Trends in Human Leptospirosis in Denmark, 1980 to 2012

L B van Alphen^{1,2,3}, A Lemcke Kunoe⁴, T Ceper⁵, J Kähler⁶, C Kjelsø^{4,7}, S Ethelberg⁴, K A Krogfelt (kak@ssi.dk)¹

1. Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark

2. European Programme for Public Health Microbiology (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

3. Current address: Department of Medical Microbiology, Maastricht University Medical Center, Maastricht, the Netherlands

4. Department of Infectious Diseases Epidemiology, Statens Serum Institut, Copenhagen, Denmark

5. Department of Microbiological Diagnostics and Virology, Statens Serum Institut, Copenhagen, Denmark

6. Department of Health Analysis, Statens Serum Institut, Copenhagen, Denmark

7. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Citation style for this article:

van Alphen LB, Lemcke Kunoe A, Ceper T, Kähler J, Kjelsø C, Ethelberg S, Krogfelt KA. Trends in Human Leptospirosis in Denmark, 1980 to 2012. Euro Surveill. 2015;20(4):pii=21019. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21019>

Article submitted on 07 April 2014 / published on 29 January 2015

Leptospirosis in humans is a mandatory notifiable disease in Denmark. To identify changing trends in human leptospirosis in Denmark, we analysed data from the passive laboratory surveillance and clinical notifications from a 32-year period (1980–2012). In that period, 584 cases of leptospirosis were laboratory-diagnosed, an average annual incidence rate of 0.34 cases/100,000 population (range: 0.07–1.1/100,000 population). Seventy per cent of patients were male. Overall, Patoc was the predominant serogroup diagnosed (32%) but over time, the *Leptospira* serogroup distribution has changed. In recent years Icterohaemorrhagiae and Sejroe have been diagnosed most frequently, in contrast to Patoc and Sejroe in earlier years. Notification data for 170 cases showed that work-related exposures were reported in 48% of infections, with fish farming (44%) and farming (22%) as the most frequently mentioned professions. Other common exposures were related to travel (13%), recreation (8%) and sewage (7%). Geomapping of cases showed a geographical clustering for some exposures. Future preventive measures could include raising awareness among clinicians about the risks and prevention of exposure in specific groups (fish farmers, farmers and travellers) to reduce leptospirosis in Denmark.

Introduction

Leptospirosis is a serious, acute febrile disease caused by spirochaetes from different species of pathogenic *Leptospira* bacteria. Leptospirosis is recognised as an emerging public health problem worldwide [1]. It is considered a zoonotic disease, as pathogenic *Leptospira* live in the kidneys of many host animals, including livestock and rodents. In Denmark, rats and mice are the most common carriers of leptospires, but many other animals, including cows and dogs can carry the bacteria. It has also been shown that in 2006 and 2007 in certain suburban sewage areas of Copenhagen, the

prevalence of *Leptospira* spp. in rats ranged between 48% and 89% [2]. The bacteria are shed into the environment via urine and can survive in fresh water like rivers and lakes, but not in sea water. The optimal environment for *Leptospira* are warm and humid conditions, but they survive in temperate climates as well. Infection in humans can occur through direct contact with an infected animal or its excrements (primarily urine) or through contaminated fresh or sewage water [3].

A *Leptospira* strain with unique antigens is termed a serovar, and several serovars with related antigens are placed in a serogroup. Approximately 30 serogroups are recognised containing ca 300 different serovars [4]. *Leptospira* spp. serovars are often specific to particular hosts and can therefore indicate a probable source of infection in humans [4,5], e.g. Icterohaemorrhagiae from rats, Sejroe and Saxkoebing from mice, Canicola from dogs, Hardjo from cows, and Pomona and Bratislava from pigs.

The disease has a mild and a severe form. Most commonly, the symptoms are non-specific and include fever, abdominal and chest pain and nausea, but can in severe cases lead to renal failure and haemorrhage, known as Weil's disease. As the disease resembles several other acute infections, the differential diagnosis includes influenza, viral meningitis, acute abdominal infection, glomerulonephritis [4], but also other zoonotic diseases occurring in the same epidemiological and ecological context, such as hanta virus infection, brucellosis and Q-fever, should be considered. For correct diagnosis it is essential to focus on the patient's travel history, activities, and exposure to animals. Culturing can be difficult, time consuming and requires specialised growth media, and is therefore not recommended for a quick routine diagnosis, but

it remains essential for a direct diagnosis of leptospirosis as well as for surveillance of resistance profiles. Leptospirosis diagnosis without culturing is conventionally performed serologically [6] and, more recently, by PCR [7,8].

