]. The comparatively higher incidence in Greece may be the result of a number of factors, including for example warm climate, a high background of canine leishmaniasis and changes in agricultural pesticide practices that in the past contributed to sandfly population suppression [13]. In particular, high prevalence of canine

leishmaniasis, is becoming a crucial risk factor for leishmaniasis in humans [14], while serological screening in canine populations is thought to generally underestimate the existing prevalence of the infection [15].

Comparisons between countries are of limited value if not accompanied by an estimation of the magnitude of underreporting. In Greece, the magnitude of underreporting remains unknown, being crudely estimated by WHO as mild (1.2–1.8 fold) [16]. In this study, between 2004 and 2009, the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine of the Medical School of the University of Crete and from the Reference Laboratory for Opportunistic Infections of the Department of Parasitology, Entomology and Tropical Diseases of the National School of Public Health reported 101 leishmaniasis cases in addition to the 260 reported by the mandatory notification system. The total number of 361 cases during the 2004 to 2009 period corresponds to approximately 1,4 fold the number of cases reported to the mandatory system. Considering that the laboratories reporting these extra cases are two of the biggest performing *Leishmania* identification in the country, the value of 1.4 fold, as an estimation of the magnitude of underreporting seems to be consistent with the WHO estimation.

The annual incidence rates of the reported visceral leishmaniasis cases are presented starting from 1998, the year of the first reform of the Hellenic surveillance system. With the exception of the year 2003, where a low in the incidence rate (0.17 per 100,000 population) could be attributed to the reporting healthcare workers adapting to the newly introduced, redesigned notification forms, the annual incidence rate of the reported cases remained in general stable, with a decrease occurring after 2007 followed by a slight re-increase in 2011.

Only 40 (7%) of the 563 cases reported from 1998 to 2011 were cutaneous leishmaniasis cases. It is notable that a considerable number of cutaneous leishmaniasis reported cases (16 of 40) were of foreign origin, with a possibility of being imported.

Data from the mandatory notification system cannot be considered a reliable source of information regarding the responsible pathogens, as the relevant field on the mandatory notification form is rarely completed by the reporting physicians. In a survey conducted in the island of Crete, covering the period from 1986 to 2010, all isolated strains (n=16) from visceral leishmaniasis patients were of *L. infantum* type, while isolated strains from cutaneous leishmaniasis patients (n=5) were of *L. infantum* (n=3) and *L. tropica* type (n=2) [5].

Regarding visceral leishmaniasis, all age groups were affected, with 375 of 500 (75%) of the cases being older than 14 years-old. This is a finding that does not seem to conform to the findings of studies in other Mediterranean countries, such as Turkey and Malta, where the majority of visceral leishmaniasis cases is below this age [17,18]. Compared to every other age group, the age group comprising less than four year-olds in Greece had a statistically significantly higher mean annual incidence rate. Cutaneous leishmaniasis infection is reported to be more frequent in the age groups of five to14 and 15 to 24 year-olds, a finding that seems to be in line with data from Turkey, where the infection is reported to be more frequent in the age group of 10 to 19 year-olds [18].

During the period from 1998 to 2011, Attica concentrates almost half of the reported visceral leishmaniasis cases, with western Attica and western Athens presenting incidence rates above 12.00 per 100,000 population, whereas their incidence rate was lower in the first study period from 1981 to 1997. This observed cumulative incidence rate increase between the two periods could be probably explained by an increase in seroprevalence in dogs in Attica [19]. In the island of Kefallonia in the Ionian Sea and in the island complex of Samos in the Aegean Sea, stable cumulative incidence rates of the disease's reported cases of above 8.00 per 100,000 population are observed across the two study periods, a finding that should be interpreted with caution, as in the case of rare diseases, areas with small populations appear to have high incidence rates even when a small number of cases occurs. Another similar example is that of the Evritania prefecture, with a population of approximately 20,000 people. Although Evritania has a zero cumulative incidence rate after 1998, it appears as a high cumulative incidence rate prefecture before 1998, although only three cases of the disease were reported since 1981.

A cumulative incidence rate increase after 1998 is observed in the prefectures of the island of Crete, which could be explained by an increasing tendency in seroprevalence and incidence in dogs in Crete [20]. Another possible explanation could be a reporting and diagnostic bias, due to the location in the island of the Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, which was established there in the early 1990s.

Finally, comparing visceral and cutaneous leishmaniasis geographical distribution during the period from 1998 to 2011, it is notable that cutaneous leishmaniasis has a rather sporadic geographical distribution, with a large number of prefectures appearing free of cases.

In 2003, notification forms were redesigned to include laboratory data and risk factors related to leishmaniasis infection. Being immunocompromised was reported by 15% of the cases for which immune status was known, although no data is collected through the mandatory notification system regarding co-infection with human immunodeficiency virus. 75% of cases for which relevant information was available, reported presence of stray dogs in the proximity of their residence, whereas the percentage of cases owning a dog was lower (67%).

As stray dogs live outdoors, the possibility of exposure and infection is expected to be much higher than in pets that are used to stay indoors [17]. According to data from the Hellenic Veterinary Association, the total number of owned dogs in Greece is estimated to be around 500,000, leading to a crude estimation of approximately 15% of the general population having a dog at home. Analytical studies could shed more light regarding interdependence between presence of dogs and acquisition of human infection.

The emergence of leishmaniasis in non endemic European countries as well as the re-emergence of the disease in the Mediterranean region of Europe have recently been identified as possible scenarios, whereas there are indications that the disease has been more or less neglected at the public health policy level [6,18]. In order to be able to perform an effective risk assessment at the European level, the availability of data about leishmaniasis and its spatial distribution in Europe and the Mediterranean region is crucial. Having robust and effective national surveillance systems is an important step in this direction and efforts to improve surveillance should be systematic and continuous. Linking laboratory data with clinical surveillance, as well as coordinating the exchange of information between the human public health and the veterinary public health sector are some of the challenges that the Greek surveillance system has to meet in the future.

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Asymptomatic *Leishmania infantum* infections in humans living in endemic and non-endemic areas of Croatia, 2007 to 2009

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The prevalence of asymptomatic leishmaniasis in the general population of Croatia has not been studied to date. To assess the prevalence of *Leishmania infantum* specific IgG antibodies among immunocompetent residents of Croatia, sera from 2,035 persons (eastern coast of Adriatic Sea, n=1,186; Adriatic islands, n=653; mainland, n=196), were tested by an enzyme immunoassay. A total of 231 (11.4%) persons had anti-*Leishmania* antibodies. Multivariate analysis revealed that seropositivity was associated with geographic location and age. Residents of coastal areas and islands were significantly more seropositive than mainland residents (odds ratios (OR) 20.37 to 28.51). Moderate to high anti-*Leishmania* seroprevalence was found throughout the eastern Adriatic coast and islands (4.0% to 22.2%) including the sites previously considered non-endemic. A highly endemic focus was identified in central coastal Dalmatia (seroprevalence 22.2%; OR: 1.72; 95% confidence interval (CI): 1.33-2.22). Regarding age, children aged 0-9 years were the most vulnerable group for asymptomatic *Leishmania* infection (OR: 2.19; 95% CI: 1.16-4.14).

Introduction

Leishmaniasis is caused by *Leishmania* spp., hemoflagellate protozoa belonging to the family Trypanosomatidae. Infected phlebotomine sandflies serve as vectors for the transmission of all *Leishmania* species. Human leishmaniasis can be divided into three main disease manifestations: (i) visceral leishmaniasis (VL), (ii) cutaneous leishmaniasis (CL) and (iii) mucocutaneous leishmaniasis (ML). The strain of the infecting organism and the host's immunologic status greatly influence clinical manifestations.

Zoonotic VL caused by *Leishmania infantum* is endemic in Mediterranean countries of Europe and domestic dog is the main reservoir [1-3]. During the last decade, there have been reports of spreading leishmaniasis northward into previously non-endemic areas of central and northern Europe [1-3]. It is well known that, besides clinical cases of VL, asymptomatic infections are common in endemic areas [2,3].

Croatia encompasses 56,538 km2. According to the 2011 census, it had a population of 4,284,889 [4]. Geographically, Croatia is composed of three areas: the Adriatic coastal zone with islands in the south, the Pannonian Plain in the north, and the mountainous region in-between. The Croatian littoral comprises a relatively narrow land belt with islands along the eastern coast of the Adriatic Sea (Figure). The littoral is traditionally divided into two large areas based on geography, ecology and cultural heritage: the northern (Istria and Primorje counties) and the southern part (Dalmatia) which is further subdivided into northern, central and southern Dalmatia. The Adriatic islands and the coastal zone are characterised by Mediterranean climate which provides good living conditions for sandfly vectors of *Leishmania* [5,6]. Continental Croatia has temperate continental or continental climate and is usually considered free of phlebotomine vectors of *Leishmania*.

Human VL and canine VL has been reported in central and southern coastal and insular Dalmatia (from Split to Dubrovnik) since 1930 [7]. From 1931 to 1957, 398 human VL cases were diagnosed in this region [8]. A case of CL was firstly recorded in 1945 and 201 CL cases were recorded by 1957. After the late 1950s, the number of VL cases declined, probably because of mass spraying with antimalarial insecticides [8]. Since 1990, studies have identified re-emerging foci of both human and canine VL in central and southern Dalmatia [9-11].

In Croatia notification is compulsory for both VL and CL, although these diseases are not included in the decisions of the European Parliament for reporting communicable diseases [12, 13]. Medical practitioners are notifying human leishmaniasis cases through

Croatia's health information system or directly to the epidemiologist in the regional public health institutes. Cases are defined as *probable* which is a case clinically compatible in endemic region or with epidemiological connection to a *confirmed* case, and confirmed that is a case laboratory-confirmed by positive parasitological (microscopy or cultivation) and serological (IFA, ELISA) tests. Case definitions for CL are similar, excluding the serological tests [13]. According to data periodically published by the Croatian National Institute for Public Health there were between one and four new cases of VL reported each year in Croatia in the last decade [14]. The estimated mean annual incidence of human leishmaniasis is 0.4 per 100,000 population [5]. Leishmaniasis in Croatia is described as predominately paediatric disease: almost half of VL patients are children up to the age of 10 years and the disease is more often found in men [9,10]. Most of the reported cases occurred among inhabitants of the Croatian coast and islands [9,10]. Besides this, few VL cases were diagnosed in Austrian [15] and Hungarian [16] tourists after returning from the Dalmatian littoral.

Recently, three *Phlebotomus* species, known to serve as vectors for *L. infantum* were found in central and southern coastal and insular Dalmatia [5]. A veterinary seroepidemiologic survey conducted in central Dalmatia among apparently healthy dogs using dot-ELISA, found canine seroprevalence ranging from zero to 42.85%, which was in accordance with previous recognition of central and southern coastal and insular Dalmatia as a high endemic foci of *L. infantum* for dogs [11]. In contrast to high seroprevalence ratios in dogs, no information about the prevalence of the infection in otherwise healthy human inhabitants of different Croatian regions is available.

The aim of this study was to assess the prevalence of IgG antibodies to *L. infantum* among healthy people living in different regions of Croatia, and to compare the seroprevalence in endemic regions with that in non-endemic regions. This is the first investigation on asymptomatic leishmaniasis in residents of Croatia.

Methods

The target population for our study was the apparently healthy general population in previously recognised endemic and non-endemic areas for leishmaniasis in Croatia. The studied areas known as endemic areas: insular Croatia, and central and southern Dalmatia, as well as areas previously considered nonendemic but with favourable Medieranean climate: northern Dalmatia, Istria and Primorje. Two counties in northern Croatia with continental climate where sandflies were considered rare or absent: Brod-Posavina County (centre Slavonski Brod) and the most north-western Međimurje County (centre Čakovec), were also included.

Serological survey

Serum samples were collected from 2007 to 2009 to examine the prevalence of IgG antibodies to *L. infantum* in Croatia. We used 'residual' sera of apparently healthy, immunocompetent individuals who did not show any symptoms of leishmaniasis and came to hospitals or clinics for routine laboratory check-ups or for blood donation. Participating hospitals' or clinics' laboratories were selected according to their geographic location: 14 served population along the Adriatic coast and two were from non-endemic parts of Croatia. Laboratories provided data on age, sex and site of residence for the study participants.

