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).

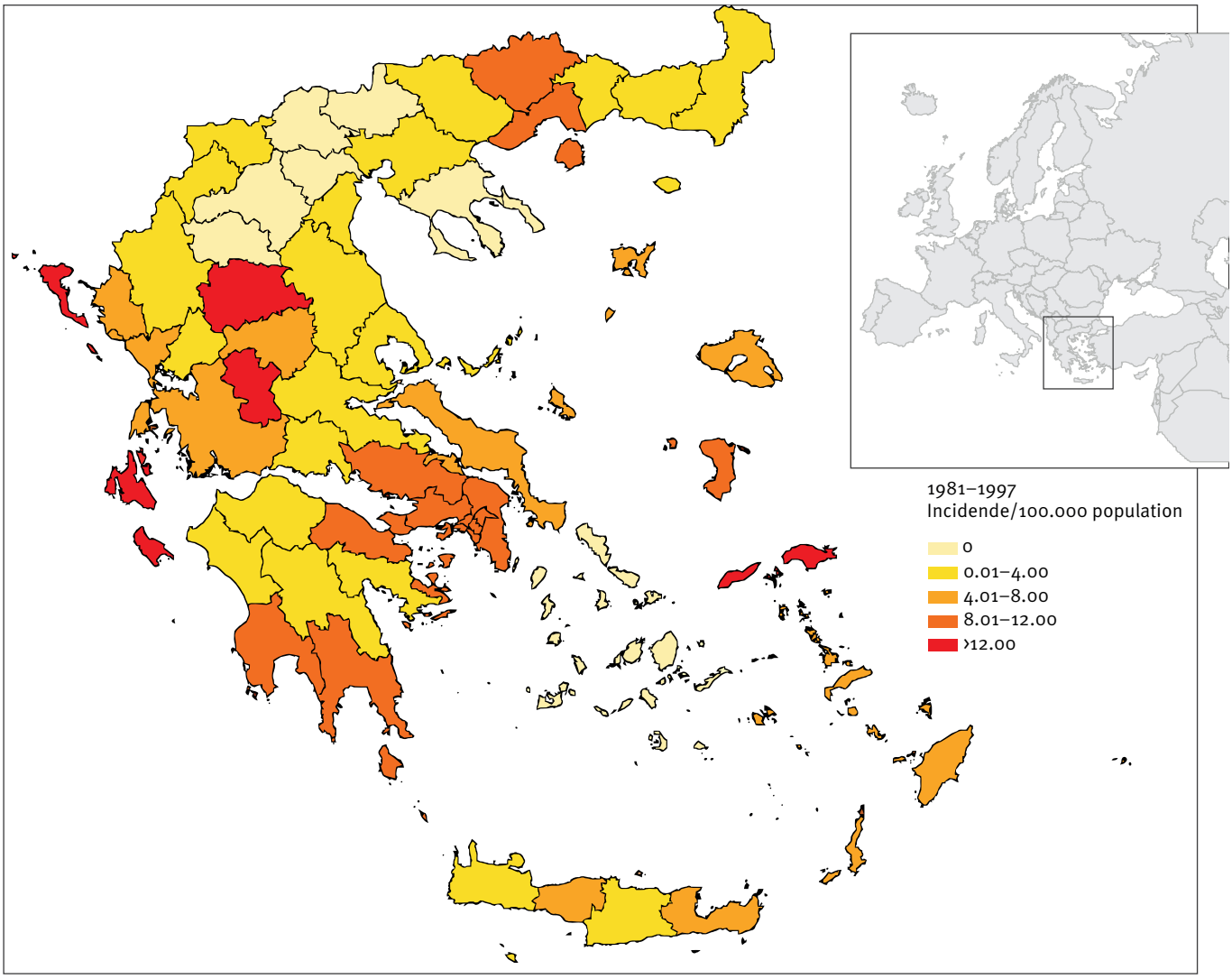
Methods

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.