Leptospirosis has been studied and diagnosed in Europe for a long time and historical reviews from Germany, France, Portugal and the Netherlands [9-12] have provided insight into the epidemiology of leptospirosis. The aim of this study was to describe the incidence of human leptospirosis in Denmark over time and analyse possible sources of exposure.

Methods

Laboratory diagnosis

Culturing for *Leptospira* is normally performed from blood, urine and spinal fluid in either Korthof medium or EMJH medium (DIFCO). In this study, patient sera were tested for specific antibodies by microagglutination test (MAT) with a variety of *Leptospira* strains which represented the strains Danish patients are typically infected with [4,5]. Over time, the serovars included in the MAT have changed. The MAT included *Leptospira* spp. of serovars: Ballum, Bataviae, Bratislava, Canicola, Grippotyphosa, Hardjo, Hurstbridge, Icterohaemorrhagiae, Poi, Pomona, Saxkoebing and Sejroe from 1980 to 2011 and included locally selected strains. The use of serovar Poi was discontinued in 2008 because of poor growth of the strain. In order to apply the World Health Organization standards and to be able to compare the Danish data with other European reference laboratories, serovars included in the MAT were switched in 2012 to the strains ordered from the Royal Tropical Institute in Amsterdam and now include Autumnalis, Bataviae, Cynopteri, Canicola, Castellonis, Copenhageni, Grippotyphosa type Moskva, Hardjo, Hurstbridge, Icterohaemorrhagiae, Javanica, Pomona, Sejroe and Tarassovi. In addition to the mentioned serovars, the sera were also tested against the non-pathogenic strain Patoc, which has antigens common to many serovars in the *Leptospira* family. Patoc does not cause leptospirosis in humans, but agglutination with Patoc acts as a marker for an infection by a pathogenic *Leptospira* strain. The following dilutions of sera (in titre) 1:30, 1:100, 1:300, 1:1,000, 1:3,000, 1:10,000, and 1:30,000 were tested by MAT. The test was considered positive if the highest observed titre was $\leq 1:100$ for at least one of the serovars [4,13]. The infecting serogroup was deduced from the highest titre against at least one serovar in the MAT. Cross reactions between serovars are known [14], therefore we report the infecting serogroups. Since 2009, leptospirosis has also been diagnosed by an in house PCR test using an improved method of DNA extraction [8,15].

Data collection

Data from all cases of leptospirosis diagnosed from 1 January 1980 to 31 December 2012 in Denmark were retrieved from Statens Serum Institut (SSI), the sole

diagnostic laboratory in the country. As cases we included only patients with a laboratory confirmation of leptospirosis and living in Denmark. Clinical diagnosis and detection of *Leptospira* and/or specific antibodies against *Leptospira* is notifiable under Danish law to the local medical officer of health (embedslæge) and to the Department of Infectious Diseases Epidemiology, SSI. The notification includes information on the infected person, location and timeframe of disease onset, documentation of admission to the hospital, as well as data pertaining to the patient's profession and/or workplaces, travel abroad, and any specific information referring to the source of infection.

Population data for the five Danish administrative regions including sex and age group distribution were provided by Denmark's Statistics and the population as of 1 January of each year (www.dst.dk) was used for analysis. For the years before 2007, population data per county (in 2007, 16 counties became five regions) was acquired and calculated into population per region.