Table 1

Sex distribution in the study population (n=2,035) compared with that of the total Croatian population

	Individuals tested	Male	Percentage (%)	(95% CI)		Census population	Male	Percentage (%)
All								
Adriatic coast	1,186	571	48.15	45.30%	50.99%	1,247,133	605,605	48.56
Adriatic islands	653	268	41.04	37.27%	44.81%	113,875	56,658	49.75
Croatian mainland[a]	196	137	69.90	63.48%	76.32%	2,923,881	1,404,072	48.02
Region/Site of residence								
Adriatic coast								
Istria and Primorje	117	47	40.20	(31.32%	49.08%)	467,678	226,115	48.35
Nothern Dalmatia	159	84	52.80	(45.04%	60.56%)	255,158	124,849	48.93
Central Dalmatia	571	293	51.30	(47.20%	55.40%)	419,131	203,513	48.56
Southern Dalmatia	339	147	43.40	(38.12%	48.68%)	105,166	51,128	48.62
Croatian mainland								
Brod-Posavina county[a]	107	96	89.70	(84.0%	95.5%)	158,575	77,115	48.60
Međimurje county	89	41	46.10	(35.7%	56.4%)	113,804	55,601	48.90

CI: confidence interval.

[a] Healthy donor effect bias present in Brod-Posavina county as blood donors constituted the majority of the sample.

To determine the target sample size for each region we used published statistical tables, which provide minimal sample sizes that are necessary for given combinations of precision, confidence levels, and variability.

A minimum of 1,100 individuals were selected as the target sample size for the coastal region with previously recognised endemic foci in order to reach the precision level of ±3% under assumption of the confidence level of 95%, p=0.5 and the size of population greater than 100,000 inhabitants. For islands, the targeted precision on population with more than 100,000 inhabitants was ±5% resulting in a minimum sample size of 400, whereas for the reference non-endemic continental region, under the assumption of maximum seroprevalence of 2%, the targeted sample size was 84 to reach the ±3% precision level.

Convenience sampling was used to select respondents in the most economically, technically, and operationally feasible method [17].

To estimate whether the study sample represents the research setting population, we compared the percentage of men estimated from the study sample with the 2011 census data (Table 1).

We classified sites based on their seroprevalence as 'high endemic focus' and 'moderate seroprevalence'. We derived these definitions combining the data from the literature with the results of this study. In particular, in a study by Federico et al. a seroprevalence of *L. infantum* infection of 4% in the Rome and Caltanissetta area was marked as 'moderate' [18] whereas Marty et al. detected a seroprevalence of 38% in the high endemic focus of Alpes-Maritimes, using Western blot technique, which is more sensitive than the technique used in this study [19]. The seroprevalence rate in the majority of our coastal areas was around 7% and defined as 'moderate seroprevalence'. The one area with a seroprevalence of 22% had a significantly higher seroprevalence in comparison with surrounding areas, all of which were classified as 'moderate seroprevalence'. Therefore, that site was defined as a 'high endemic focus' and the definition was further supported by epidemiological data since the majority of cases of VL in Croatia are reported from 'high endemic foci' [5,9-11].

Sera were tested for the presence of IgG antibodies to *L. infantum* by commercial enzyme-linked immunosorbent assay (NovaLisa *Leishmania infantum* IgG, NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany), according the manufacturer's instructions. Declared specificity and sensitivity of the test were 85% and 91% respectively. A serum was considered positive when the ratio between the optical density (OD450) value of the serum and the cut-off was >1.1.

The study was approved by the ethics committees of the Split University School of Medicine and of the Split University Hospital. Individual informed consent was not required according to the ethical committee.

Statistical analyses

Data were analysed with statistical package SPSS 19.0 (SPSS; Chicago, Illinois, US). Associations of seropositivity with sex or geographic region were tested by Pearson's chi-square test whereas the significance of difference in median age of participants by seropositivity was tested by Mann-Whitney U test. The strength of association between seropositivity and the site of residence was estimated by odds ratio (OR), 95% CI and p-values. For each site observed seroprevalence was compared to unweighted average rate in the accompanying region (i.e. coastal or continental) and ORs were calculated from the standard 2x2 table.

A multiple logistic regression analysis was used to evaluate the potential risk factors associated with *Leishmania* infection, including age as categorical variable, sex, and region of residence. Significance level was set at 0.05. In case of multiple testing, we adjusted the p-values with the Bonferroni correction.

Results

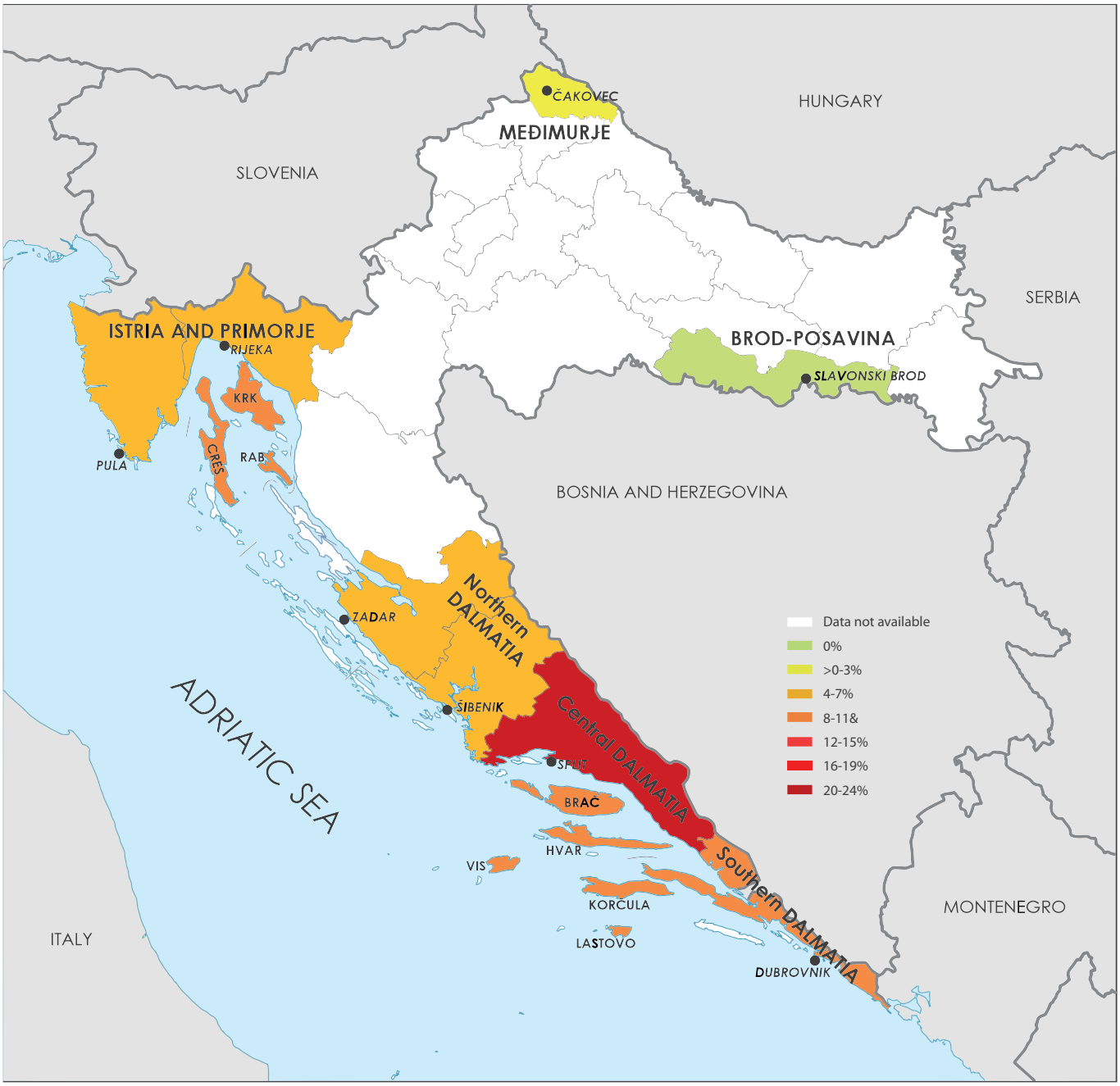
Blood specimens from 2,035 persons (975 men and 1,060 women) were collected. These included 1,186 persons in the coastal area from Istria to the Montenegro border, 653 on Adriatic islands and, for comparison, 196 residents of two northern continental Croatian counties (Table 2). The survey enrolled persons from all age groups, ranging from eight months to 88 years, with the median of 42 years (interquartile range (IQR): 21-59). The large deviation from the census data was observed only in the reference site in Brod-Posavina County, in which blood donors were overrepresented.

Of the total of 2,035 sera of healthy residents, anti-*L. infantum* IgG antibodies were found in 231 (11.4%). Seroprevalence differed significantly among Croatian sites ranging from 0.0% in Brod-Posavina County to 22.2% in central Dalmatia ($x2=112.24$; $df=13$; $P<0.001$) (Figure, Table 1). Overall, according to the place of residence, we found a strong association between seropositivity and residence in island, coast, or continental areas ($x2=35.41$; $df=2$; $P<0.001$). Post hoc chi-square analysis revealed that inhabitants of coastal areas had significantly higher seroprevalence than islanders ($x2=9.27$; $df=1$; $Pcorr=0.007$) or those from the two continental regions ($x2=29.43$; $df=1$; $Pcorr<0.001$). Furthermore, islanders had significantly higher seroprevalence than continental inhabitants ($x2=17.37$; $df=1$; $Pcorr<0.001$).

To analyse the spatial distribution pattern of *Leishmania* infection in more detail, we estimated strength of associations of seropositivity with the site of residence in particular regions: coastal, continental, or islands (Table 3). Only in Adriatic coastal counties seroprevalence of sites differed significantly from the average regional rate. While central Dalmatia inhabitants had

Figure

Geographic distribution of seroprevalence for anti-*Leishmania* IgG in asymptomatic healthy individuals, Croatia, 2007–2009 (n=2,035)



the highest prevalence of antibodies and the highest risk for *Leishmania* infection (OR: 1.72; 95% CI: 1.33 to 2.22; p<0.001), residents of the rest of coastal areas had lower risk (OR: 0.27 to 0.52; p-value: 0.002 to 0.006) as compared to the average rate in the coastal region. Although these results clearly indicated central Dalmatia as a high endemic focus for *Leishmania* infection, the observed seroprevalence in other coastal areas (ranging from 4.3% to 8.0%) also indicated these areas as endemic sites where the seroprevalence was higher than in continental Croatia.

Regarding sex and age as potential risk factors, we found no association of seroprevalence with sex (x2=0.11; df=1; p=0.739). Among 975 men, antibodies were found in 113 (11.6%) sera, while among 1,060 women, antibodies were found in 118 (11.1%) sera. This finding was further confirmed by the multivariate model with seropositivity as dependent, and age, sex and geographic location as independent variables. Multivariate logistic regression analysis of *Leishmania* seropositivity in association with age, sex and geographic location is shown in Table 4.

In contrast to sex, we found significant differences in the median age of seropositive and seronegative persons: 40 years (IQR: 16-58), and 42 years (IQR: 22-60), respectively (Mann-Whitney U test, p=0.039). This indicated age as a risk factor for *Leishmania* infection. Additionally, in the multivariate model with age, sex and geographical region as covariates, age was a significant predictor of seropositivity (overall significance p=0.022). The rates of seroprevalence for each age group of participants as well as the associated OR with 95% CI adjusted for covariates are shown in Table 4. Results show that *Leishmania* seropositives are most likely in 0 to 9 year-olds (17.5%; OR: 2.19; 95% CI: 1.16–4.14). It is noted that anti-*Leishmania* antibodies were found in nine of a subgroup of 71 children under the age of four, including one girl aged one year old. The data also show that *Leishmania* seropositivity does not continuously change with age. Instead, a bimodal distribution is indicated with comparable high risk of asymptomatic infection in those aged 0 to 9, 10 to 19 and 40 to 49 years (OR: 1.84 to 2.19; all p-values <0.05). In contrast, people of all other age groups had similar risk for *Leishmania* infection to those in the 30 to 39-year-old reference age group (OR: 1.00 to 1.33; p-values: 0.374 to 0.992).

