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Human leptospirosis in Portugal: a retrospective study of eighteen years

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Summary
Introduction: Leptospirosis, an under-recognized public health problem, needs to be confirmed through specific laboratory diagnosis.
Design: We describe herein a series of 4618 symptomatic patients for whom a microagglutination test (MAT) serology was available, representing a unique picture of human leptospirosis in central mainland Portugal and the Azores islands of São Miguel and Terceira, over eighteen- and twelve-year periods, respectively.
Results: The distribution of the 1024 (22%) cases identified was an average 57 cases per year, with higher frequency in males (67%). These represent the majority of leptospirosis notifications in Portugal, with a higher annual incidence rate in the islands, compared to the central mainland (11.1 and 1.7/100 000 population, respectively). Middle-aged adults (25—54 years) were most frequently infected (45%). Cases occurred mainly in December and January. Serovars from nine presumptive serogroups caused infection, with a predominance of Icterohaemorrhagiae, Pomona, and Ballum, accounting for 66% of cases.
Conclusions: Seropositivity was associated with both anicteric and icteric leptospirosis. Several risk factors and a higher transmission risk in certain areas were emphasized. Leptospirosis was confirmed as a steadily increasing public health problem; good surveillance, communication, and laboratory support are thus necessary to reduce the impact of leptospirosis in areas at risk.

Introduction

Leptospirosis is a globally important zoonotic disease that affects humans on all continents in tropical and temperate climates, now being identified as one of the emerging infectious diseases.[1—3] Bacteria from the family *Leptospiraceae* are the causative agents.

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The natural reservoirs for leptospires are wild animals, particularly rodents, and domestic animals, commonly bovines and pigs, which, after infection, can become chronic renal carriers. Human infection is accidental, usually occurring after direct or indirect contact with urine from leptospiruric animals. Indirect exposure through water, soil or vegetation contaminated with urine accounts for most sporadic cases in occupational groups such as farmers, sewer workers and military personnel, while direct exposure occurs in veterinarians and farmers working with livestock.[4]

Previously regarded as an occupational illness, its epidemiological pattern is changing and now there are increasing reports of leptospirosis contracted during accidental events in waters polluted with leptospires and during recreational activities.[5,6] In addition, a number of retrospective epidemiological studies have recently been published in countries of temperate zones[7,8] and tropical areas,[9,10] aiming to summarize the regional features and trends in human leptospirosis cases.

In Portugal, the first isolation of the agent of Weil's disease was obtained in 1931.[11] However, regular research on human *Leptospira* infection (International classification of diseases (ICD), 10th revision: 100–100.9) started in the 1980s,[12] becoming a notifiable disease in 1987 for all leptospires; Icterohaemorrhagiae cases have been notifiable since 1950.

So far, the number of officially reported cases has been low in mainland Portugal,[13,14] when compared with the increasing number of cases in São Miguel and Terceira islands (the Azores), where more fatal cases have occurred in recent years.[14,15] In fact, leptospirosis is still not always notified[16] nor even diagnosed, due to both its marked clinical polymorphism and to the difficulties involved in standard bacteriological and/or serological diagnoses.

The purpose of this retrospective seroepidemiologic community-based study in the central and southern mainland regions, and the São Miguel, Terceira and Madeira islands, was to summarize incidence, clinical symptoms, prevalent serovars, and occupational risk of infection in laboratory-confirmed human leptospirosis cases in Portugal. Data were collected during eighteen years at the Unit of Leptospirosis and Lyme Borreliosis (ULBL) at the Institute of Hygiene and Tropical Medicine (IHMT) of the New University of Lisbon, one of the two governmental institutions where diagnostic testing for leptospirosis takes place.

Patients and methods

Study areas

In addition to the central and southern regions of mainland Portugal, with populations of ~1 944 000 and ~3 705 000, respectively (average of the censuses of estimated resident population for 1981, 1991 and 2001),[17] the Azorean islands of São Miguel and Terceira were also included in the study (Table 1). These two islands belong to the Archipelago of the Azores, which is composed of seven volcanic islands, administrated autonomously, and situated in the middle of the North Atlantic Ocean approximately 1500 km from the European coast and 3900 km from the North American coast. Nowadays, over 237 000 people live on these islands,[18] which cover a total area of 2322 km^2. About 77% of the inhabitants live on the islands of São Miguel (~128 000) and Terceira (~55 000).[19]

In addition, the island of Madeira, with a total area of 741 km^2 and a population of ~245 000,[19] was analyzed from 2001.