Geomapping and geocoding

QGIS 1.8.0_Lisboa (www.qgis.org) was used for the spatial analysis of leptospirosis cases and plotting of incidence per region and per county. A geographical database with county and region borders in vector format (SHP file) was obtained from the Danish Geodata Agency (GST). Leptospirosis cases were geocoded using the Central Population Registry (CPR registry) and the geocoding of addresses from GST. The address data used for the study originated from the Danish Geodata Agency and were built on Official Standard Addresses and Coordinates (OSAK). The standard addresses from the public information server (den offentlige informationsserver, OIS, the basis for the OSAK addresses) are constructed from address data that the Danish municipalities provide. The addresses from all municipalities are gathered in OIS. In OSAK, these data are supplied with extra information such as

FIGURE 1

Annual incidence rate of leptospirosis, Denmark, 1980–2012

FIGURE 2

Geographical distribution of leptospirosis over time, Denmark, 1980–2012

postal codes and are therefore well suited for national use. An OSAK address consists of an address with an address point attached, which is defined with a set of Universal Transverse Mercator (UTM) coordinates. Furthermore, the register contains information about road code, road number and municipality code [16]. Addresses were joined to cases based on the date of disease notification or the date of diagnosis.

Results

Occurrence and incidence of leptospirosis

From 1980 to 2012, 584 Danish cases of leptospirosis were diagnosed in Denmark. The annual number of leptospirosis cases in Denmark peaked in 1981 with 55 cases, and then decreased until the early 1990s. Since then, the annual number of cases has varied from four to 32 cases per year. Over the whole period from 1980 to 2012, the average annual number of cases was 17.

This corresponds to an average annual incidence rate of 0.34/100,000 inhabitants, with the highest annual incidence in 1981 (1.1/100,000) and the lowest annual incidence in 2009 (0.07/100,000) (Figure 1).

Analysis of the incidence rate per county was possible for the period from 1980 to 2006, before Denmark has been organised in five regions. This showed that Ribe county had the highest incidence of 0.91/100,000 population, followed by Viborg county (0.56/100,000) and Ringkjøbing county (0.50/100,000), which are all located on the western coast of Jutland (Figure 2A). The incidence over time showed that Ribe county consistently had the highest incidence (Figure 2 B–C), while no cases were diagnosed in Viborg county between 1990 and 1999 (Figure 2B). Analysis of the incidence rate per region showed that the incidence rate was highest in the region South Denmark (0.44/100,000) and lowest in the region North Denmark (0.26/100,000) and the Capital Region (0.27/100,000) (Figure 2D). The highest annual incidence rate was observed in 1981 in Central Denmark (1.62/100,000).

Male patients accounted for 70% of all infections. Data on the age of the patient was available for 582 cases (99.7%) and the median age was 49.0 years (range: 0–87 years). The incidence rate per age group showed a clear peak for men in the age group 50–59 years, with 0.84/100,000 as a maximum, while the incidence rate for women increased with age, with a maximum incidence rate of 0.47/100,000 in the age group ≥70 years (Figure 3).

Leptospirosis diagnostic tests

The number of tests performed and persons tested could be obtained for the years 2005 to 2012. In those years, 4,438 tests had been performed at SSI on samples from 3,364 individuals. Some testing is performed on persons not residing in Denmark; between 2005 and 2012, 169 persons (5%) tested were not

Danish residents and were therefore excluded from the study. The annual number of tests performed per year increased from almost 400 per year in the period 2005 to 2007, to just below 600 in the period 2008 to 2010. In 2011, the number of tests increased abruptly to 1,018 after media reporting of a death due to leptospirosis. In 2012, the number of tests was 642. This shows that the number of samples submitted for testing has been stable over recent years.

Serogroups over time

Among all 584 diagnosed cases, the most commonly diagnosed serogroups were Patoc (n=187; 32.0%), Icterohaemorrhagiae (n=168; 28.8%) and Sejroe (n=146; 25.0%). However, the distribution of serovar over time has changed. Patoc has become less dominant among the serogroups; while it contributed 50.2% between 1980 and 1989, only 14.7% of the cases were identified as serogroup Patoc in the past 13 years (Table 1). At the same time, Icterohaemorrhagiae has become predominant: The contribution of this serogroup increased from 14.9% between 1980 and 1989 to 47.1% of the cases between 2010 and 2012. In recent years, more cases have had an unknown serogroup, following the introduction of PCR diagnostics in 2008. From all patients diagnosed by PCR, additional samples are requested for serogroup identification by MAT, but samples are not always available.

