LABORATORY DIAGNOSIS OF LYME BORRELIOSIS AT THE PORTUGUESE NATIONAL INSTITUTE OF HEALTH (1990-2004)

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Lyme borreliosis is considered to be an emerging infection in some regions of the world, including Portugal. The first Portuguese human case of Lyme borreliosis was identified in 1989. Since 1999, this disease is considered a notifiable disease (DDO) in Portugal, but only a few cases are reported each year, which does not allow consistent analysis of risk factors and the impact on public health. In this study the authors analyse the data available at the Centre for Vectors and Infectious Diseases Research (CEVDI) laboratory, at the Instituto Nacional de Saúde Dr. Ricardo Jorge (National Institute of Health, INSA) during the past 15 years (1990-2004) and evaluate them against the registry of national reported cases (1999-2004). Serological tests were the basis for laboratory diagnosis. Data on year of diagnosis, sex, age, geographical origin and clinical signs are available for 628 well documented Portuguese positive cases. The number of cases per year varied between 2 and 78, with the highest number of cases reported in 1997. Of the positive cases, 53.5% were female and the age group most affected was 35-44 years old. Neuroborreliosis was the most common clinical manifestation (37.3%). Human cases were detected in 17 of the 20 regions of Portugal, and the highest number of laboratory confirmed cases were from the Lisbon district. The comparison of the number of notified cases and the number of positive cases confirmed by our laboratory show that Lyme borreliosis is clearly an underreported disease. Due to the scattered distribution of the positive cases and the low prevalence of the tick species *Ixodes ricinus*, the most effective prevention measure for Lyme borreliosis in Portugal is education of the risk groups on how to prevent tick bites.

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Introduction

Lyme borreliosis has been reported throughout Europe where it is the most common tickborne infection, as it is in the United States [1].

Clinically, it shows up as a multisystemic disease, presenting dermatological, rheumatic, neurological and cardiac manifestations.

The first reported human case of Lyme disease in Portugal was identified in 1989 [2]. Diagnosis is preformed by the Centre for Vectors and Infectious Diseases Research (CEVDI) at the Instituto Nacional de Saúde Dr. Ricardo Jorge (National Institute of Health, INSA), using several techniques including culture, PCR, and antibody detection. The first strains of *Borrelia burgdorferi* sensu lato were isolated from ticks captured in the south of Portugal [3] and the study showed that they belong to a new species, *B. lusitaniae* [4]. Subsequent studies confirm the presence of several *B. burgdorferi* s.l. species (*B. lusitaniae*, *B. afzelii*, *B. garinii* and *B. valaisiana*) in ticks and the infection prevalence could vary between: studies have found prevalences of 11.9% (n= 234, collected in several regions), 11.8% (n= 2806, Mafra region), 34.7% (n=206, Grandola region); and 31.2% (n=285, the island of Madeira) [5, 6, 7, 8]. In all the studies made so far, *B. lusitaniae* is the most prevalent borrelia species. Recently, a strain of this species was isolated from a human sample, indicating that it could cause disease in humans [9]. Other species of borrelia, *B. garinii*, *B. afzelii B. burgdorferi* sensu stricto and *B. valaisiana* have already been detected in mainland Portugal and/or the island of Madeira [5, 10]. Since 1999, Lyme borreliosis has been a mandatorily notifiable disease in Portugal, but only a few cases are reported each year, which does not allow consistent analysis of risk factors and the impact on public health. The aim of this study was to contribute to a more precise evaluation of the epidemiological situation of Lyme borreliosis in Portugal, analysing the data available at the CEVDI's laboratory concerning the serological diagnosis of this disease and data available on the statutory notifiable disease register.

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Material and methods

The results of previous testing of all the sera and/or cerebrospinal fluid (CSF) of patients with clinical suspicion of Lyme borreliosis received at CEVDI's laboratory between 1990 and 2004 were analysed retrospectively. The antibodies were detected by indirect immunofluorescence in-house assay using a strain of *B. garinii* and a cut-off of 1:256 for IgG in sera and 1:4 in CSF were adopted. All borderline and positive samples were confirmed by immunoblot assay also an in-house test, using a strain of *B. garinii*. The interpretation was done according to the European group recommendations [11].

All the positive sera were tested to *Treponema* spp. and rheumatoid factor and all sera with a positive result were considered to be false positives for Lyme borreliosis. The laboratory definition of a positive case is when we detected a seroconversion (significant change in levels of the specific antibodies IgG and/or IgM in two samples), or when we detected a positive titres of specific antibodies in one sample, in patiens with clinical suspicion of Lyme borreliosis [12].