The basic demographic and environmental features of these islands have been described previously.[20,21] Azores residents are predominantly rural workers who earn money through cattle-associated activities, whereas in Madeira, the major economic activity is tourism.

Hospital patients

Between January 1986 and December 2003, a total of 6702 serum samples from 4618 patients presenting to hospitals with symptoms consistent with leptospirosis, from urban and rural regions, were analyzed. Sixty five per cent were males and the overall age range was 25–74 in 2027 (77%) out of the 2632 whose age was available.

An individual standardized questionnaire was used, including identification, onset of symptoms, clinical, and occupational and social exposure. Until 1992, the patient reports came only from mainland hospitals – central (57%) and southern (29%) – because those from the islands were only submitted to the IHMT from 1992 for São Miguel, 1993 for Terceira, and 2001 for Madeira. Considering the need to obtain more epidemiological data (namely onset of symptoms and age) and more than one serum sample per patient, complementary information was always requested

Table 1 Regional distribution of 1024 cases of leptospirosis in Portugal (1986–2003)

	Population[a]	No. tested	No. cases (%)	Mean annual incidence (/100 000)		
				Annual mean	SD	Range
Central mainland (1986–2003)	1 944 353	2629	604 (23.0)	1.7	0.62	0.7–2.6
Southern mainland (1986–2003)	3 704 668	1348	163 (12.1)	0.3	0.13	0.0–0.5
Azores islands (São Miguel and Terceira) (1992–2003)	182 900	594	244 (41.1)	11.1	6.34	2.7–23.5
Madeira islands (2001–2003)	245 011	47	13 (27.7)	1.8	1.34	0.8–3.3
Total (%)		4618	1024 (22.2)			

[a] Average of Territorial censuses of estimated resident population from 1981, 1991 and/or 2001 according to the study period per region (Instituto Nacional de Estatística – INE).

from the Health Services, especially for the leptospirosis-confirmed cases with a serological follow-up. Nevertheless, this laboratory information is absent for a significant number of mainland and Azores confirmed cases, and more recently for Madeira seropositives. For this reason, these incomplete individual questionnaires were only considered for regional incidence rates and gender distribution analyses. This latest group includes all the Madeira patients, for whom there are no data other than the patient's name.

Laboratory tests

Sera were examined by the microagglutination test (MAT) for the presence of antibodies against pathogenic *Leptospira*, using the reference technique described previously.[4,22] Briefly, 19 serogroups, represented by a battery of 26 reference serovars (including the five local circulating strains), were used as live culture antigens. These were Australis (Australis serogroup); Autumnalis (Autumnalis); Ballum, Arborea and Castellonis (Ballum); Bataviae (Bataviae); Canicola (Canicola); Celledoni (Celledoni); Cynopteri (Cynopteri); Grippotyphosa (Grippotyphosa); Hebdomadis (Hebdomadis); Copenhageni and Icterohaemorrhagiae (Icterohaemorrhagiae); Javanica (Javanica); Louisiana (Louisiana); Mini (Mini); Panama (Panama); Mozdok and Pomona (Pomona); Pyrogenes (Pyrogenes); Hardjo, Saxkoebing, Sejroe and Wolffi (Sejroe); and Tarassovi (Tarassovi). The saprophytic serovar Patoc (serogroup Semaranga) was used as an internal control. Samples were screened at 1:40 dilution and reacting sera were further double-titrated to the end point, defined as the highest dilution that agglutinated 50% or more of the leptospires.