The monthly distribution of the cases with the most dominant serogroups Icterohaemorrhagiae, Sejroe and Patoc showed an incidence peak in the months August to November for Icterohaemorrhagiae, while the Sejroe and Patoc cases were scattered throughout the year (Figure 4).

Notifications, hospitalisations and deaths

Leptospirosis is a notifiable disease in Denmark and between 1980 and 2012, 170 of 584 diagnosed (30%) leptospirosis cases were notified to the Department of Infectious Diseases Epidemiology at SSI. In recent years, the proportion of notified cases among all diagnosed cases has increased, as between 2004 and 2012, on average 53% of the annual laboratory-diagnosed cases were notified.

For analysis of exposure, hospitalisations and deaths, the information provided in the notification was used. Among the 170 notified cases, 139 (82%) were hospitalised and four deaths (2.3%) were reported between 1980 and 2012.

Source of infection and serogroup

The notification data included information on the most likely source of infection. Among the 170 notified cases, 19 (11%) were female and 151 (89%) male. The sources of exposure are grouped into work (82 cases), travel abroad (22 cases), recreation (14 cases), sewage water (11 cases) and other (10 cases). The likely source of exposure was unknown for 31 notified cases (Table

FIGURE 3
Leptospirosis incidence rate by age and sex, Denmark, 1980–2012 (n = 582)

TABLE 1

Serogroup distribution of leptospirosis cases, Denmark, 1980–2012 (n = 584)

Serogroup	1980–89		1990–99		2000–09		2010–12		Total	
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%
Ballum	1	0.4	0	0.0	2	1.1	1	2.9	4	0.7
Bataviae	0	0.0	1	0.9	0	0.0	1	2.9	2	0.3
Bratislava	11	4.2	8	7.1	6	3.4	0	0.0	25	4.3
Canicola	0	0.0	0	0.0	2	1.1	0	0.0	2	0.3
Grippotyphosa	0	0.0	0	0.0	3	1.7	0	0.0	3	0.5
Hardjo	0	0.0	1	0.9	2	1.1	0	0.0	3	0.5
Hurstbridge	0	0.0	2	1.8	7	4.0	0	0.0	9	1.5
Icterohaemorrhagiae	39	14.9	52	46.4	61	34.5	16	47.1	168	28.8
Patoc	131	50.2	25	22.3	26	14.7	5	14.7	187	32.0
Poi	5	1.9	5	4.5	11	6.2	0	0.0	21	3.6
Pomona	1	0.4	0	0.0	4	2.3	0	0.0	5	0.9
Sejroe	73	28.0	18	16.1	52	29.4	3	8.8	146	25.0
Unknown ^a	0	0.0	0	0.0	1	0.6	8	23.5	9	1.5
Total	261	100	112	100	177	100	34	100	584	100

^a Unknown serogroups are cases diagnosed by PCR only, since 2008.

2). Work-related cases accounted for almost half of the notified leptospirosis cases. The professions that were notified most frequently were fish farmers (45% of work-related cases), farmers (22%) and sewage workers (11%). Not work-related exposure to sewage water, e.g. cleaning of flooded areas after heavy rainfall, was also reported as exposure (6.5%). Among travel-related cases, Asia was the most common destination, but travel in Europe was also reported. In the group with recreational exposure, most exposures were related to fresh water activities.

FIGURE 4

Monthly distribution of leptospirosis cases of the three most dominant serogroups, Denmark, 1980–2012 (n=501)

Although the numbers per group are small, we analysed whether certain serogroups were more common in connection with a certain type of exposure. This showed that fish farmers in 27 of 37 cases were infected with serogroup Icterohaemorrhagiae, while farmers were infected in 10 of 18 cases with serogroup Sejroe. Sewage workers showed a variety of serogroups, while persons exposed to sewage at home were in nine of 11 cases infected with serogroup Icterohaemorrhagiae. Cases related to travel abroad showed more variety of serogroups, although Icterohaemorrhagiae and Sejroe were most commonly reported. Persons exposed during recreation in Denmark were infected with Icterohaemorrhagiae in eight of 14 cases.