The data from the laboratory confirmed positive cases were compared with the available data from the cases of Lyme borreliosis notified during the period of 1999-2004. The notification of human cases of Lyme borreliosis was done directly by the clinician to the competent health authority, the Direcção Geral de Saúde (DGS), at the health Ministry. The case definition establish to the clinicians by the health authority must fit the following criteria. Confirmed case: Erythema migrans confirmed by laboratory findings or at least one of the late manifestations of Lyme borreliosis with laboratory confirmation.

Results

Among 12 535 biological samples taken for analysis from patients with clinical suspicion of Lyme borreliosis, 628 (5%) tested positive using the EUCALB diagnostic criteria.

In patients with neurological symptoms, CSF was sometimes sent for analysis (21%). Data is available describing the 628 Portuguese patients, 129 of whom tested positive for both CSF and sera. The remaining 499 patients were diagnosed based in the result of sera analysis, with the observation of seroconversion. The number of cases per year varied between 2 and 78, with the highest number of cases in 1997 [FIGURE 1].

The geographical distribution of the positive cases, based in the patients' home addresses, shows that Lyme borreliosis infection has been seen in 17 of the 20 districts of Portugal [FIGURE 2].

There were slightly more female patients (53.5%) than male patients (46.5%).

FIGURE 1

Number of samples received at CEVDI/INSA, and percentage of Lyme borreliosis cases found to be positive, Portugal, 1990-2004

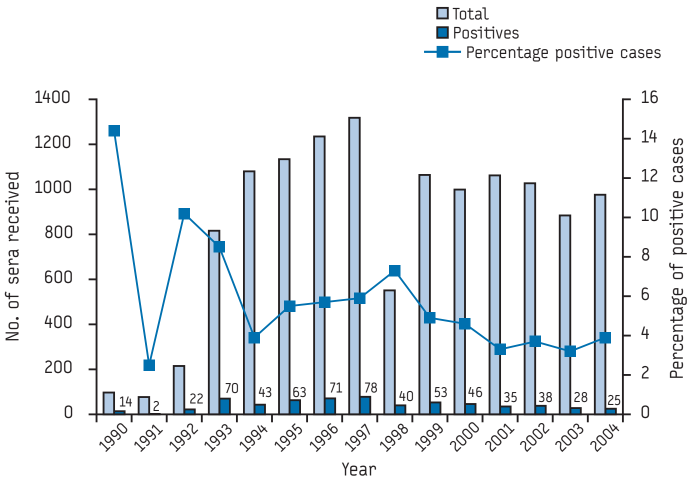
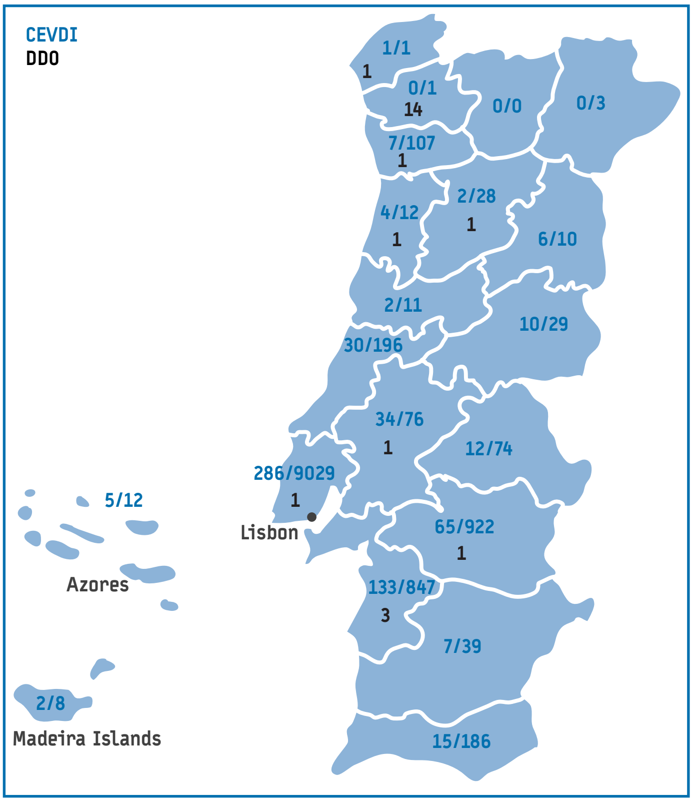


FIGURE 2

Geographical distribution of the positive cases by cases studied at CEVDI (1990-2004) and number of notified cases, Portugal, 1999-2004



The notification forms were frequently not filled in completely, which may have caused some distortion in the data analysis of age and clinical manifestations. Information on patient age was available on only 62.3% of the forms. Analysing the available data, the mean age was 44 years old (range: 2 months to85 years) and the age group most affected was 35-44 years old (21.3%) [FIGURE 3].