Sera were frozen immediately ($-20\,^{\circ}C$) for further processing if serological examination did not take place within a few days of arrival at the laboratory. Sera were retested whenever anomalous results were observed. In a very few cases clinical specimens were cultured in EMJH semi-solid medium for primary isolation as described previously.[23,24]

Leptospirosis case definition and diagnostic criteria

A laboratory-confirmed case of leptospirosis in this long-term series was defined as a hospitalized patient meeting clinically compatible symptoms (fever, myalgia, headache, nausea/vomiting, prostration and/or jaundice), with laboratory criteria for confirmation: (i) significant MAT titers ($\geq$1:160) in one or more serum specimens; (ii) four-fold or greater increase in MAT titer(s); (iii) seronegative conversion to a minimum agglutination titer of 160; or (iv) isolation of *Leptospira*. The use of this cut-off for positivity (instead of the well known 1:100 in non-endemic areas)[4] resulted from our epidemiological situation, which is characterized by some hospitalized confirmed cases with low antibody titers, even in areas of moderate endemicity like the Azores. A laboratory-confirmed probable case was defined as having a MAT titer of 80 in a single serum sample from a symptomatic patient.

To designate the presumptive serogroup of the infecting serovar, unique titers of 160 or higher were always considered as significant in both the single serum samples or in the latest patient sample in the convalescent phase. When the highest titer was the same with two or more serogroups, the final result was considered as 'serogroup not determined'.

Data analysis

Data for the last eight years were analysed using SPSS software for Windows (version 11.5). In some cases, this eight-year period was compared to the previous ten-year period with the assumption that the lack of two years does not influence the comparison.

Geometric mean titers (GMT) of MAT results were calculated according to Sokal and Rohlf.[25] Statistical analysis was performed with the Pearson Chi-square test, with a CI of 95%, using the same SPSS software.

Results

Regional and annual distribution

During the 18-year study period, a total of 1024 (22%) symptomatic patients were confirmed to have a current *Leptospira* infection. The mean annual incidence rate in the Azores islands was much higher (11.1 per 100 000 population) when compared to the other regions (Table 1). As regards the annual distribution of leptospirosis confirmed cases (Table 2), an average of 57 cases per year (range 16 to 93 cases, SD 23.85) was observed overall. Although the total number of cases for the Azores was smaller than that for the mainland (Table 2), the annual mean frequency of 20 cases was significantly ($p < 0.001$) greater than the equivalent number of 45 cases for the mainland in the same twelve-year period.

Regarding the overall seropositivity rates, a significantly higher percentage (27%; 609/2273) was observed in the most recent eight-year period when compared to the percentage previously obtained (18%; 415/2345) between 1986 and 1995 ($p < 0.001$). This finding appears to be the result of the increased laboratory diagnosis of leptospirosis cases in certain regions, with significantly higher seropositivity rates compared to previous years, namely, in the central mainland, with 30% (324/1072 patients) and 18% (280/1557) seropositives, between 1996–2003 and 1986–1995, respectively ($p < 0.001$), and in the Azores islands with 44% (126/286), 45% (82/182) and 29% (36/126) seropositives between 2000–2003, 1996–1999 and 1992–1995, respectively ($p = 0.006$).

During the eight-year study period 1996–2003, 2273 patients were examined with a total of 3324 serum samples. These numbers were close to the 2345 patients and 3378 sera observed for the previous ten-year period 1986–1995. In addition, a more detailed analysis of the individual number of serum samples in these two periods showed a clear reduction of sera examined per patient in recent years. Sixty five per cent of the patients (1468 out of 2273) had only single samples when compared to only 10% (235 out of 2345) with unique serum samples previously. Except for those 17 leptospirosis cases where a long-term follow-up of the humoral immune response was undertaken between 1996 and 2003, with an average of six serum samples per case during three to four years, the remaining patients observed during this period yielded two (27%; 620 patients), three (6%; 143)

Table 2 Annual distribution by gender of leptospirosis cases in mainland Portugal, the Azores (São Miguel and Terceira) and the Madeira islands