Mapping of place of residence at the time of disease onset or of diagnosis was possible for 335 (57.4%) cases. Figure 5 shows the cases in Denmark and indicates the source of exposure. Fish farmers mostly resided in Ribe county and the rest of Jutland where most fish farms are located, while the cases with non-work related exposure to sewage were mainly located in the Copenhagen area (eight of 11 cases). Farmers as well as cases related to travel and recreation were more spread out over the country.

Discussion

Overall, leptospirosis incidence in Denmark has not changed remarkably in the past 32 years. About a third of all leptospirosis cases from 1980 to 2012 were female, similar to what is reported in Portugal (67% male cases) [12], while in the Netherlands, less than 10% of the cases are female [11]. The reported incidence ratio of male vs female cases was 5:1 in Germany

TABLE 2

Sex, possible exposures, and *Leptospira* serogroups of notified leptospirosis cases, Denmark, 1980–2012 (n = 170)

Exposures	Total (%)	Sex		Serogroup				
		M	F	IH	Sejroe	Patoc	Other	Unknown (PCR only)
Work	82 (48.2)	81	1	45	17	1	17	2
Fish farmer	37	37	0	27	2	1	6	1
Farmer	18	18	0	6	10	0	2	0
Sewage worker	9	9	0	2	2	0	4	1
Hunter	3	3	0	3	0	0	0	0
Mink farmer	2	2	0	1	0	0	1	0
Other	13	12	1	6	3	0	4	0
Travel abroad	22 (12.9)	16	6	8	6	1	3	4
Asia (Malaysia, Nepal, Thailand, Bali, Cambodia)	15	13	2	4	6	1	1	3
Europe (Italy, Poland, Spain)	4	1	3	2	0	0	1	1
Central America (Tobago, Venezuela)	2	2	0	1	0	0	1	0
World	1	0	1	1	0	0	0	0
Recreation in Denmark	14 (8.2)	14	0	8	1	2	3	0
Canoe/kayak	4	4	0	2	1	0	1	0
Fishing	3	3	0	2	0	0	1	0
Other water	4	4	0	2	0	1	1	0
Hunting and Fishing	1	1	0	1	0	0	0	0
Other (snake owner, orchard)	2	2	0	1	0	1	0	0
Sewage-related	11 (6.5)	9	2	9	0	0	0	2
Cleaning (after flooding)	9	7	2	7	0	0	0	2
Cleaning (not specified)	2	2	0	2	0	0	0	0
Other exposures	10 (5.9)	6	4	3	1	1	5	0
Farm (living/visiting)	9	5	4	3	0	1	5	0
Combination travel/sewage worker	1	1	0	0	1	0	0	0
Unknown exposure	31 (18.2)	25	6	12	9	4	5	1
Total	170 (100)	151	19	85	34	9	33	9

F: female; IH: Icterohaemorrhagiae; M: male.

and 10:1 in France and Italy [17]. Whether these numbers reflect the true number of cases in these countries is unclear, as it has been reported that clinical leptospirosis is typically more severe in men, which may lead to systematic underinvestigation and undertreatment of female cases [18]. As leptospirosis is known as a neglected disease we analysed whether the number of tests performed had dropped over recent years, but no decline has been observed in Denmark since 2005. In contrast, a strong increase in the number of tests occurred after media reporting on a death due to leptospirosis in 2011.

The trend in *Leptospira* serogroup distribution has changed over the past 32 years. In recent years, Icterohaemorrhagiae has replaced serogroup Patoc, which cross-reacts with most pathogenic *Leptospira* not included in the MAT panel. In Denmark, Icterohaemorrhagiae and Sejroe have become the predominant serogroups during the past 12 years,

while in France and the Netherlands, the two most predominant serogroups are Icterohaemorrhagiae and Grippotyphosa [10,11] and Ireland reports Icterohaemorrhagiae and Hardjo as the dominant serogroups [19]. Unfortunately, since the introduction of PCR diagnostics, we have seen an increase in cases without a known serogroup, as MAT is not performed for all PCR-positive cases.

Overall in Denmark, work-related exposure comprises a larger part of the leptospirosis cases than in other countries, where travel and recreational activities are far more important exposures [9,11,19]. The work-related exposure has decreased compared with an earlier report from Denmark [20], partially due to an increase in travel-related exposure, as could be expected due to an increase in international travel in recent years. Travel-related exposure was seen in 13% of our cases and is observed in other European countries, as illustrated by a recent report of two confirmed

FIGURE 5

Geographical mapping of leptospirosis cases by exposure, Denmark, 1980–2012 (n = 335)

cases after travel in Spain [21]. A recent review also shows a clear increase in the proportion of travel-associated leptospirosis over time [22].