Discussion

To our knowledge, this is the first study of the prevalence of asymptomatic *Leishmania* infection in the general population of Croatia. In order to determine the *Leishmania* infection distribution, residents of various geographical and ecological areas were included in a serological survey. The findings of this study reveal a strong association of seropositivity with geographic region (east Adriatic coastal and islands areas) and age group. The presence of seropositive people in northwestern coastal and island regions (Istria and

Table 2

Age and sex and seropositivity for anti-*Leishmania* IgG of study population by area of residence, Croatia, 2007–2009 (n=2,035)

	Number of sera	Participant age groups (years)							Information on age missing	Age range	Male (%)	Seropositive (%)
		0-9	10-19	20-29	30-39	40-49	50-59	60-69	>70			
Total	**2,035**	**177**	**272**	**250**	**246**	**242**	**315**	**205**	**290**	**38**	**0 - 88**	**975 (47.9)**	**231 (11.4)**
Region/Site of residence												
Adriatic coast	1,186	149	206	133	131	146	189	97	107	28	0 - 86	569 (48.0)	169 (14.2)
Istria and Primorje	117	12	13	11	27	17	17	10	10	0	0-83	47 (40.2)	5 (4.3)
Northern Dalmatia	159	10	29	19	15	21	36	15	14	0	3-84	84 (52.8)	10 (6.3)
Central Dalmatia	571	112	117	74	59	63	67	31	33	15	0-82	293 (51.3)	127 (22.2)
Southern Dalmatia	339	15	47	29	30	45	68	42	50	13	0-86	145 (42.8)	27 (8.0)
Adriatic islands	653	17	41	70	61	69	110	97	181	7	1-88	268 (41.0)	61 (9.3)
Croatian mainland	196	11	25	47	54	27	17	10	2	3	0 -72	137 (69.9)	1 (0.5)
Brod-Posavina county	107	0	12	25	34	19	10	4	0	3	18-62	96 (89.7)	0 (0.0)
Međimurje county	89	11	13	22	20	8	7	6	2	0	0 -72	41 (46.1)	1 (1.1)

Table 3

Seroprevalence of anti-*Leishmania* IgG by residence compared with unweighted average in the associated region, Croatia, 2007–2009, (n=2,035)

	p-value	OR	95% CI
Region/Site of residence			
Adriatic coast	Reference: seropositivity 14% (95% CI, 12-16%)		
Istria and Primorje	0.002[a]	0.27	0.11-0.67
Northern Dalmatia	0.006[a]	0.4	0.21-0.78
Central Dalmatia	<0.001[a]	1.72	1.33-2.22
Southern Dalmatia	0.002[a]	0.52	0.34-0.80
Adriatic islands	[b]seropositivity 9% (8-11%)		
Croatian mainland	Reference: seropositivity 0.2% (0.1-0.9%)		
Brod-Posavina county	0.758	0	NA
Međimurje county	0.849	2.22	0.14-35.83

CI: confidence interval; NA: not applicable; OR: odds ratio.

[a] Significant at level 0.01.

[b] Site samples were too small for reliable comparison.

Primorje) where cases of VL have not been reported yet, suggests that *Leishmania* transmission toward north may have occurred.

A highly endemic focus in central Dalmatia was confirmed in accordance with previous reports of both canine and human leishmaniasis in central and southern Dalmatia's coast and islands [5, 7-11]. However, the northwestern part of the Adriatic littoral, including Istria, Primorje, and northern Dalmatia, was considered a non-endemic region [5, 7-11]. In 2002, a case of VL was diagnosed in a patient who had no history of travel to known endemic regions and apparently had contracted the infection during his stay on the Velebit Mountain in northern Dalmatia [20]. Our results confirm this observation as they indicate that asymptomatic *Leishmania* infection is found throughout the eastern Adriatic coast and islands. Depending on the geographical location, a moderate to high (4%-22%) prevalence of asymptomatic infection has been observed.

Data from seroepidemiological studies conducted in other Mediterranean countries have also shown a variable prevalence (from 0.5 to 56%) of *Leishmania* infections depending on the geographic regions studied and on the test used for detection [3,21]. In our study we used a commercial ELISA as a relatively simple method for testing a large number of sera. Convenience sampling was used for selecting respondents. Despite some of its limitations, in a study by Kelly et al. it was shown that a convenience sample of sera from diagnostic laboratories was an appropriate sampling strategy to provide population immunity data to inform country's health policies [17]. Although the healthy donor effect bias was present in Brod-Posavina County, blood donors represented only a minority in other sites and are not expected to have influenced results.

The highest prevalence of 22.2%, significantly higher than in other coastal zones, was found in central Dalmatia. In comparison with other coastal areas, central Dalmatia has a high background of canine leishmaniasis and sandflies [5,6,11] which are likely to be associated with high prevalence of asymptomatic *Leishmania* infection among residents in this area. Central Dalmatia is also an active focus with the highest number of human VL cases in Croatia [9,10,14]. Most of VL cases diagnosed in Croatia during the study period from 2007 to 2009 occurred in central Dalmatia: seven cases were diagnosed with a mean annual incidence of 0.5 per 100,000 population (unpublished data). In southern Dalmatia, *Leishmania* exposure was higher than expected (8% seroprevalence), despite the lack or small number of clinical cases and apparently lower risk of infection than observed for central Dalmatia.

As expected, the seroprevalence was significantly lower in residents of continental areas of Croatia; in fact all but one person (a 40 year-old woman) were seronegative. As these regions are not considered to be endemic for sandflies, we cannot exclude the possibility that the one seropositive person could have been infected while traveling to the Croatian littoral where people from continental areas often spend their summer vacations.

The same possibility of travel-acquired infection with consecutive seroconversion has to be taken in account when the seemingly northward spread of asymptomatic leishmaniasis is interpreted. One could also speculate that autochthonous *Leishmania* infection may occur due to the observed spread of sandflies to

Table 4

Seroprevalence of anti-*Leishmania* IgG by age, sex and region of residence, Croatia, 2007–2009 (n=2,035)

Age group (years)	Sera tested	IgG positive (%)	p value	OR	95% CI
0-9	177	31 (17.5)	0.016[a]	2.19	(1.16-4.14)
10-19	272	39 (14.3)	0.048[a]	1.84	(1.01-3.38)
20-29	250	18 (7.2)	0.992	1.00	(0.50-2.01)
30-39	246	17 (6.9)		Reference	
40-49	242	37 (15.3)	0.015[a]	2.13	(1.16-3.93)
50-59	315	30 (9.5)	0.621	1.17	(0.63-2.19)
60-69	205	19 (9.3)	0.631	1.18	(0.60-2.36)
>70	290	30 (10.3)	0.374	1.33	(0.71-2.51)
Sex					
Men	975	113 (11.6)	0.61	1.08	(0.81-1.44)
Women	1,060	118 (11.1)		Reference	
Region					
Adriatic islands	653	61 (9.3)	0.003[a]	20.37	(2.78-149.2)
Adriatic coast	1,186	169 (14.2)	0.001[a]	28.51	(3.95-205.79)
Continental sites	196	1 (0.5)		Reference	

CI: confidence interval; OR: odds ratio.

[a] Significant at level p<0.05.

some regions at the North of Croatia [1,2], especially in light of the recent finding of *Phlebotomus* spp. in southern Hungary near the Croatian border [16].

In agreement with other reports [21-24] our study shows that there is no difference in the prevalence of asymptomatic *Leishmania* infection between men and women.

In respect to age, our prevalence results differ from findings in other studies. In the present study a bimodal distribution of *Leishmania* seropositivity by age, with peaks in young (0-19 years) and middle-aged (40-49 years) population, was suggested for asymptomatic population. Several studies reported the age distribution of seroprevalence to *Leishmania* in a healthy human population so far. The authors mainly noted a higher prevalence in older people suggesting that susceptibility to *L. infantum* infection increases with age [21-23] or they claimed no association with age [24,25]. Furthermore, Davies et al. suggested seroprevalence drops rapidly with age [26]. In some of these studies [21,23] age groups under 18 years were not included and/or samples were not equally distributed by age, resulting in different precision of seroprevalence estimate between different age groups. A study in Brazil, using a non-commercial ELISA found that 28.5% sera of 638 tested children aged between 0 and 5 years were positive, and concluded that infection is associated with the age of ≥2 years [27]. In our study, seropositives were most likely to be aged 0 to 9 years. Since in Croatia VL is still predominantly paediatric disease [6,7] it cannot be excluded that some of the seropositives in this age group might become symptomatic, therefore further differently designed studies with follow up of such participants are needed.

It can be concluded that compared to other parts of Croatia, seroprevalence is significantly higher in central and southern coastal as well as insular areas. This indicates the presence of asymptomatic *L. infantum* infection in humans and confirms the endemicity of these areas. This finding may be of particular importance in light of the increasing popularity of the Croatian coast and islands, from Istria to Dubrovnik, as a holiday destination for travellers from *Leishmania*-free areas or countries. Our data should alert physicians to consider leishmaniasis in the differential diagnosis of conditions such as unexplained febrile illness especially in immunocompromised subjects returning from these endemic areas. In addition, seropositivity observed in non-endemic areas and the higher seroprevalence in children should be investigated in the future. Therefore, further studies including clinical, parasitological, epidemiological and entomological investigation are required for elucidating the cycle of transmission, the maintenance and the role of *Leishmania* in human and animal health in different Croatian regions.

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The burden of visceral leishmaniasis in Italy from 1982 to 2012: a retrospective analysis of the multi-annual epidemic that occurred from 1989 to 2009

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Starting from 1989 Italy experienced an increase of visceral leishmaniasis (VL) cases over a baseline of 10 to 30 cases reported annually. The number of cases peaked in 2000 and 2004 with more than 200 cases/year, and subsequently declined to reach on average one third of the 2000 peak value in the period after 2010. A retrospective analysis from 1982 to 2012 showed that the multi-annual epidemic consisted of major components including (i) an outbreak involving infants and immunocompetent adults in parts of the Campania region (southern peninsular Italy) and that appears to have declined naturally, (ii) a second outbreak affecting human immunodeficiency virus (HIV)-infected individuals throughout the country, that declined owing to the use of highly active antiretroviral therapies (HAART), (iii) a generalised increase of VL cases in immunocompetent individuals and patients affected by associated conditions other than HIV from endemic regions of peninsular and insular Italy (other than Campania), which was due to a geographical spreading of VL foci, with no major case-clusters or outbreak features. A minor component consisted in the appearance of a few autochthonous cases in formerly non-endemic areas, starting from the early 1990s. Epidemic determinants and reasons for VL decline in the Campania region remain largely unexplained, despite the information available on canine reservoir and phlebotomine vectors in Italy.

Introduction

Visceral leishmaniasis (VL) is a protozoan disease transmitted by phlebotomine sandflies and caused by members of the *Leishmania donovani* complex. The disease results from the systemic intracellular infection of the macrophage-rich organs. The incubation period is long (an average of 3–8 months, reaching up to >10 years in case of reactivation from latent infections) and the chronic appearance of signs and symptoms makes a clinical suspicion difficult in low or non-endemic areas. Epidemiological features and determinants of VL outbreaks are largely diverse. Epidemics of anthroponotic VL caused by *L. donovani sensu stricto* (a species recently introduced in the Mediterranean [1]) have long been known to occur as multi-annual waves with, as seen in outbreaks in India and East Africa, thousands of cases in wide areas, followed by inter-epidemic periods of five to 20 years [2-5]. On the other hand, zoonotic VL, a disease caused by *L. infantum* for which domestic dogs act as the main reservoir hosts, exhibits a typical pattern characterised by isolated or small localised clusters of cases (usually less than 10), with large epidemics uncommon in the Mediterranean area [6]. The first documented outbreak of zoonotic VL occurred within the 1971 to 1972 period, near Bologna, Italy, with 60 clinical cases (13 deaths) diagnosed from villages where only a total of four cases had been documented in the previous 50 years [7]. Determinants of this outbreak have remained unexplained. Investigations led to the discovery and first worldwide description of asymptomatic VL cases [8]; indeed, chronic *L. infantum* asymptomatic infections have been shown thereafter to be widespread in southern European countries [9]. Very recently (2010–2012) another localised VL outbreak with more than 100 cases has occurred near Madrid, Spain, where major determinants have been identified in environmental changes and the unusual role of hares as reservoir hosts [10].