Year	Total tested (no.)	Total cases (no.)	Mainland (center + south)						The Azores (São Miguel and Terceira)						The Madeira islands					
			Males			Females			Males			Females			Males			Females		
			Tested (no.)	Cases (no.)	%	Tested (no.)	Cases (no.)	%	Tested (no.)	Cases (no.)	%	Tested (no.)	Cases (no.)	%	Tested (no.)	Cases (no.)	%	Tested (no.)	Cases (no.)	%
1986	162	16	89	9	10.1	73	7	9.6												
1987	154	42	84	27	32.1	70	15	21.4												
1988	246	64	134	39	29.1	112	25	22.3												
1989	289	53	187	31	16.6	102	22	21.6												
1990	271	27	193	15	7.8	78	12	15.4												
1991	248	23	181	10	5.5	67	13	19.4												
1992	267	46	131	21	16.0	124	19	15.3	12	6	50.0									
1993	288	57	147	33	22.4	109	19	17.4	29	5	17.2	3	0	0.0						
1994	232	40	114	16	14.0	74	16	21.6	37	7	18.9	7	1	14.3						
1995	188	47	102	20	19.6	48	10	20.8	33	17	51.5	5	0	0.0						
1996	229	65	122	25	20.5	70	16	22.9	33	24	72.7	4	0	0.0						
1997	250	84	134	33	24.6	67	24	35.8	43	25	58.1	6	2	33.3						
1998	274	81	135	33	24.4	82	29	35.4	48	16	33.3	9	3	33.3						
1999	223	37	118	10	8.5	66	15	22.7	36	12	33.3	3	0	0.0						
2000	260	85	127	34	26.8	84	27	32.1	41	22	53.7	8	2	25.0						
2001	326	78	138	24	17.4	86	16	18.6	64	27	42.2	12	4	33.3	19	6	31.6	7	1	14.3
2002	397	86	189	38	20.1	121	17	14.0	69	27	39.1	8	1	12.5	6	3	50.0	4	0	0.0
2003	314	93	135	25	18.5	84	22	26.2	76	43	56.6	8	0	0.0	9	3	33.3	2	0	0.0
Total	4618	1024	2460	443	18.0	1517	324	21.4	521	231	44.3	73	13	17.8	34	12	35.3	13	1	7.7

Table 3 Age distribution of 672 (87.6%) out of 767 confirmed leptospirosis cases in mainland Portugal and the Azores (1992–2003)

Age group	Population[a]	Mainland (center + south)		Age group	Population[a]	The Azores (São Miguel and Terceira)	
		Cases (no.)	Cases/100 000			Cases (no.)	Cases/100 000
1–14	352 388	6	0.1	1–14	41 603	1	1.3
15–24	322 118	16	0.4	15–24	32 780	36	9.2
25–34	328 648	44	1.1	25–49	65 936	133	16.7
35–44	327 952	63	1.6	50–64	23 243	40	14.2
45–54	292 573	91	2.6	≥65	20 615	19	7.7
55–64	268 040	100	3.1				
65–74	259 147	96	3.1				
≥75	197 531	27	1.1				
Total		443				229	

[a] Territorial census of 2001 with available data for age range.

and four (1%; 25) serum samples per patient. In the first ten years, the majority of the patients provided two (54%; 1266 patients) and three (27%; 633) sera per patient.

Regarding the average number of days to the first blood sample in the 1468 (65%) out of 2273 patients between 1996 and 2003, a similar annual distribution was observed, with an overall mean of 7.4 days (range 1–15, SD = 4.23) for mainland and 7.2 days (range 2–15, SD = 3.20) for the two Azores islands.

Case distribution

Six hundred and eighty six (67%) of 1024 leptospirosis cases, including 12 of 13 seropositives from Madeira, were males (Table 2), with a clear predominance in the Azores ($p < 0.001$) compared with the central and southern mainland ($p = 0.01$).

According to the available data for the past twelve years, age was known in 672 (88%) of 767 laboratory-confirmed cases (443 from mainland and 229 from the Azores) with the following distribution: 1% under 14 years of age, 8% were in the 15–24 age group, 70% in the 25–64 age group, and 21% in the 65 and over age group (Table 3). In mainland Portugal, the highest incidences of the disease were observed between the ages of 25 and ≥75 years (range 1.1–3.1/100 000 population) whereas in the Azores (Table 3), younger people (from 15 years) were more affected (range 7.7–16.7/100 000), with a significantly higher seropositivity rate compared to the mainland cases for the same age group (15–24 years; $p < 0.001$).