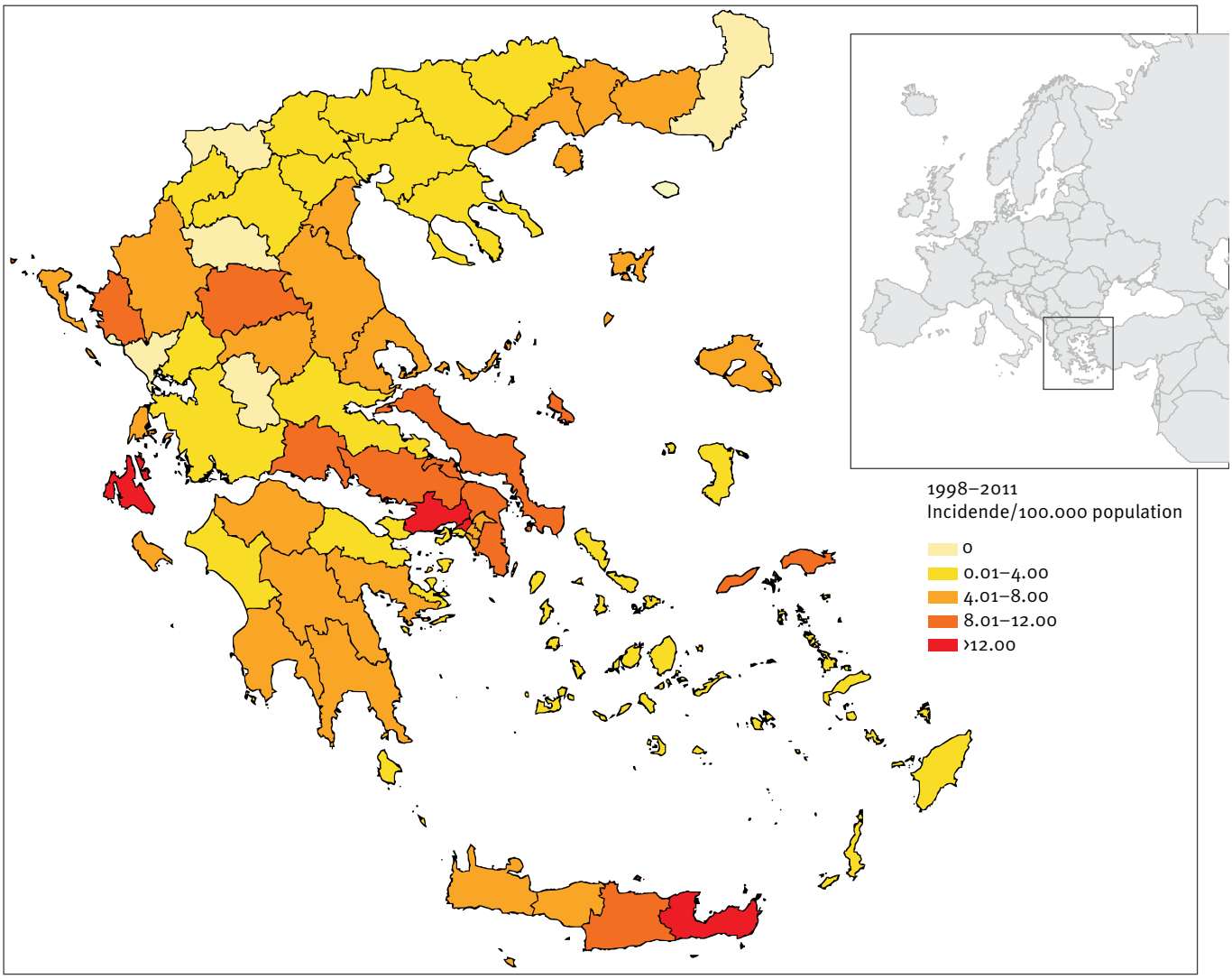
According to the Hellenic mandatory notification system, a confirmed visceral leishmaniasis case is an individual with clinical manifestations compatible with visceral leishmaniasis (persistent fever, splenomegaly, substantial weight loss, anaemia, lymph node enlargement) and laboratory confirmation 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.

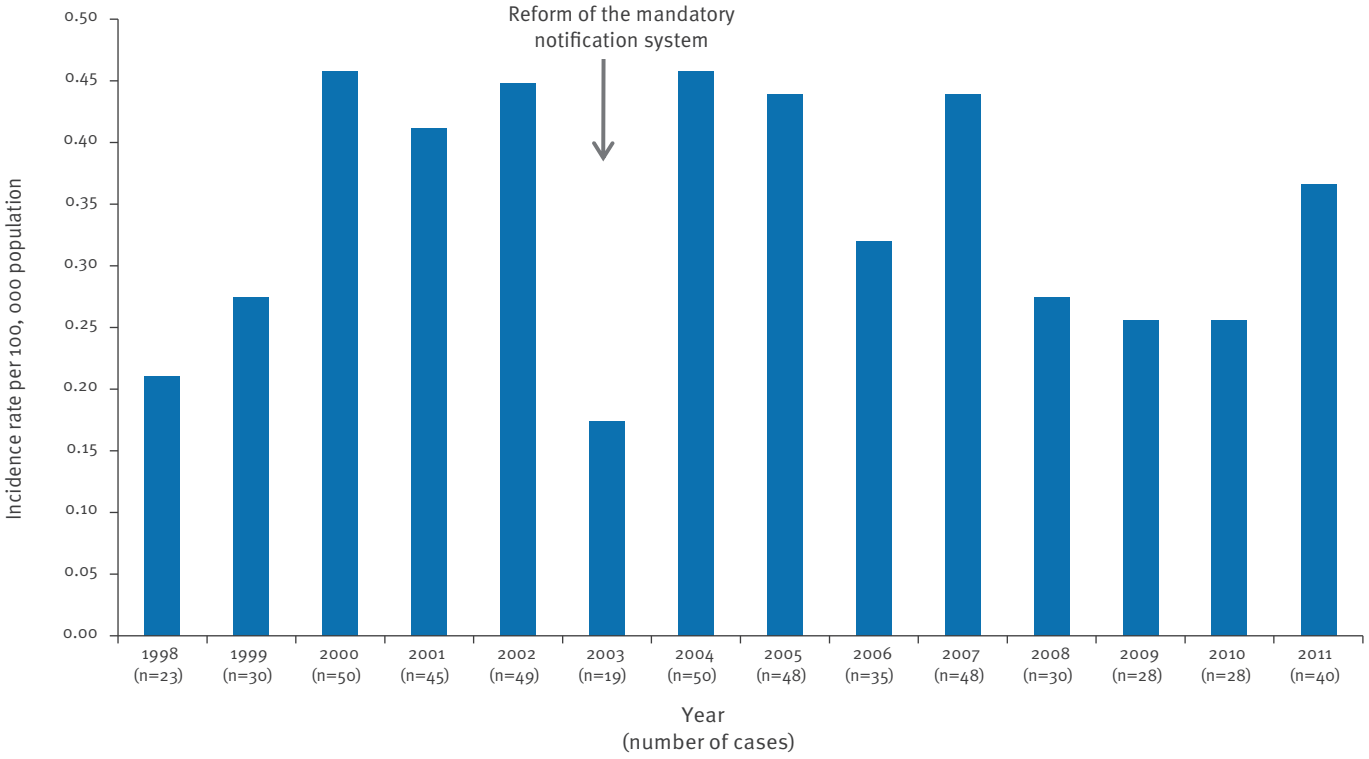
Age and sex distribution and origin of leishmaniasis cases