Between 1989 and 2009, Italy experienced an increase in the number of VL cases over a baseline of about 10 to 30 cases reported annually since the 1950s. The number of cases peaked in 2000 and 2004, when more than 200 cases/year were observed and thereafter began to decline. The bell shape of the epidemic wave, of 20-years in width, and the substantial lack of aggressive control programmes that could justify the general decline of VL incidence, strongly resemble in trend the historical *L. donovani* multi-annual epidemics, although the number of cases involved is dramatically lower. Indeed, there is still not enough knowledge on the epidemiology of *L. infantum*, and our surveillance data show that epidemics can present in a different way than expected. In this paper, we have retrospectively analysed both published and unpublished

information collected by our unit within the frame of VL epidemiological research and surveillance in Italy, in order to identify possible determinants explaining the recent trend of the disease in the country.

Methods

Study design

Human VL data collection was based on available notifications, review of published literature and unpublished information. Basic data for all patients included at least the year of diagnosis and the patient's residence at the level of the first administrative unit (region). More detailed information regarding selected patient groups were analysed from both published and unpublished data collected by our unit since 1982. Canine reservoir data collection was based on the review of published literature and unpublished information available at our unit or obtained from public veterinary reference centres in Italy.

Human data

VL is a compulsory notifiable disease in Italy since 1956. Diagnosed cases are recorded at the provincial local health units to which hospitals belong; notifications are gathered at regional level and subsequently centralised at the Ministry of Health. However under/late reporting may occur from some provinces or when VL is diagnosed in patients suffering from associated conditions such as human immunodeficiency virus (HIV) co-infection, or organ transplant.

Online notifications centralised at the Italian Ministry of Health (last update: 2009 [11]) and notifications available upon request at the regional or provincial local health units registries until 2012, were integrated with information stored in the database of our unit at Istituto Superiore di Sanità, which holds detailed patient's information from an average of 50% (annual range: 43–69%) of cases annually notified in Italy, thanks to centralised VL diagnosis and medical surveillance activities performed by our laboratories [12]. Diagnosis in clinically-suspected patients was routinely performed on clinical samples sent by hospitals (paediatrics, internal medicine, and infectious diseases wards) from throughout the country. Serology (immunofluorescent antibody test, IFAT) and microscopy, culture and polymerase chain reaction (PCR) (the last technique being employed routinely since 1997) on bone-marrow and/or peripheral blood (buffy coat) material were used in combination as recommended by World Health Organization guidelines [13,14]. If VL was confirmed, relevant information on patients was obtained, which included demographic data, place of hospitalisation, putative place of infection, concomitant/underlying conditions, drug regimens used and post-therapy results. Isolation of parasites from VL clinical samples was also routinely performed, resulting in a large collection of 871 *Leishmania* strains from different Italian areas and patient categories. Parasites were identified by multi-locus enzyme electrophoresis (MLEE) following the standard Montpellier (MON) nomenclature [15], and/or by molecular techniques suitable for *L. infantum* identification including PCR-restriction fragment length polymorphism (RFLP) of a repetitive DNA sequence [13] or by internal transcribed spacer 1 (ITS-1) nested-PCR-RFLP [16].

Data on canine leishmaniasis

Besides the analysis of published literature, that was employed to map historical canine leishmaniasis (CanL) seroprevalence rates, recent information on diagnosis in dogs was obtained for the period from 2005 to 2011 (and updated in 2012) from the network of 10 institutes for zooprophylaxis as well as from six collaborating faculties of veterinary sciences. The main objective, to be accomplished in the frame of the European seventh framework programme (FP7) project EDENext, consisted in the country-wide mapping of communes (lowest administrative level) with undisputable presence of autochthonous CanL as an indicator of endemic transmission.

Results

General trend of visceral leishmaniasis in Italy

Laboratory-confirmed recorded cases of VL in Italy from 1982 to 2012 are shown in Figure 1. Between 1950 and 1981, the number of annual cases ranged between 10 and 30 cases/year [17]. Starting from 1989 (44 cases), a steady increase was observed with a peak in 2000 and again in 2004 involving 215 and 204 cases, respectively. A progressive decline in the annual number of cases occurred during the subsequent eight years, reaching on average one third of peak cases in the period between 2010 and 2012.

Major components of the epidemic trend

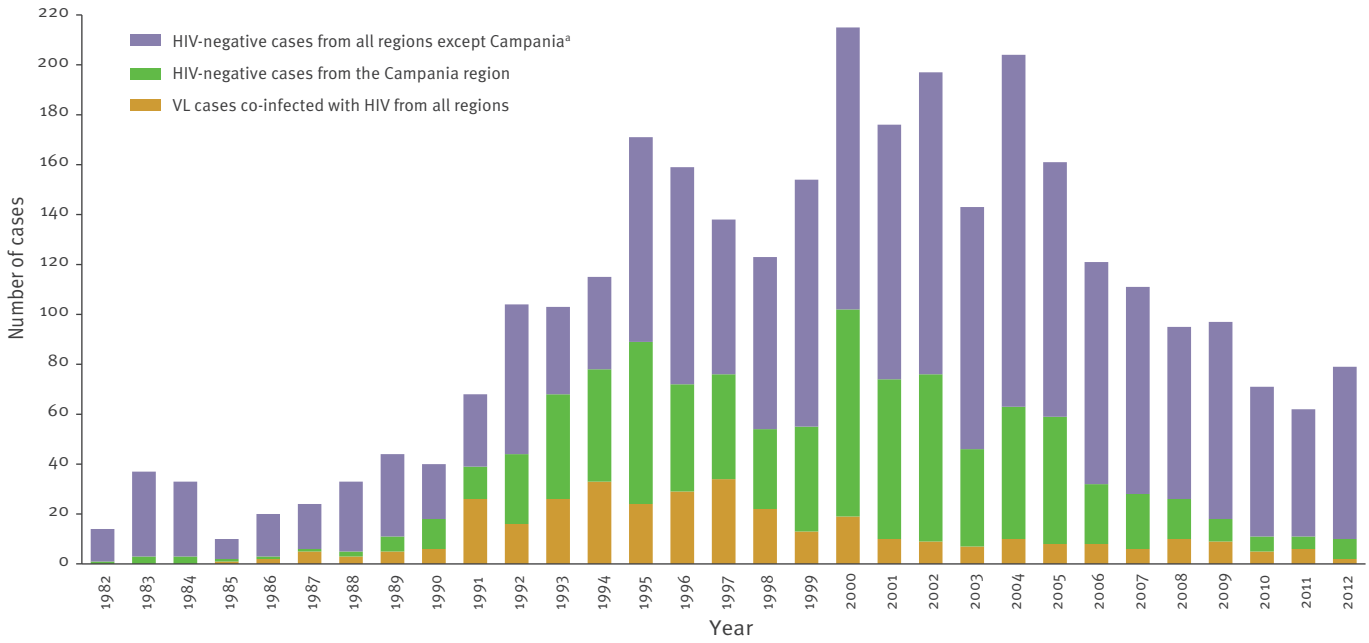
Three major components of the epidemic curve were identified including (i) an outbreak involving parts of three provinces of the Campania region, (ii) an HIV–VL co-infection epidemic and (iii) a generalised increase of VL in regions of peninsular and insular Italy.

Epidemic involving parts of three provinces of the Campania region

Starting from 1989, an increase of VL cases occurred, resulting in not only major hospitals with experience in VL management, receiving cases. As some cases were misdiagnosed and/or received inappropriate treatment [18], a dedicated surveillance was implemented in the Campania region consisting in VL testing centralised at Istituto Superiore di Sanità in Rome. The surveillance disclosed the beginning of an epidemic trend that, from 1989 to 1990, involved areas of three provinces, namely Naples (Vesuvius area), Caserta (inland area) and Salerno (coastal area). Case clusters were identified in several villages or peri-urban districts reaching an incidence of two VL cases/1,000 population in some years. A striking feature was that this epidemic was unrelated to HIV co-infections [19], which were occurring also in

Figure 1

Distribution of annual number of laboratory-confirmed visceral leishmaniasis cases in Italy, 1982–2012 (n=3,122)



HIV: human immunodeficiency virus; VL: visceral leishmaniasis.

[a] Each year the largest part (>90%) of the HIV-negative cases from all regions other than Campania, included patients from regions of peninsular and insular Italy, the remaining cases being from regions of northern continental Italy.

the form of an epidemic in other Italian regions in the same period. Parasite identification carried out on 225 strains up to the year 2003, revealed that about half of the cases (110 strains) were due to a novel zymodeme of *L. infantum* (MON-72) found also in dogs and in phlebotomine vectors from Campania foci [20,21]. The analysis of clinical records did not suggest any particular virulence of this zymodeme as compared to the commonest agent of Mediterranean VL, zymodeme MON-1.

Altogether 789 cumulative cases were diagnosed during the long outbreak period from 1989 to 2008 (Figure 1). After a peak of 83 cases in 2000, the annual number of cases dropped slowly to less than 10 cases/year after 2009. Since, the disease has become sporadic in all age groups, including children, and disappeared from most of the former 'outbreak villages and districts' although it emerged with a few cases in the remaining two provinces of Campania region (Avellino and Benevento).

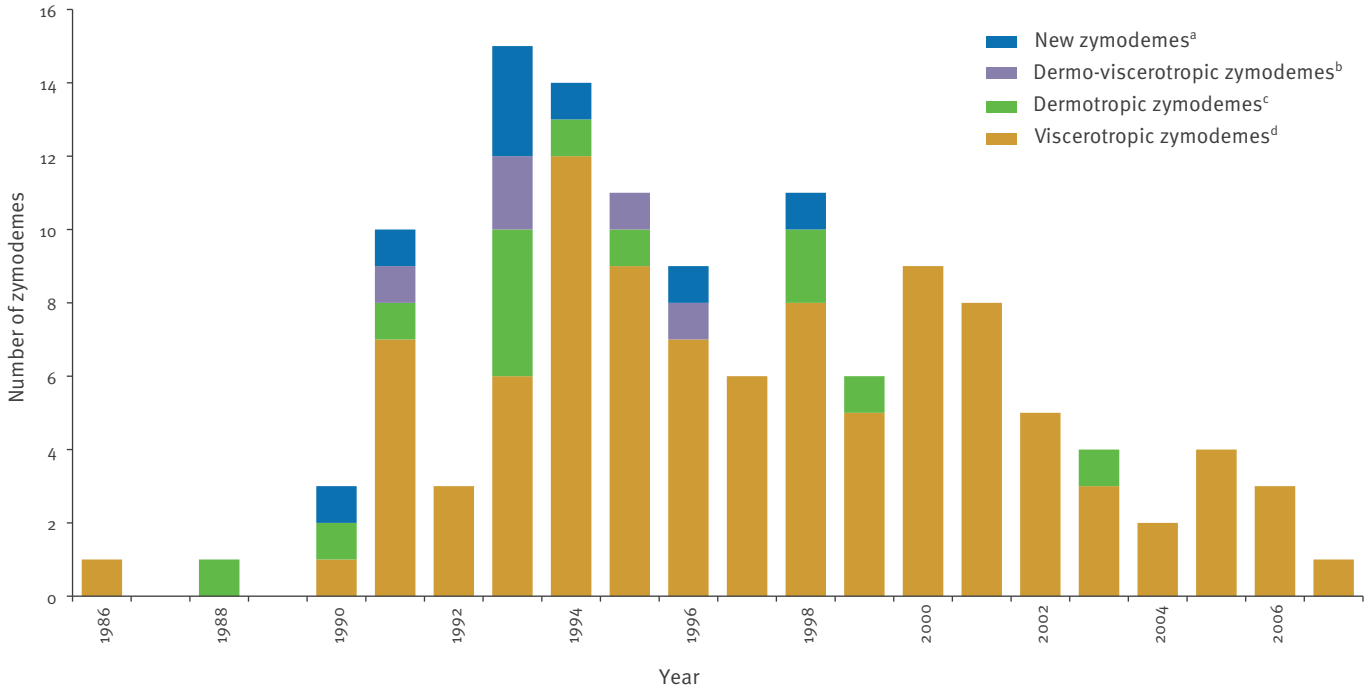
Human immunodeficiency virus–visceral leihmaniasis co-infection epidemic

A dedicated surveillance network centralised at World Health Organization headquarters, Geneva, was established after a meeting held at Istituto Superiore di Sanità, Rome, in 1994. From the beginning, our unit participated in the network by reporting information collected through a national monitoring system [22]. First HIV–VL cases were recorded in 1985. A sharp increase in the annual number of cases was observed in 1991 followed by two peaks in 1994 (33 cases) and 1997 (34 cases). The introduction and generalised use of highly active antiretroviral therapy (HAART) for HIV infection at a time between 1997 and 1998 resulted in a clear decrease in the incidence of HIV–VL co-infections. Since 2001, a plateau of about 10 cases/year or less has been observed until present (Figure 1). Cases were recorded from several regions of Italy, with higher incidences in Sicily, Lombardy and Latium. In all the epidemic phases, clinical and epidemiological features of HIV–VL were very similar to those of other Mediterranean affected countries, such as Spain and France, and have been reviewed for all cases recorded up to 2006 [23]. VL relapses, which occurred invariably in acquired immunodeficiency syndrome (AIDS) individuals before the HAART era, became less frequent in these patients following the epidemic decline. Of note, these relapses still characterise a number of co-infected patients, resulting in a significant impact on the public healthcare system.