Table 4 Annual distribution of mandatory notifications and number of deaths between 1986 and 2003 in Portugal for leptospirosis

Year	Notifications[a]			Deaths[b]			Deaths/notifications
	Males	Females	Total	Males	Females	Total	Ratio
1986			n.a.	4	1	5	n.a.
1987			n.a.	1	0	1	n.a.
1988			n.a.	7	3	10	n.a.
1989			n.a.	2	1	3	n.a.
1990	n.a.	n.a.	25	12	0	12	48.0
1991	18	8	26	n.a.	n.a.	7	26.9
1992	12	8	20	n.a.	n.a.	12	60.0
1993	23	15	38	n.a.	n.a.	14	36.8
1994	25	13	38	n.a.	n.a.	3	7.9
1995	31	6	37	5	4	9	24.3
1996	32	7	39	15	4	19	48.7
1997	53	14	67	15	4	19	28.4
1998	71	21	92	15	4	19	20.7
1999	31	20	51	6	7	13	25.5
2000	25	20	45	7	2	9	20.0
2001	62	18	80	14	4	18	22.5
2002	27	10	37	7	3	10	27.0
2003	58	14	72			n.a.	n.a.
Total	468	174	667	110	37	183	27.4

n.a.: not available.
[a] Data from Direcção Geral Saúde (DGS) between 1991 and 2002.
[b] Data from Instituto Nacional de Estatística (INE) and Direcção Geral Saúde (DGS) between 1986 and 2002.

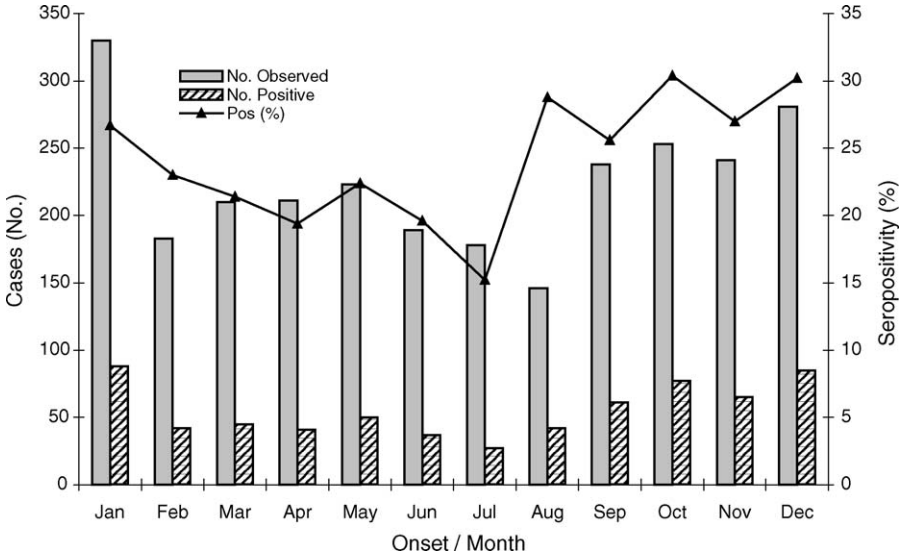

Figure 1 Distribution and seropositivity according to month of leptospirosis onset in 660 (88%) out of 751 MAT-confirmed cases (1992—2003).

Considering the influence of both age and sex on the case distribution, older women and men (≥65 years), with or without rural activity, had similar infection risk ($p = 0.74$). Males from the Azores were more affected than females for the remaining age groups.

Leptospirosis was the reported cause of death in 183 patients and of mandatory notification in 667 patients (Table 4). All patients were hospitalized, but died despite medical support. From the available data, between 1996 and 2002, the highest case-fatality rate for leptospirosis was observed in the 60—69, 40—49 and 70—79 age groups, with 35, 18 and 17 cases, respectively, out of 107 death notifications.[26] Overall, the fatality rate was higher among males (75%). The reported notifications of disease, between 1991 and 2003 (Table 4), followed the same pattern as fatal cases regarding gender (males 73%), and age, with the highest disease frequency (112 out of 521 cases) in the 60—69 age group. Of the remainder, most of the reported cases were in the 50—59, 40—49 and 70—79 age groups with 90, 89 and 80 cases, respectively.[26]

Occupation and risk factors

Information concerning at least one of the following data (county, profession or contact with animals) allowed for an exposure classification in 880 (86%) out of the 1024 confirmed cases. The distribution of seroprevalence during the eighteen-year period was affected by the rural origin of the majority of the leptospirosis cases (695 out of 880, 79%), and thus with a significant link to professional activities, and/or contact with rodents and domestic animals. The likely source of infection was investigated for 596 (58%) of the leptospirosis cases with this type of information. A significant association ($p < 0.001$) was found with each of the following risk sources for leptospirosis: untreated water, cattle, pigs, dogs and rodents. Recreational risk factors were not investigated because only four positive cases reported leisure activities associated with bathing in the central region of the country.