No clinical symptoms were reported in 237 (37.7%) of the 628 positive cases [FIGURE 4]. Analysis of the information provided by the physician in the remaining 391 cases showed that the most frequently reported manifestations were neurological, reported in 146 patients (37.3%), followed by nonspecific symptoms in 109 cases (27.8%). Five of the cases with nonspecific symptoms had hepatic symptoms (4.5%), nine had myalgia (8.3%), 19 had optical symptoms (17.4 %) and 76 reported only fever (69.7%).

The evaluation of the number of cases reported nationally between

FIGURE 3

Number of Lyme borreliosis cases by age group, Portugal, 1999-2004

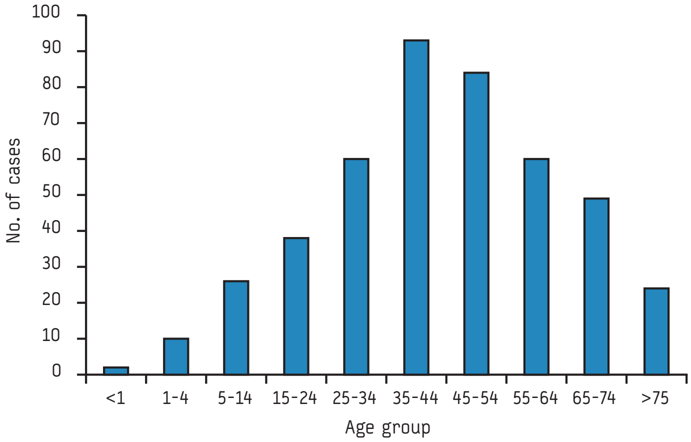
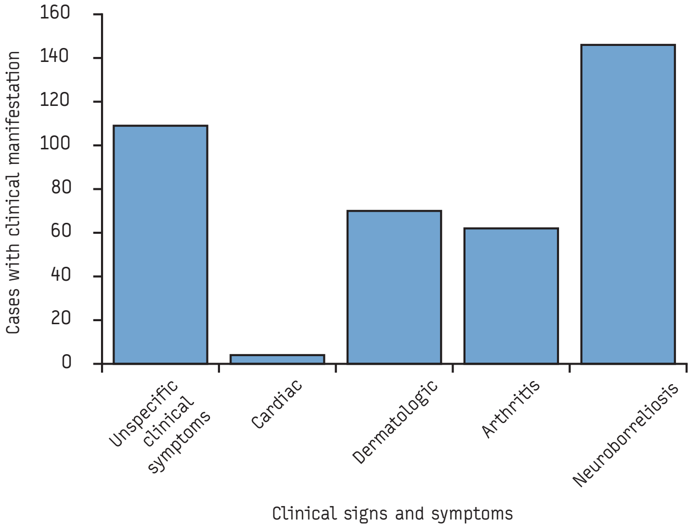


FIGURE 4

Distribution of positive Lyme borreliosis cases, by clinical signs and symptoms, Portugal, 1999-2004



1999 and 2004 (n=24) [13] and the number of positive cases confirmed by our laboratory (n=225) during the same period, show that is clearly an underreported disease. The annual incidence, estimated on the basis of the statutory notifiable disease is 0.04 per 100 000 inhabitants. However, when laboratory data are taken into account, we assume that this rate could be on average 10 times higher, 0.4 per 100 000 inhabitants [FIGURE 5].

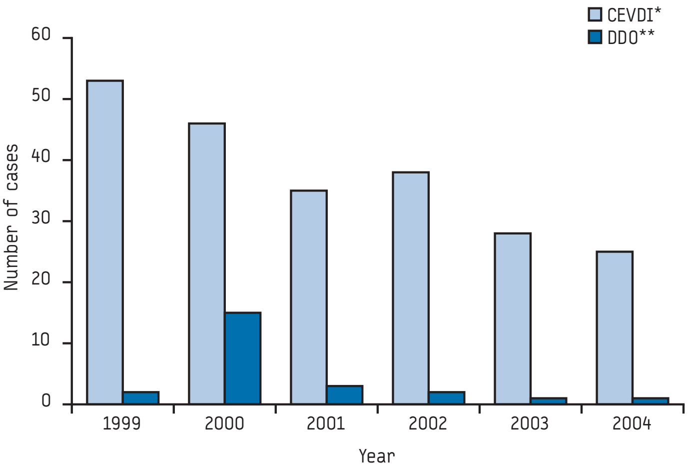
Discussion/Conclusion

Although Lyme borreliosis is a mandatorily notifiable disease in Portugal, the evaluation of CEVDI data concerning human cases of Lyme borreliosis and the number of notified cases during the same period (1999-2004) shows that this disease, like other vector borne diseases, such as boutonneuse fever (the most prevalent tick borne disease in Portugal), is clearly underreported in our country [14]. According to our data, between 1999-2004 we detected an average of 35 new cases of Lyme borreliosis each year. Other diseases such as AIDS and tuberculosis have a bigger impact on public health and the general impression gained is that Lyme borreliosis cases are not considered important enough to notify and to publish.