The characterisation of *L. infantum* strains isolated from HIV-patients in Italy until 1998 has been extensively reviewed [15], and shows the high level of genetic polymorphism of these strains. In subsequent years, 51 new strains were isolated and identified by MLEE and/or molecular methods. Altogether, 206 strains (163

Figure 2

Yearly composition of zymodemes of *Leishmania infantum* isolated from human immunodeficiency virus-infected patients with visceral leishmaniasis (primary infection), Italy, 1986–2007 (n=126)



MON: Montpellier nomenclature.

[a] New zymodemes are represented by MON-136, MON-185, MON-188, MON-190, MON-201, MON-228, MON-183 var. MDH100 and MON-189 var. NH140 (1 strain each).

[b] Dermo-viscerotropic zymodemes are represented by MON-34 (4 strains) and MON-80 (1 strain).

[c] Dermotropic zymodemes are represented by MON-11 (1 strain), MON-24 (6 strains), MON-29 (2 strains), MON-78 (2 strains) and MON-189 (1 strain).

[d] Viscerotropic zymodemes are represented by MON-1 (102 strains) and MON-72 (1 strain).

from primary infections, the remaining from disease relapses) were typed from cases which occurred during the 1986 to 2007 period. *L. infantum* was confirmed as the main agent responsible of the co-infections, whereby only one case of co-infection with *L. donovani* was recorded and this case was imported. The annual composition of zymodemes from primary infections was more diversified in the group of strains obtained before HAART therapy introduction than in the group obtained after HAART. The pre-HAART group was composed of 84 strains collected up to 1998, and the post-HAART group included 42 strains typed between 1999 and 2007 (MLEE analysis data are not available after this year). As shown in Figure 2, the former group consisted of 16 zymodemes, dominated by the viscerotropic MON-1 (60/84, 71%). The remaining zymodemes, represented by one to six strains each, showed variations in one to four enzyme patterns and were identified as the already known dermotropic (n=4), viscerotropic (n=1) and dermo-viscerotropic (n=2) zymodemes, and eight new zymodemes never isolated from HIV-negative leishmaniasis patients. Conversely, the post-HAART group consisted only in three zymodemes, mainly composed by MON-1 (40/42, 95%) and by two known dermotropic zymodemes (1 strain each). The difference in zymodeme composition between the two strain groups scored as difference in the proportion of strains with the MON-1 zymodeme 'MON-1' versus those with zymodemes other than MON-1 'non-MON-1' in each group (60 vs 24 strains, and 40 vs 2 strains in pre-HAART and post-HAART period, respectively) was highly significant (Fisher exact test, $P<0.001$). Of note, HIV–VL cases were referred to our unit from the same hospitals distributed throughout the country during the whole period from 1986 to 2007. These findings suggest that HAART therapy, while conferring an immune restoration, may also operate a negative selection towards less virulent agents of VL in treated immunocompromised patients.

Generalised increase of visceral leishmaniasis in regions of peninsular and insular Italy

Taking the Campania region aside, the peninsular regions of the Tyrrhenian, Ionian and Adriatic coast, as well as Sicily and Sardinia islands (all historically endemic for VL) showed a generalised increase of VL in HIV-negative individuals, which was remarkable from 1995 (79 cases) to 2007 (68 cases) with a peak of 130

Table

Visceral leishmaniasis cases recorded among human immunodeficiency virus-negative individuals in regions other than the Campania region, Italy, 1995–2007 (n=1,032)

Region	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Liguria	8	6	1	1	6	5	6	3	5	4	6	3	9
Tuscany	1	3	3	2	2	5	4	9	8	10	6	4	10
Latium	13	18	15	17	16	27	23	21	23	23	29	22	23
Abruzzo	0	1	2	2	2	5	0	1	2	5	6	3	0
Apulia	5	11	10	2	12	11	17	6	4	7	1	6	2
Calabria	10	4	6	4	6	1	7	7	2	6	5	9	6
Sardinia	7	13	2	5	4	8	5	9	5	5	2	4	3
Sicily	33	27	17	24	26	36	27	52	29	43	25	18	7

Visceral leishmaniasis cases recorded in human immunodeficiency virus-negative individuals in the most endemic regions of peninsular and insular Italy – other than the Campania region – in 1995–2007, corresponding to the period of highest incidence among this population. Cells reporting the two highest values of numbers of cases recorded by region are shaded in grey, showing the uneven distribution of the hot spots.

cases in 2004 (Figure 1). Case analysis showed that such increase was not associated to large case clusters in particular geographical areas, but rather to a generalised spreading of transmission foci involving one or more endemic regions depending on the years (Table) and affecting different provinces.

Minor component of the trend: visceral leishmaniasis in northern continental Italy

Starting from 1990, regions from northern continental Italy traditionally considered free from *Leishmania* transmission had become focally endemic for *L. infantum*. This was definitely shown through active investigations involving canine serosurveys and phlebotomine sandfly monitoring during the 2003 to 2006 period in six regions (Piedmont, Valle d'Aosta, Lombardy, Veneto, Trentino Alto-Adige and Friuli-Venezia Giulia) [24]. With regard to human VL, cases had been regularly diagnosed between 1982 and 2012 in residents from all the above regions (a range of 5–15 cases/year, including HIV-co-infected individuals) mainly from cities of Lombardy and Piedmont. Travel histories were not available for cases notified to the Ministry of Health and most of them should be considered as imported from highly endemic southern regions of Italy or other Mediterranean counties as the consequence of travels during summer holidays. On the other hand, a few indisputable cases classified as autochthonous have been identified during the above active investigations [24] and also diagnosed thereafter by our laboratories, consisting of HIV-negative individuals from newly endemic areas of Piedmont, Lombardy and Veneto.

Individual factors: age and associated conditions

To investigate further on determinants of the Italian epidemic trend, we analysed the age distribution and underlying/concomitant conditions associated to VL. Full data were available for patients diagnosed from 1987 to 2005, thus representing both pre- and late epidemic phases. Records from 1,296 patients were considered. The resulting population is shown in Figure 3, distributed by arbitrarily 'VL-driven' age groups. 755 cases (about 60%) were adults (›17 years of age), however infants (‹ 2 years-old) were the most numerous homogeneous group (329 cases; 25% of all cases, 61% of paediatric ones, i.e. 0–16 years-old). Cases in preschool and young school children (3–6 years-old) and in children grouped with young adolescents (7–16 years-old) were considered separately to show the relatively decreasing risk for VL associated to age increase in childhood (indeed the two age-groups have 100 cases each, yet the 3–6 years-old group spans only a total of 4 years of age, compared to 10 years spanned by the 7–16 years-old group). Adults and middle aged individuals (17–50 years-old) were considered as a single group because these formed the age group at higher risk for HIV infection, and accounted for 575 patients (44% of all cases, 76% of adults›17 years-old). The age group between 51 and 70 years accounted for 140 cases, and the elderly group (›70 years-old, including several individuals over 80 years of age) for 40 cases.

Patients without any recorded underlying/concomitant conditions represented the greatest majority of the population, including 1,049 cases (81%). 179 HIV co-infected patients were recorded, representing 14% of total cases (n=1,296) and 31% of the 17–50 age-group patients (n=575). One hundred and ninety-seven (99%) of these HIV patients were adults. Finally, associated conditions other than HIV infection were recorded in 68 patients representing 5% of total cases. Fifty-seven of the 68 patients were adults, representing 8% (57/755)

of any adults. Twenty-three underlying/concomitant conditions other than HIV infection were recorded. They were single in 54, or associated in 14 patients. The most frequent conditions were organ transplantation (14 cases), hepatitis C and liver cirrhosis (12 cases each), pregnancy (9 cases), leukaemia (7 cases), lymphoma (5 cases), hepatitis B and systemic lupus erythematosus (4 cases each), thymoma and diabetes (3 cases each), and haemophagocytosis (2 cases). Conditions found in one case each were chronic glomerulonephritis, multiple sclerosis, pneumonia, primitive CD4 deficit, infection with cytomegalovirus, Wegener's granulomatosis, rheumatoid arthritis, thalassaemia, splenectomy, pericarditis and chronic renal failure.

Our findings point out that even if 'physiologically less immunocompetent' groups like infants and the elderly were excluded (for a total of 369 patients), the large majority of remaining cases (680/927, 73%) still consisted of immunocompetent individuals for whom susceptibility to VL disease remains unexplained.

Canine leishmaniasis data

General distribution

CanL is widespread in southern Europe, but the highest values of predicted seroprevalence are found in the Italian peninsula. The median prevalence calculated from 377 canine serosurveys performed in Italy from 1971 through 2006, involving about 424,000 dogs, was 18% with a range of 11–21% in different decennia when the surveys were performed [25]. Of 494 *L. infantum* canine strains characterised by MLEE, the greatest part belong to zymodeme MON-1 (457 strains), found distributed throughout the country; a large group of strains (n=36) from the Campania region was found to belong to the variant zymodeme MON-72. These homogeneous groups of canine zymodemes did not reflect the elevated zymodeme polymorphism detected among the agents of human disease (both VL and cutaneous leishmaniasis), leaving the role of dogs as reservoir for all *L. infantum* zymodemes endemic in Italy unexplained.