Seasonal occurrence

Distribution according to month of leptospirosis onset in 660 (88%) out of 751 MAT-confirmed cases (1992—2003) is shown in Figure 1. A winter distribution is evident, with the peak months being October and December, besides the months of August and September, with a significant percentage of cases in the summer time. The month of exposure was taken as the month of onset of illness, except when the onset date was between the 1st and 7th days. In these cases exposure was assumed to have occurred in the preceding month. Monthly case numbers varied from 27 cases in July to 88 in January. The average for the wetter months (October—April) was 63 cases and for the drier months (May—September) 43 cases.

Clinical presentation

From the clinical data available for 2855 (62%) of 4618 examined patients, numerous combinations of signs and symptoms were reported on admission (Figure 2). The most common presentation involved nonspecific clinical manifestations, including fever (58%; 2678 patients), myalgia (21%;

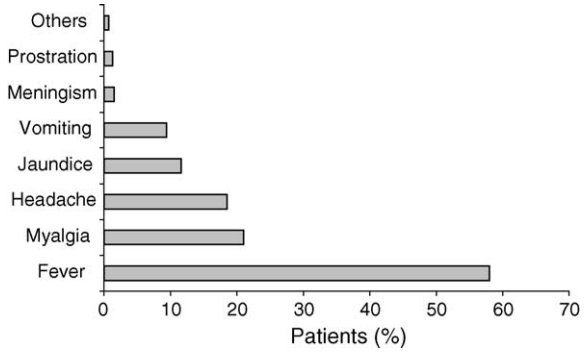

Figure 2 Clinical criteria on admission in 2855 (62%) out of 4618 examined patients (1986—2003).

Table 5 Presumptive serogroup distribution of the 1024 out of 4618 leptospirosis cases by study period

Leptospira interrogans serogroup	1986—1995	1996	1997	1998	1999	2000	2001	2002	2003	Total	%
Icterohaemorrhagiae	88	14	19	11	6	10	9	11	9	177	17.3
Pomona	91	6	11	9	2	4	2	2	5	132	12.9
Ballum	43	10	21	9	2	6	3	4	18	116	11.3
Sejroe	33	7	6	9	3	3	3	3	0	67	6.5
Australis	44	1	2	1	0	2	1	1	1	53	5.2
Grippotyphosa	27	0	2	3	0	2	0	1	0	35	3.4
Canicola	10	6	0	0	0	0	0	0	0	16	1.6
Panama	12	0	0	0	0	0	0	1	0	13	1.3
Louisiana	0	0	0	2	0	0	0	0	0	2	0.2
Others[a]	9	5	4	0	4	3	2	2	5	34	3.3
Cross-agglutination	58	16	19	37	20	55	58	61	55	379	37.0
Total	415	65	84	81	37	85	78	86	93	1024	

[a] Tarassovi (9), Autumnalis (6), Hebdomadis (6), Mini (5), Pyrogenes (4), and Bataviae, Cynopteri, Javanica and Panama (1 each).

969) and headache (18%; 854). Twelve per cent had jaundice (535 patients) and 2% (61) of these had meningism. A minority of patients (0.7%; 31) had other features, namely, conjunctival suffusion, exanthema and vasculitis.

In the patients testing negative (1467 out of 2855), the main clinical diagnosis was flu-like illness (55%). As regards the clinical diagnosis of the 1024 MAT-confirmed leptospirosis cases, seven main categories were observed. Anicteric and icteric leptospirosis accounted for the majority (49%) of the cases (330 and 115, respectively), followed by pyrexia of unknown origin (22%; 197 cases), hepatitis (8%; 68), acute kidney failure and meningitis (6% each; 56 and 53), and pneumonia (5%; 43).

Laboratory confirmation

Between 1996 and 2003, 517 (85%) of 609 patients were diagnosed serologically by the detection of a significant titer ($\geq$1:160) at the first serum sample, with a geometric mean titer (GMT) of 801 ranging from 1125 in 1996 to 427 in 2002. Of the remaining 92 cases, 12% seroconverted from negative and 88% showed a four-fold increase or greater in MAT doubtful or positive titers in paired serum samples, with a mean GMT of 804. From the 241 (47%) of the 517 cases with a positive MAT titer in the first serum sample, 88.4% had unique titers of 1:320 or greater.