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.

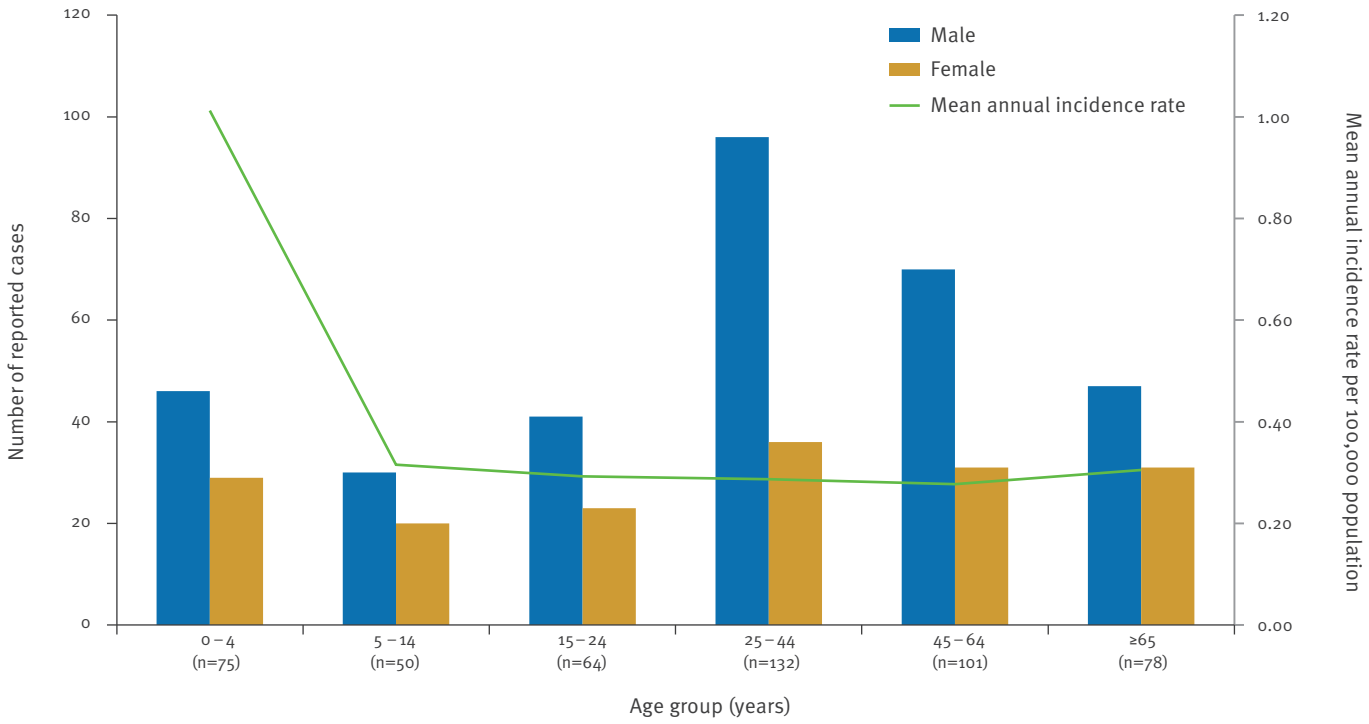
Clinical manifestations, laboratory findings and risk factors for leishmaniasis

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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