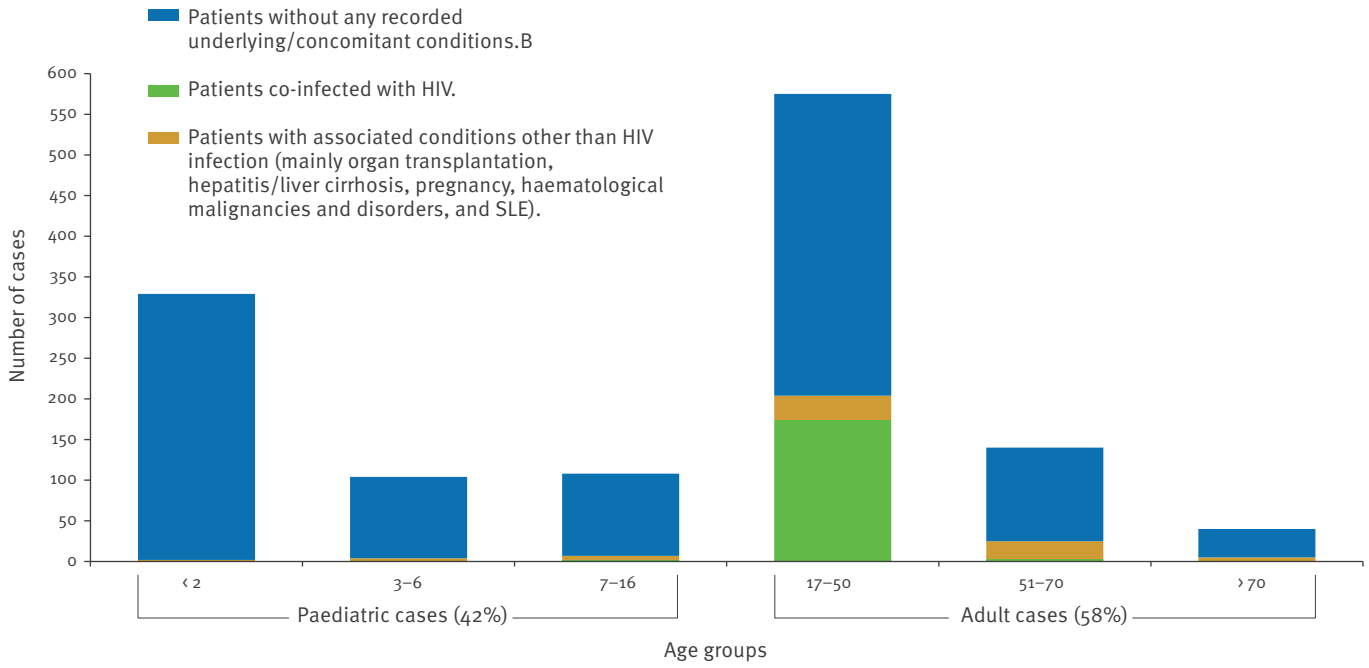
A 2005 to 2012 data mapping on the presence of autochthonous CanL among approximately 8,100 Italian communes is shown in Figure 4. Although still incomplete because of the lack of investigations in several areas, about 2,700 communes (33%) have been found endemic for CanL so far.

Canine reservoir in the Campania region

CanL has long been known to be endemic at high prevalences in the Campania region. In 1999, one year before the peak of 83 VL cases recorded in the region, the local institute for zooprophylaxis had examined 1,675 dogs and found 251 seropositives at IFAT titres ›1/80, corresponding to a seroprevalence of 15% [26].

Figure 3

Age distribution and underlying/concomitant conditions recorded in 1,296 patients affected by visceral leishmaniasis in Italy, 1987–2005



HIV: human immunodeficiency virus; SLE: systemic lupus erythematosus.

In the 2005 to 2009 period, corresponding to the drop of VL incidence in that region, the same Institute reported 9,723/70,557 seropositive dogs at the same IFAT titre range, corresponding to a seroprevalence of 14% i.e. very similar to the value recorded in 1999 (2010, data from Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples). These findings suggest that canine seroprevalence rates cannot per se explain the epidemic trends of human VL.

Discussion

At country-wide level, leishmaniasis epidemics may consist of an increase of disease cases over the expected baseline in the general population throughout the territory, or represent the additive outcome of different outbreaks occurring in particular areas and/or affecting specific groups of individuals. Epidemic trends have a typical bell shape where the increasing slope represents the natural epidemic onset, whereas the decreasing slope may either be natural or due to aggressive human interventions to control the disease. Our analysis indicates that the VL epidemic in Italy was a complex phenomenon, in which more than one component was represented: (i) an outbreak involving infants and immunocompetent individuals in parts of the Campania region, that appears to have declined naturally; (ii) a second outbreak affecting HIV-infected individuals throughout the country, that declined via the use of HAART therapies, which also probably applied a negative selection pressure on less virulent *Leishmania* genotypes originally involved in the epidemic. Hence, the contribution of the HIV–VL epidemic to the general VL trend in Italy has been limited in time, so that the two trends peaked and started to decline in different years. As mentioned above, the HIV–VL trend did not affect greatly the Campania VL trend, since the occurrence of co-infected cases in this region was negligible.; (iii) a generalised increase of cases due to disease spreading within traditionally endemic areas (a major contribution in cases) as well as the appearance of cases in previously non-endemic areas (a minor contribution in cases).

While the appearance of autochthonous VL in northern continental Italy could reasonably be explained by the de novo colonisation of these areas by phlebotomine vectors along with the frequent importation of Leishmania-infected dogs from the endemic south [24], and the generalised increase of cases in endemic areas by an increase in transmission potential due to changes in vector density, both the onset and the natural decline of the outbreak in the Campania region, traditionally endemic for VL, will probably remain unexplained. One reason for the decline could be searched in the immunity levels against *Leishmania* acquired by the population during the epidemic peaks, however this hypothesis is difficult to verify because of the sporadic nature of the disease that would require large population cohorts to be examined prospectively.

Figure 4

Map (ArcView GIS 10) showing the distribution of communes (in blue) where autochthonous cases of canine leishmaniasis have been recorded, Italy, 2005–2012

GIS: geographic information system.

Other investigated epidemiological compartments of the zoonotic VL cycle, the canine reservoir and phlebotomine vectors, did not help in elucidating the causes of the Italian epidemic. First of all, no aggressive programmes to control the increase of VL, that may justify the general drop in incidence, were ever put in place at country level. Indeed, only some regions have implemented guidelines and rules to monitor infections in pets and/or kennelled stray dogs, recommending drug treatment of infected animals and the use of topical insecticides against sandflies. However, as far as pet dogs are concerned, both measures were left at the owners' expenses resulting in the limited coverage of the dogs to control. Because canine topical insecticides have an impact on CanL incidence only after repeated mass use [27] and drug treatments may have some efficacy in decreasing transmission only when administered during the sandfly season, the measures actually undertaken were most probably not very effective in reducing the *Leishmania* transmission potential.

Incidence of CanL may vary considerably within endemic areas, with focal distribution. To date there is no clear evidence for a direct association of CanL prevalence values and incidence of human VL disease in a given territory. Indeed, several examples are available in literature where very elevated prevalences recorded in dogs did not result in human VL cases at all [28]. It implies that although dogs are efficient sentinel hosts for the *Leishmania* transmission in a given territory, and hence the finding of autochthonous CanL cases in previously non-endemic areas can be of value to predict the occurrence of human cases, the prevalence rate of canine infections does not appear an useful parameter to explain determinants of human VL trends in endemic areas, like we observed in Campania region.

With regards to the phlebotomine vectors, Italy is endemic for four Phlebotomus species proven to transmit *L. infantum*, *P. perniciosus* being the most widespread and efficient vector. A recent atlas based on validated bibliographical records on phlebotomine sandflies from 1985 to 2009 reported the presence of this species in 134 of approximately 8,100 Italian communes throughout the country. Among the other vector species, *P. perfiliewi* was recorded in 50 communes of peninsular Italy, *P. neglectus* in 41 communes from southern and northern, but not from central Italy, and P. ariasi in four communes at the French border [29]. Hence, considering the huge disproportion with available CanL data, information on phlebotomine vector distribution in Italy is still largely incomplete. On the other hand, like for CanL, to date there is no clear evidence for a direct association of phlebotomine vector presence and the incidence of human VL in a given territory. Indeed, many parameters necessary to a robust definition of vectorial capacity in phlebotomine species, such as standardised density measurements, dog- versus man-biting rates, number and frequency of blood meals/ovodepositions, infected vs infectious sandfly ratio, are still lacking from the scientific literature worldwide. Hence, while the presence of competent vectors in a given territory can be predictive for the occurrence of human VL when CanL cases are found too, current entomological data are still of low informative value for the analysis of epidemic VL trends.

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Article Type

Surveillance of leishmaniases in France, 1999 to 2012

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Leishmaniasis is endemic in the south of France, where autochthonous disease is caused by *Leishmania infantum*, and affects both humans and dogs. The prevalence of canine leishmaniasis is between 3 and 66% depending on the region and the methods used. Human leishmaniases are also imported into France, mainly from French Guiana and North Africa. The surveillance of autochthonous and imported human leishmaniases is based on passive notification to the National Reference Centre for Leishmaniases (NRCL) created in 1998. Between 1999 and 2012, 317 autochthonous and 1,154 imported cases were notified to the NRCL. The average number of autochthonous cases notified per year was 22.6, mainly cases of visceral leishmaniasis (84.5%). All cases were infected in the south of France. Leishmaniasis incidence is 0.22 per 100,000 inhabitants in the endemic area. Imported cases were more frequent (annual mean of 82.4 cases) and consisted predominantly in cutaneous leishmaniasis (CL) cases (91%), essentially *L. major* CL imported from Maghreb and Sub-Saharan Africa, and *L. guyanensis* CL from French Guiana. This national notification system allowed a better understanding of the incidence and distribution of the disease; it is also useful to assess the temporal-spatial evolution of the disease in France, which appears relatively stable.

Introduction

In Europe, leishmaniasis is a zoonosis endemic in countries surrounding the Mediterranean Basin. In France, the French Ministry of Health supported the creation of the National Reference Centre for Leishmaniases (NRCL) in 1998 in Montpellier (http://www.parasitologie.univ-montp1.fr/english_vers/en_index.htm), with the aim of better understanding the epidemiological situation of the human disease at the national level. Its true incidence was unknown and the suspected increase of imported cases and of cases in immunocompromised patients needed to be confirmed. In this context, one of the first activities of the NRCL was to set up a system for notifying autochthonous and imported human leishmaniasis cases in France. This retrospective study reports the results of fourteen years of this surveillance.

Epidemiological situation of leishmaniases in France

In France, the endemic area of leishmaniases is restricted to the south of the country. Several foci are clearly identified along the Mediterranean coast from the Spanish to the Italian border: the eastern Pyrénées, the Cévennes, the Provence, the Alpes-Maritimes and Corsica. The transmission is generally rural but two large cities, Nice and Marseille, are known to comprise endemic foci within their boundaries [1,2]. Dogs constitute the main reservoir of the pathogen, and *Leishmania infantum* is the species responsible for all autochthonous cases. Human cases due to this species are reported every year. However, symptomatic visceral leishmaniasis (VL) human cases represent only 'the tip of the iceberg' [3]. Indeed, individuals living in endemic areas of *L. infantum* are frequently exposed to biting by the sandfly vector. Epidemiological studies conducted worldwide in endemic areas of *L. infantum* and using leishmanin skin test, serology, blood cultivation or polymerase chain reaction (PCR), strongly suggest that the frequency of asymptomatic carriers is high [reviewed in 3].

Before 1999, there was no established notification of human leishmaniasis cases to the French health system. It was therefore difficult to have a precise picture of the incidence and prevalence of leishmaniases in France. Yet, the mean annual incidence of autochthonous VL in France was estimated for the years from 1989 to 1995 at around 1.3, 0.66 and 0.22 cases per

100,000 inhabitants for the foci in the Pyrénées-Orientales, the Alpes-Maritimes and the Cévennes, respectively [1,4]; however, these values do not reflect the strong variations between micro-foci, which can be evidenced using classical [5] or modern [2, 6, unpublished data] epidemiological tools.

The phlebotomine sandfly vector

In France, the disease is spread by sandflies of the genus *Phlebotomus*, specifically *P. perniciosus* and *P. ariasi*, which have a seasonal activity, generally from May/June to September/October [7,8]. In southern France, *P. perniciosus* represents the most common vector species: it is mainly present in rural and in peri-urban areas and preferentially at altitudes less than 600 m above sea level. *P. ariasi*, in contrast, is found preferentially in rural areas at altitudes between 200 and 1,400 m above sea level; it represents the main vector in the Cévennes and Pyrénées-Orientales foci [9, reviewed in 10].

The canine reservoir

Canine leishmaniasis, affecting essentially the domestic dog (Canis familiaris), is endemic in the regions confined by a triangle of which the apex corresponds to the departments (French administrative territorial divisions) of Ardèche and Drôme and the base to the Mediterranean coast. Two national surveys, performed in 1987 and 2004 and exclusively based on reports from veterinary clinics [11,12], led to the creation of a map displaying the endemic geographical areas and the changing profile of the disease, by comparing maps over an interval of almost 20 years. The information was completed by a retrospective database search and mapping about canine leishmaniasis covering the period 1965 to 2007 [13]. The results show that the disease is still prevalent in southern France, including Ardèche, and that new endemic areas emerge, contiguous to pre-existing endemic foci. Overall, 25 of 95 departments are affected; but for several of them, very low numbers of cases were reported [13,14], questioning the endemic nature of the disease in these areas and reducing the main endemic region to the 12 departments closest to the Mediterranean Sea. The seroprevalence in dogs in the latter ranges from 8.1 to 28% [11, reviewed in 1].