There was presumptive serologic evidence of nine major infecting serogroups with Icterohaemorrhagiae the largest serogroup followed by Pomona and Ballum (Table 5). Cross-agglutination with at least two serogroups occurred in 403 cases with sera showing co-agglutinins in single or paired acute stage samples at significant titers, in particular, in more recent years. Cross-agglutination was found particularly between Icterohaemorrhagiae and Ballum (18%), Icterohaemorrhagiae and Sejroe (17%), or Pomona and Sejroe (16%).

Discussion

Leptospirosis has been confirmed as a steadily increasing public health problem in Portugal, particularly in the central mainland and São Miguel and Terceira islands, over periods of eighteen and twelve years, respectively. The present series of 1024 cases is unique in presenting regional information on leptospirosis in two of the most affected areas.

There has been a significant increase in case-frequency reporting since 1997, due to the compulsory notification system of Direcção Geral de Saúde (General Directorate of Health)[13], with the highest numbers in 1998 and 2001 (92 and 80 cases, respectively). Moreover, between 1996 and 2003, only 135 cases are known to have been officially reported in other regions (including 45 in the north of Portugal) when compared to the 134 and 211 notified cases, respectively, for the Azores and the central mainland region in the same period (Figure 3).[26]

The semi-tropical Azores islands, with a mean annual incidence (1992—2003) of 11.1 cases per 100 000 population, had the same high risk of exposure as some tropical countries such as Tahiti (11.3), the French West Indies (8.9) and La Réunion Mayotte (7.4).[27] Furthermore, the mean annual incidence (1986—2003) of 1.7 cases per 100 000 in mainland Portugal was much higher than the reported incidence of 0.4 cases per 100 000 in France, assumed to be one of the highest in Western Europe.[28] These data likely reflect a true increase in cases mainly due to global climate changes and the consequent increasing contact with contaminated water and rats.

A seasonal winter distribution was evident, as reported elsewhere.[1,2,29] In fact, climatic conditions in our country are favorable for *Leptospira* transmission, particularly on the Azores islands,[30,31] where the level of humidity is high (77% average/year), and precipitation, although regular and well distributed throughout the year, has a greater abundance during the winter months.[21] The observed global higher incidence rates in wetter years, namely 1988/1989 and 1997/1998,[13,26] demonstrated the recognized importance of variation in rainfall in human transmission.[29] Moreover, the well-known warming effects of the Gulf Stream in recent years with a mean air temperature around 13 °C during the colder months (January/February), and a mean value of 23 °C in the warmer months (July/August), may also have contributed to the greater survival time of viable leptospires in surface waters.

Besides vaccination of domestic animals and rodent control, the prevention of occupational human exposure is recognized as a basic control measure. In this case, the

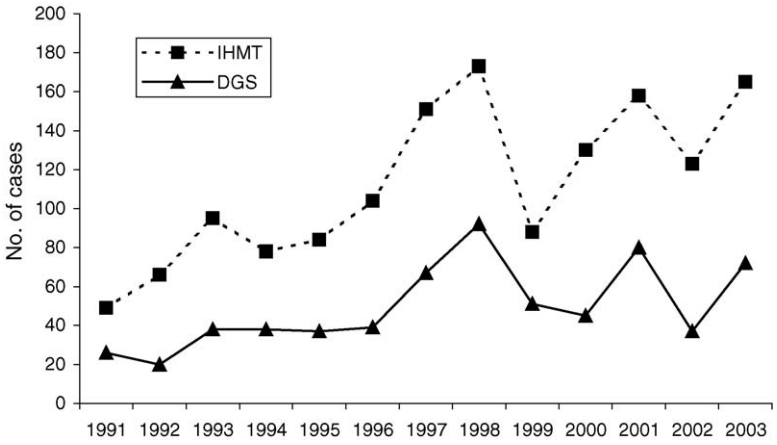

Figure 3 Distribution of 642 mandatory notifications from Direcção Geral de Saúde (DGS), and 822 laboratory-confirmed cases of leptospirosis at the Institute of Hygiene and Tropical Medicine (IHMT).

use of protective clothing, avoiding fresh water ponds, washing hands after a risk contact with contaminated material, and early reporting of symptoms and signs compatible with a differential diagnosis of leptospirosis, should be widely implemented in the risk-areas.