The major problem of underreporting is the impossibility of realise an epidemiological analysis of Lyme borreliosis in Portugal. For example, according to the notification data, Lyme borreliosis is more common in the Braga district (n=14) in northern Portugal, but when the results are analysed, the only sample from this district to be sent for analysis was negative and the districts showing higher number of confirmed cases are Lisbon (n=286), Setubal (n=133) and Evora (n=65)

FIGURE 5

Number of notified Lyme borreliosis cases and number of cases diagnosed at CEVDI during the period 1999-2004, Portugal



* CEVDI : Centro de Estudos de vectores e doenças infecciosas

** DDO : Doença de Declaração Obrigatória (Mandatory notification of disease)

districts located in central and southern Portugal. It is also possible that the results have been influenced by the proximity of the CEVDI's facilities to these regions, and the hospitals and physicians located at Northern regions of Portugal may usually send their samples to other regional laboratories that also perform these tests. For example, if sufficient samples from Braga district and other northern regions were sent to our laboratory, perhaps the proportion of positive cases in these regions would increase. Also, if we analyse not only the number of positive cases but also the proportion of it, the district of Lisbon is simultaneously the district with a higher number of positive cases and one of the districts with a lower proportion of positive cases.

As the laboratory data are not cross-checked with the official data, it is impossible to know which cases detected at CEVDI were reported to the health authorities, which laboratories performed the laboratory testing and why the clinicians did not notify the positive cases that they diagnose. Also, the fact that some of the positive cases may have been in patients who acquired their infections in districts or countries other than their area of residence should be considered, although patients in Portugal usually use the health facilities in their area of residence. In our experience, fewer than 10 patients during the time period considered (1999-2004) mentioned the possibility that they may have acquired their infection outside of their area of residence. However, the number of positive cases of Lyme borreliosis detected is undoubtedly higher than the number of cases reported. The reported incidence of Lyme borreliosis in Portugal is among the lowest reported in Europe. However, if we analyse the proportion of positive cases detected during this study (5%), we can see that this value is similar to the detected in other studies of seroprevalence in risk populations performed in several European countries [15]. After 15 years performing laboratory diagnosis, even knowing the limitations of laboratory results and being aware that the diagnosis of Lyme borreliosis should be always established by the clinician, these data, could contribute to the better understanding of the epidemiology of Lyme borreliosis in our country. To improve the notification of this disease, a network should be established to link all laboratories performing Lyme borreliosis diagnosis, aggregating all laboratory detected cases. This would allow the competent health authority to compare this information with the cases notified by clinicians and to make a more accurate analysis.

The distribution of positive cases is influenced by clinicians' awareness of vector borne diseases, but the size of the *I. ricinus* population and the prevalence of infected ticks are also contributory factors to the incidence of the disease. The estimated annual incidence for Lyme borreliosis in Portugal is 0.04 per 100 000 inhabitants. A higher estimated can be obtained if we take laboratory data into consideration (0.4 per 100 000 inhabitants). However, as other laboratories also perform this test, it seems likely that underreporting is even higher, and consequently the true incidence of Lyme borreliosis in Portugal should be similar to the published values detected in other countries such as Scotland (0.6 per 100 000 inhabitants), United Kingdom (0. 3 per 100 000 inhabitants) and much lower than that detected in countries such as France (16 per 100 000 inhabitants), Germany (17.8-25 per 100 000 inhabitants), Bulgaria (55 per 100 000 inhabitants), Slovenia (120 per 100 000 inhabitants) and Austria (130 per 100 000 inhabitants) [1, 16, 17]. It would be interesting to compare the incidence detected in Portugal with geographical areas such as southern Spain, Morocco, Tunisia and Algeria but, to our knowledge, there are no available data published concerning the incidence of the disease in the these regions. All these areas share with Portugal some eco-epidemiological aspects such as vector population abundance and prevalence of infection, lack of information about the vertebrate reservoirs and the presence of the different *Borrelia burgdorferi s.l.* strains with particular relevance to *B. lusitaniae*., During the past five years, the number of human cases detected each year at CEVDI seems to have stabilised at approximately thirty five cases per year. This reduction may perhaps be explained by the increased number of other laboratories performing this diagnosis. Also, due to the diversity of the possible clinical presentations of Lyme borreliosis that may be confused with other aetiologies, the benign course of the majority of clinical cases, and the usually very positive response to the timely application of antibiotics, a large percentage of cases are never sent to the laboratory to confirm a clinical diagnosis. In this study, the positive cases which mention erythema migrans are very rare, probably because many clinicians are aware that this stage frequently does not evoke an antibody response and that laboratory confirmation cannot be expected, and therefore do not request a laboratory confirmation of their clinical diagnosis. Considering that the incidence of Lyme borreliosis is directly linked to the density of the tick vector *I. ricinus*, and knowing that this species is not found in high tick population densities, we would expect the incidence of Lyme borreliosis to also be low. However, we should also consider the *I. ricinus* **has been found to exist** all over the country, but due to differing environmental characteristics, especially climate, distribution is not uniform throughout Portugal but focused in some regions where conditions are more suited to the survival of this tick species, and where this species predominates, achieving high population density.