In the Cévennes focus, a study conducted in 1997, showed that among 253 domestic dogs tested serologically, 29.6% were positive and 70% of them presented clinical signs of leishmaniasis [6]. However, in the same survey, using an ultrasensitive PCR assay, the overall prevalence of parasite carriage was found at 80%; and at least 65% of asymptomatic dogs were found harbouring circulating parasites in their blood [6]. Thus, asymptomatic dogs can act as a reservoir of the parasite and seem to allow the transmission and spread of leishmaniasis [reviewed in 15-17]. Another study modelling previous surveys of canine leishmaniasis estimated the prevalence between 5.4 and 20.3% [18].

Human cases

It is not easy to get an accurate picture of the disease progression in humans in southern France during the twentieth century [1]. According to literature data, at least 200 cases of leishmaniasis were recorded between 1918 and 1975, and a further 22 cases from 1975 to 1984 and 65 cases from 1985 to 1992 [unpublished data, 1, 4, 19]. To our knowledge, a single attempt was made in the past to prospectively record all national cases of VL during two years (1986-87) [20]: a total of 89 patients were recorded, of which 70 acquired the disease in France.

The objectives of the present study are to update these data and to report the results obtained by the NRCL in the surveillance of the human disease in France between 1999 and 2012.

Methods

All leishmaniasis cases notified in France between 1999 and 2012 were analysed according to the place of infection, clinical presentation of the disease, age and risk factors for immunosuppression. For reasons of data completeness, only the period from 2007 to 2012 was analysed for risk factors.

French Guiana (a French overseas territory located in South America) is an endemic area for leishmaniasis [21-23]. Although the Parasitology-Mycology Department of the University Hospital of Cayenne is associated with the NRCL, the focus of the analyses presented here is Europe, and thus cases diagnosed in French Guiana are not included in this study. However, cases imported from French Guiana and diagnosed in France are included.

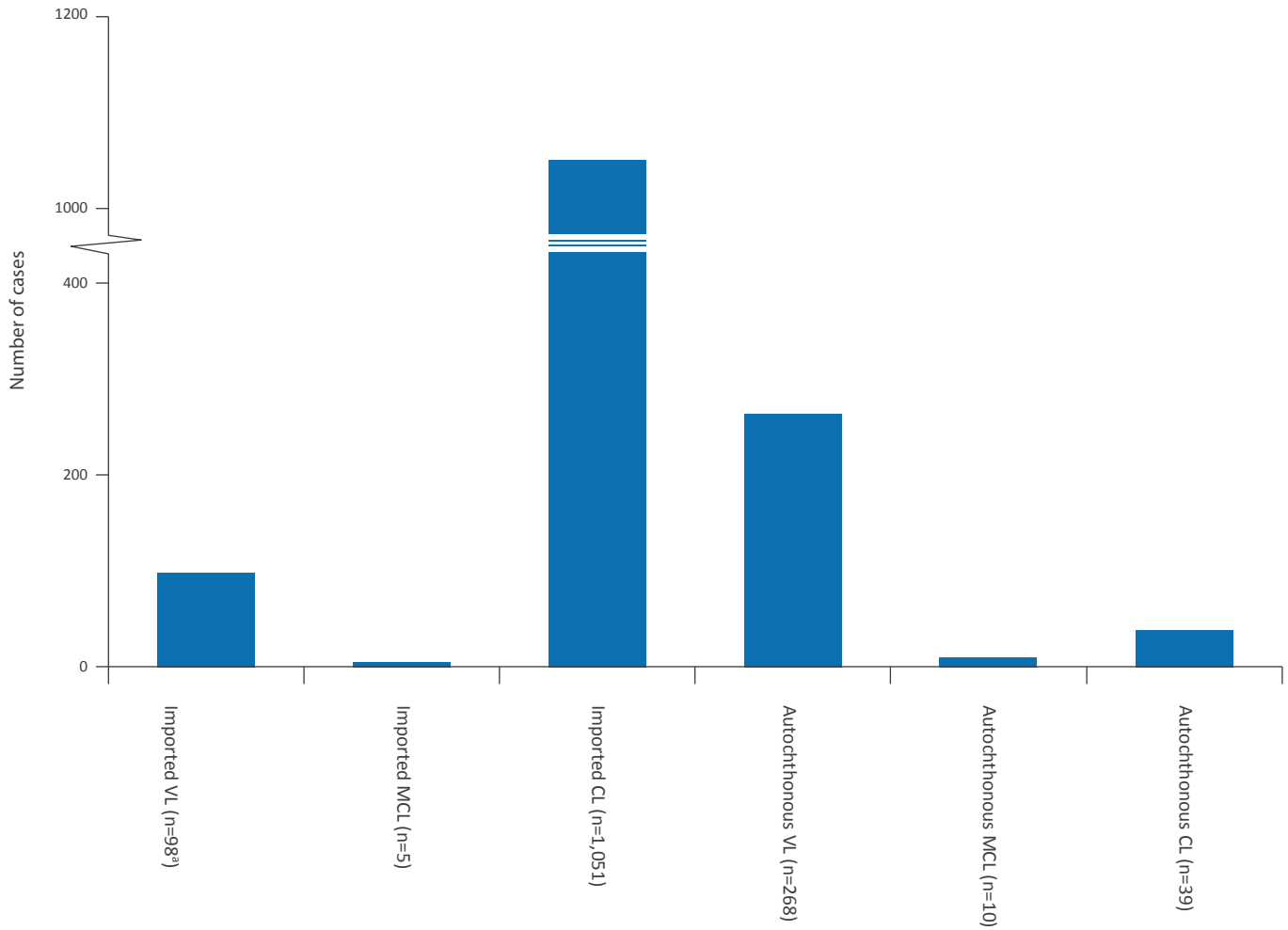
Cases confirmed by at least one specific biological test could be notified to the CNRL. Data were obtained via the standard reporting form created in 1998 at the NRCL. This form is available online (http://www.parasitologie.univ-montp1.fr/doc/Declaration_pub_2011.pdf) and can be sent back after anonymisation of the data, by mail or email but it cannot be filled online. The notification is not compulsory. It is made on a voluntary basis and relies mainly on care facilities supporting patients. The following characteristics are specified on the form: age (children defined as ‹16 years old), sex, risk factors with particular reference to immunosuppressive conditions such as organ or bone marrow transplantation, human immunodeficiency virus (HIV) infection, immunosuppressive therapy, leukaemia, solid organ cancer, clinical features (for VL: pancytopenia, splenomegaly, hepatomegaly, fever, weight loss; for CL or MCL: number of lesions, localisation, type of lesions such as ulceration, nodule) duration of symptoms, the presumed place of infection and laboratory tests performed for diagnosis.

Results

During a period of 14 years between 1999 and 2012, the NRCL received notifications of 317 and 1,142

Figure 1

Notified autochthonous and imported leishmaniasis cases, France, 1999–2012 (n=1,471)



CL: cutaneous leishmaniasis; MCL: mucocutaneous leishmaniasis; VL: visceral leishmaniasis.

[a] Including 12 VL cases of undetermined origin.

autochthonous and imported cases of leishmaniasis, respectively, as well as 12 (visceral) cases of undetermined origin (Figure 1). More than 70 health centres notified cases: they were mostly university hospital centres but also general hospital centres, the health services of the French army, and occasionally private medical clinics or even a few practitioners.

Autochthonous human leishmaniasis cases

Of the 317 cases of autochthonous leishmaniasis 268 (84.5%) were VL cases, 39 (12.3%) cutaneous leishmaniasis (CL) and 10 (3.1%) mucocutaneous leishmaniasis (MCL) cases.

The ratio of men to women was 1.8 and the disease affected mostly adults (222 cases; 70%); among those, 50 were over 60 years old; 73 patients (23%) were less than five years old; the mean age of the patients was 35.5 years and the median 39 years (range1 to 89 years).

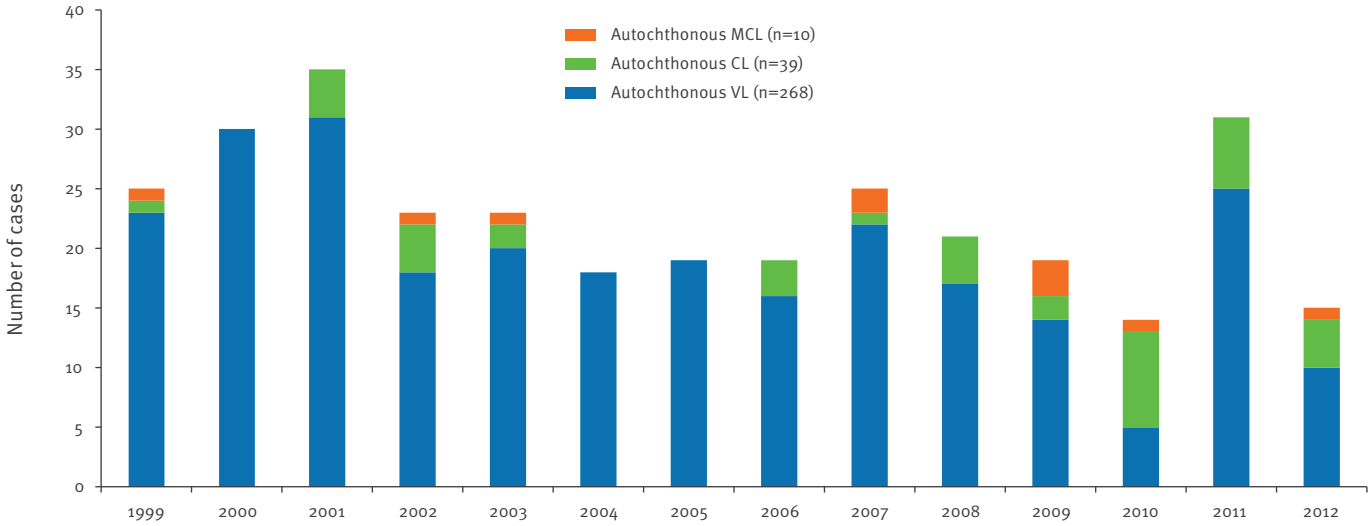
Figure 2 shows the number of autochthonous annual cases from 1999 to 2012 according to the clinical presentation. For the whole metropolitan France, the average number of notified cases per year was 22.6 (ranging from 14 in 2010 to 35 in 2001). Infection occurred in the south of France in all cases. The mean annual incidence of leishmaniasis that may be inferred from these data is 0.21 per 100,000 inhabitants for the endemic area and 0.26 for the eight most affected departments (Figure 3); it varies from 0.64 (Alpes Maritimes) to 0.01 (Aude) per 100,000 inhabitants.

Visceral leishmaniasis cases

VL was the predominant clinical presentation (268/317; 84.5%), with a mean of 19.1 (ranging from 5 to 31) cases per year, and a peak at 32 VL cases in 2001. The two

Figure 2

Annual number of notified autochthonous cases of leishmaniasis by clinical picture, France, 1999–2012 (n=317)



CL: cutaneous leishmaniasis; MCL: mucocutaneous leishmaniasis; VL: visceral leishmaniasis.

departments with the highest number of notified VL cases are the Alpes-Maritimes (97 cases) and Bouches-du-Rhône (46 cases) (Figure 3).

An analysis of our data over the period 2007 to 2012 shows that VL cases occurred in 62% (90/145) in men and more frequently (46.9%; 68/145) in the age group 20 to 60 years; 19.3% (28/145) of the cases were observed in people over 60 years (Figure 4).

Cutaneous leishmaniasis and mucocutaneous leishmaniasis cases

Since 1999, only 39 cases of CL and 10 of MCL have been reported. Autochthonous CL, with a mean of 2.8 cases (ranging from 0 to 8) per year. With respect to MCL, only 10 cases have been reported, including three from Eastern Pyrénées. MCL was essentially a primary condition of mucosae, with neither visceral, nor purely cutaneous involvement.