The disease predominantly affects active adult men, following the universal trend in temperate zones,[7,8] throughout the different age groups (15 to ≥75 years). Older women (≥65 years) were equally at risk in both regions. This finding reflects a changing social pattern over recent years, in which retired women are more likely to undertake manual work in higher risk environments.

Leptospirosis in males, being more significant in São Miguel and Terceira, can be explained by a number of potential risk factors, besides the strong environmental risk exposure with highly infected small mammals (*Mus musculus*, *Rattus rattus*/*R. norvegicus and Erinaceus europaeus*), which showed an overall Leptospira isolation rate of 53% in São Miguel and 46% in Terceira, over a three-year period.[30] In addition, a significantly high transmission risk from house mice in Terceira was also reported,[31] emphasizing the need to develop integrated control programs to reduce the risk of human disease. Such an approach has just been implemented.

The clinical picture of leptospirosis ranges from influenza-like to fulminant disease. Being highly variable and dependent on the infecting serovar, infection dose, host factors or a combination of these,[4] laboratory confirmation is important for accurate diagnosis. Fatal cases in young people progress rapidly in the first week of the disease to acute renal failure and respiratory distress syndrome, before seroconversion occurs.

According to official data, between 1986 and 2002, fewer than 19 deaths due to leptospirosis have been reported each year.[14] It is important to note that there has been increasing attention given to this zoonosis, which has resulted in an earlier recognition and presumptive clinical diagnosis, with earlier initiation of appropriate therapy, including placement in intensive care units. However, we assume that a great number of undiagnosed cases occurred in earlier times, before the recent increased awareness of leptospirosis as an important public health problem.[32]

Although isolation was not usually attempted for logistic reasons, the standard MAT was always performed. Leptospires of nine serogroups were responsible for most of the cases, but Icterohaemorrhagiae and Ballum were respectively the first and the third major infecting serogroups. These MAT results are in agreement with the previously confirmed circulating serovars Copenhageni/Icterohaemorrhagiae and Arborea/Ballum in the endemic areas, by kidney isolation from rodents (genera *Mus* and *Rattus*) and/or insectivores (*Erinaceus europaeus*).[33] As regards Pomona, the second major serogroup only identified in patients from mainland Portugal, its predominance correlates well with the field isolates of serovar Mozdok, not only in pigs[34] but also in different sylvatic species, namely the insectivore *Crocidura russula*, the field-mouse *Mus spretus* and the two rats *Rattus rattus* and *R. norvegicus*.[33] Another strain of serogroup Pomona, typed as *L. kirschneri* serovar Tsaratsovo, was also recently obtained from an abattoir horse.[35] Of the remaining serogroups, strains belonging to Sejroe (serovar Hardjo), Australis (serovar Bratislava) and Canicola (serovar Canicola) groups have all been recovered from domestic animals in Portugal as follows: from cattle,[36] from pigs and horses[34,35] and from dogs (Collares-Pereira, unpublished data), respectively.

Leptospiral MAT conducted on a large number ($n = 241$; 40%) of unique serum samples of confirmed cases between 1996 and 2003, yielded agglutination titres ≥320 (data not shown). Prior to 1996 the titres were much higher ($p = 0.001$). This difference may be attributed to earlier therapy due to the recognition of disease and support from the clinical laboratory, since there has been no change in the form of therapy.[1,4]

Infection with serovars from the Icterohaemorrhagiae serogroup has been reported to be associated with increased disease severity,[7,9] while a number of published studies have questioned the relationship between infecting serogroups and severity.[36–39] In the current study, severe cases of leptospirosis were associated with the three major infecting serogroups but Icterohaemorrhagiae was confirmed to have a clear association with the more serious forms, particularly in Terceira and São Miguel.[32]

In conclusion, the knowledge that demographic, climatic, and environmental changes can place new areas or specific

populations at increased risk for infection indicates the need for good surveillance, communication, and laboratory support to reduce the impact of leptospirosis in Portugal.

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