In the absence of publications describing clinical cases, the information available in the clinical forms is very useful because the analysis allows us to clarify some epidemiological aspects such as risk factors concerning age, sex and geographic localisation.

Other information that would help laboratory diagnosis, such as symptom onset date, information about occurrence of recent tick bites, and recent trips, are frequently unavailable. This is why collaboration and exchange of information between clinicians and laboratories are so important.

Research concerning the eco-epidemiology of Lyme borreliosis in Portugal has so far been slow to advance, and it is difficult to study the impact and risk factors. However this knowledge is essential if we are to implement adequate prevention programmes, which are currently considered the best approach to solving the problem of vectorborne diseases. Sixteen years after the report of the first human case of Lyme borreliosis in Portugal this is still a poorly understood disease in Portugal. Due to the scattered distribution of the positive human cases and the scattered nature of the tick vector distribution throughout Portugal, the most effective prevention measure for Lyme borreliosis in Portugal is probably educating risk groups about how to avoid tick bites.

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ORIGINAL ARTICLES

Surveillance report

CASE-CONTROL STUDY FOR RISK FACTORS FOR Q FEVER IN SOUTHWEST ENGLAND AND NORTHERN IRELAND

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Q fever (*Coxiella burnetti*) is thought to account for 1% (700 cases) of community acquired pneumonia in the United Kingdom each year, and can result in serious complications such as endocarditis. Although outbreaks have frequently been reported worldwide, the causes are often not clearly identified and there have been few studies of risk factors in sporadic cases.

We conducted a matched case-control study. Cases of acute Q fever in people aged over 15 years in southwest England and Northern Ireland were identified from January 2002 to December 2004. Controls were matched for age, sex and the general practice at which they were registered. Questionnaires asking about contact with animals, and leisure and work activities, were posted to cases and controls.

Questionnaires were completed by 39/50 (78%) of the cases and 90/180 (50%) of the controls. In the single variable analysis, occupational exposure to animals or animal products was the only risk factor associated with cases at the 5% level (P=0.05, odds ratio (OR) 3.4). Long term illness appeared to be significantly protective (P=0.03, OR 0.3). In multivariable analysis the strength of association between occupational exposure and illness remained high (OR 3.6, 95% confidence interval (CI) 0.9 to 14.8) and smoking emerged as a possible risk factor.

This is the first case-control study to identify occupational exposure to animals or animal products as the most likely route of infection in sporadic cases as opposed to outbreaks.

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Key words: Coxiella, Q fever, occupational exposure, case-control studies.

Introduction

Q fever is a zoonotic infection caused by the rickettsial organism *Coxiella burnetii*. In the United Kingdom it is most commonly carried, often asymptomatically, in sheep, cattle and goats, and is transmitted to humans by inhalation of aerosols. High concentrations of the organism are found in the placenta/placental fluids. Coxiellae can remain viable for months in the environment. The disease occurs most frequently in humans exposed to farm animals or in areas where animal products are handled [1]. Retrospective serological studies have shown evidence of high rates of past infection in farm workers, which suggests that many cases are often not identified at the time of illness [2].

The major clinical manifestations of Q fever are respiratory, cardiac and hepatic, although symptoms are often non-specific. *C. burnetii* is thought to account for 1% (700 cases) of community-acquired pneumonia in the UK each year, and although more serious complications such as endocarditis are rare, they do represent a significant burden of disease [3].

Although outbreaks have frequently been reported worldwide, the causes have often not been identified [4] and we have only been able to