Imported leishmaniasis cases

Between 1999 and 2012, 1,154 imported cases were reported to the NRCL, a mean of 82.4 cases (range 37 to 148) annually. Only 98 of these were VL cases, resulting in an annual mean of seven cases. CL cases represented 91% (1,051/1,154) of the total: 41.9% (440/1,051) of these were from Africa, originating from North Africa in 30.9% (325/1,051) of cases and in sub-Saharan Africa in 11% (115/1,051). Imported CL cases acquired in French Guiana represented 41.7% (438/1,051) of the notified cases (only 44 cases, 4.2% of them having been infected in the rest of Latin America). These three geographical areas correspond to the main migratory movements to metropolitan France.

Molecular strain typing from the Old World cases for the years 2009 to 2011, identified a large majority (141 cases; 88%) of *L. major*, followed by *L. tropica* (18 cases; 11%); for the New World cases, *L. guyanensis* was largely predominant (170 cases; 83%), followed by *L. braziliensis* (27 cases; 13%). It is of note that imported cases from French Guiana reported to the NRCL are not necessarily representative of the epidemiological situation of this region.

Autochthonous and imported visceral leishmaniasis cases and immunocompromising conditions

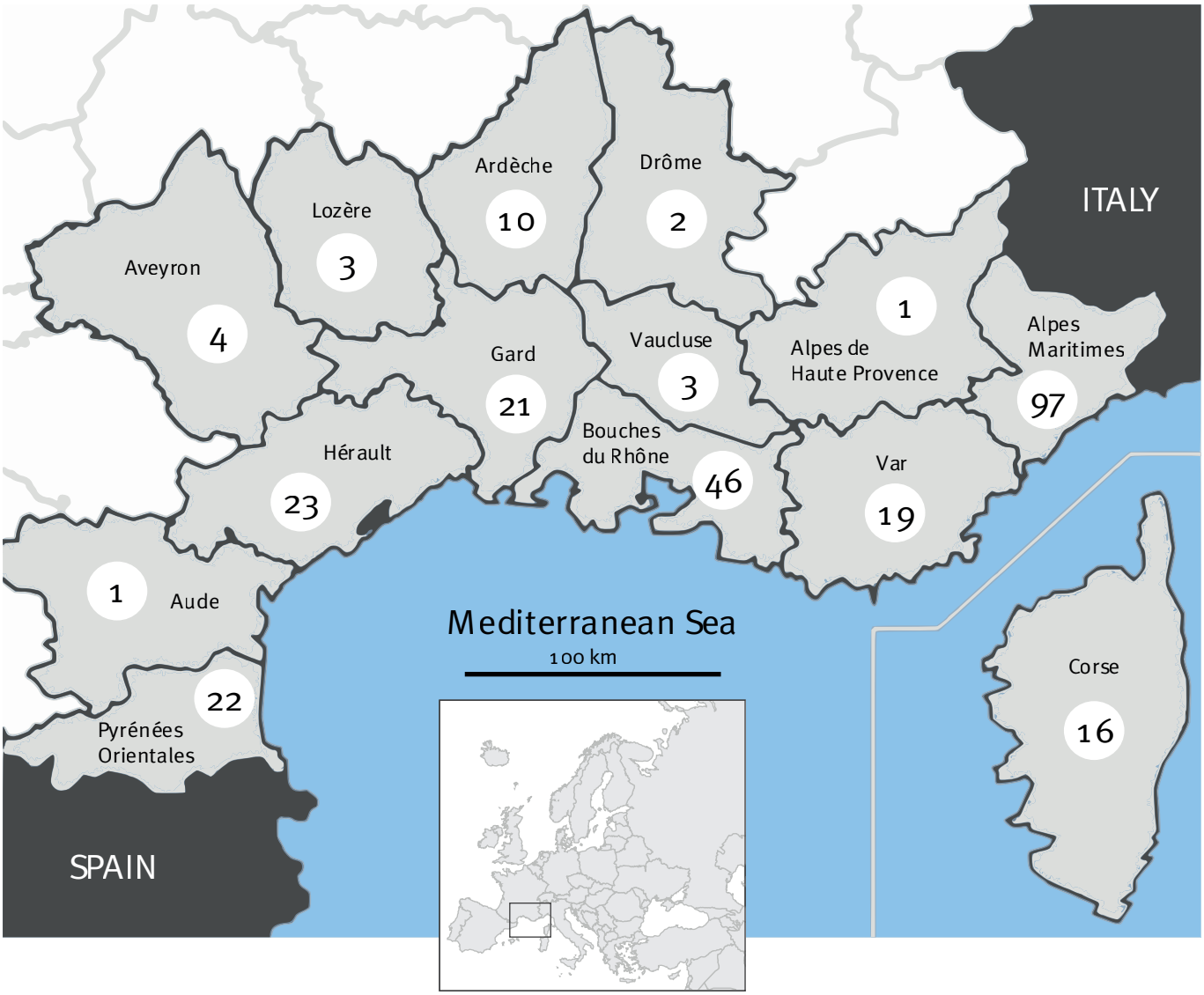
Taking into account all VL cases (n=366) reported to the NRCL from 1999 to 2012, 268 autochthonous cases and 98 imported cases, data analysis showed the association of an immunocompromising condition in 44.3% of cases (162/366): 31.4% (115/366) were HIV infected, 9.6% (35/366) had an immunosuppressive treatment and 3.3% (12/366) had received an organ or bone marrow transplant.

Discussion and conclusion

In France, autochthonous leishmaniasis is due to *L. infantum* and is endemic mainly in the Mediterranean region. Canine leishmaniasis remains widespread in these foci and is the subject of studies by the ANSES (National Agency for Health Security of Food and Environment) as well as several national veterinary (Lyon, Maisons-Alfort, Nantes) or medical (Nice, Marseille) faculties, often in collaboration with the NRCL. With respect to human cases, the creation of a notification system at the NRCL in 1999 has allowed a better understanding of the incidence and distribution

Figure 3

Cumulative number of notified autochthonous visceral leishmaniasis cases in the most affected departments in France, 1999–2012 (n=268)

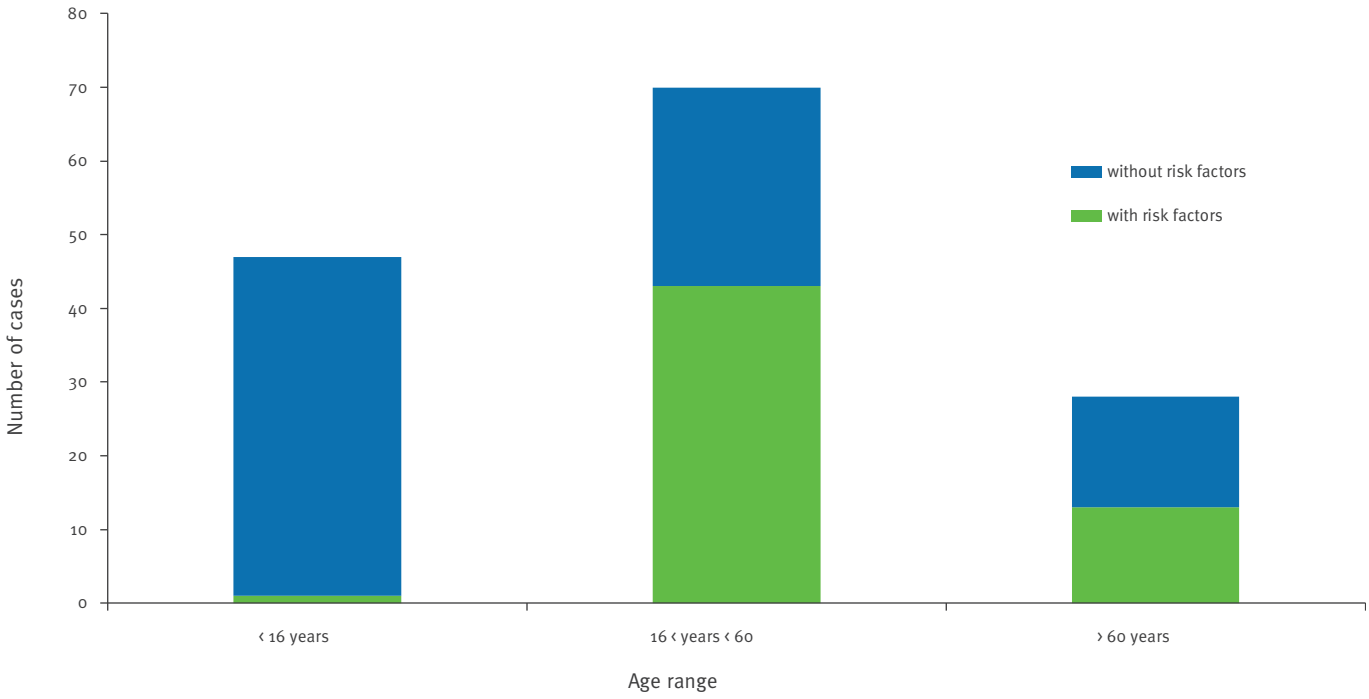


of the disease. Although an increase in incidence or an extension of the endemic areas can be anticipated in view of global warming [24,25], it appears that autochthonous cases remain relatively rare (annual mean 22.6) in France with a clear predominance of VL (84.5%).

VL has long been considered a disease of young children. The first case of Mediterranean kala-azar in a child in France was reported in 1918 [26]. A national survey conducted in 1986-87 showed a predominance of the disease in children, which constituted 51% of cases, with equal distribution between both sexes [20]. However, over the last decades, with the emergence of immunosuppression and primarily of HIV / acquired immunodeficiency syndrome (AIDS), the epidemiology of the disease has changed: in particular, the incidence in adults has increased significantly. Indeed, between 1975 and 2004, children accounted for only 30% of the cases [1]. This can be partly correlated with the increasing number of cases of leishmaniasis/HIV co-infection: HIV-positive patients are at high risk, and leishmaniasis is considered an opportunistic disease [27,28]. Between 1996 and 1998, 50% of new VL cases in southwestern Europe (including France) involved HIV-positive patients [27]. The mean age in our adult patients with VL was 36 years [28]. Nevertheless, the occurrence of cases among the elderly was not exceptional, which may be explained by ageing, declining of immune system, association with other pathologies such as tumours, inflammatory diseases, VL can also affect patients with no apparent immunosuppression nor risk factor, irrespective of their age. Overall, the incidence of VL during the study period was relatively stable, but a decrease of the incidence can be noted if compared to the period from 1993 to 1997 [1, 2], this

Figure 4

Notified autochthonous leishmaniasis cases by age group and risk factors, France, 2007–2012 (n=145)



For reasons of completeness of the data, only 2007–2012 was analysed for risk factors.

being most likely linked with the introduction of highly active antiretroviral therapy in AIDS patients.

In contrast to VL, autochthonous cases of CL and MCL are rare in France [29] and appear sporadic. CL is probably underdiagnosed and certainly undernotified, to a great extend because cutaneous lesions due to *L. infantum* are often small, painless and spontaneously self-curing within a few months; hence these benign lesions are essentially seen by general practitioners or dermatologists, which generally do not notify cases. The number of MCL cases notified to the NRCL, however, reflects almost the whole of the total seen in France, as this atypical clinical presentation necessitates the implementation of a specific laboratory diagnosis and initiation of anti-*Leishmania* drug treatment.

Compared to autochthonous cases, the number of imported cases is relatively high with an annual mean of 82.4 cases, mainly consisting in CL cases due to *L. major* and *L. guyanensis*, while for VL cases the annual mean of seven is not far from the nine cases per year reported in 1986-87 [20].

The monitoring by the NRCL is also useful to assess the temporal-spatial evolution of the disease. It is difficult to infer from these data whether the incidence of autochthonous leishmaniasis is declining in France or not: certain data sets suggest a medium-term (over decades) tendency to decline [1,2,20], but our data show that it is currently relatively stable. As to the risk of seeing the emergence of 'exotic' or hybrid parasites [30] which would be transmitted locally by permissive phlebotomine vectors, we consider that it is almost null. Indeed, (i) there is no evidence for any permissivity of *P. perniciosus* and *P. ariasi*; (ii) the probability of this occurring in nature appears extremely low, as, on top of permissivity, it requires gathering a number of factors allowing transmission (southern France, summer season, absence of treatment, etc.).

The Working Group for the Notification of Human Leishmanioses in France

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Conflict of interest

None declared.

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