The highest incidence of leptospirosis over the years was observed in Ribe county, as reported previously [5,20]. Geomapping of our cases by exposure showed that most cases in Ribe county are fish farmers. Fish farms only exist in the Danish peninsula of Jutland and constitute an attractive environment for rats. Despite the decline in fresh-water fish farms from more than 700 in the early 1980s, to 196 in 2011 [23], it is still

an important industry in Denmark. Fish farmers constituted 36% of work-related exposures between 2000 and 2009, while no cases among fish farmers have been observed since 2009. Farmers and sewage workers represent the other important work-related exposures, as has been reported from Germany and Ireland [9,19]. Analysis of *Leptospira* serogroup distribution for the predominant types of exposure showed that fish farmers were most commonly infected with serogroup Icterohaemorrhagiae, the serogroup linked to rats. In contrast, farmers were most commonly infected with serogroup Sejroe, suggesting that mice

may be involved in transmission of leptospirosis. Borg-Petersen first isolated serovar Saxkoebing of the Sejroe serogroup in Denmark from the yellow-necked mouse *Apodemus flavicollis* [24], and infection has been reported on rare occasions in man [25]. As rats are well-known carriers of leptospirosis in Denmark, we analysed whether a correlation existed between the annual number of reported rat sightings, as proxy for the rat population in Denmark, and the annual number of leptospirosis cases. Data on the rat population was provided by the Danish Ministry of the Environment in the form of the number of reports of rat sightings per year between 1996 and 2012 (personal communication: Kirsten Søndergaard, July 2014). No correlation between the annual number of rat sightings and leptospirosis cases was found (data not shown). However, as leptospirosis serogroups associated with rats and mice were most frequent in Denmark, rodent control and attention to the risk of infection from rodents' habitats could help prevent the infection.

Exposure to sewage, either work-related or at home (12% of notified cases) can also be a factor in acquiring leptospirosis in Denmark. A recent study addressing illness after cleaning of flooded areas in Copenhagen in July 2011 showed that 56 of 257 (22%) of the involved professionals developed symptoms of illness [26]. A cluster of five leptospirosis cases was detected in Copenhagen after the flooding and one person died. Although only 6.5% of the notified cases in our study were exposed to sewage or flooded areas at home, this could increase in the future, as data from the Danish Meteorological Institute indicate that rising temperatures worldwide could result in more frequent extreme rainfall and storms in Denmark, resulting in more frequent flooding and thus possible exposure to leptospirosis [27]. Reports describing a link between leptospirosis and extreme weather such as heavy rainfall and flooding have been published recently [28,29].

One limitation of our study was that only a proportion of diagnosed cases were notified clinically. Among the notified cases, only 11% were female, while overall, women comprised 30% of the cases. Furthermore, the serogroup distribution was different when comparing all cases with the group of notified cases, as serogroup Patoc was observed in only nine of 170 (5.3%) notified cases, while overall, serogroup Patoc was identified in 32% of cases, and in 14.7% of the cases in the past 13 years. This indicates that the notified cases may not give a true representation of all cases and it is possible that we lack important information on possible exposure among female patients and patients with certain serogroups of *Leptospira*. The discrepancy between the diagnosed cases and the notified cases may reflect differences in disease severity, where a clinically more severe case may be more readily notified. This hypothesis is supported by the fact that fewer women were observed in this group and serogroup Icterohaemorrhagiae was overrepresented among our notified cases. More detailed clinical information on

the severity of disease would have been very useful, but unfortunately clinical information is very limited on the notification forms used in Denmark and was not available for this study.

Leptospirosis is a serious disease, as reflected by the hospitalisation rate of 81% and four reported deaths among our notified cases. The severity of acute infection is obvious, but the long-term effects of Leptospirosis are unknown and chronic infections with *Leptospira* have been previously reported [30]. Leptospirosis has also been implicated as a cause of uveitis in humans [31]. Therefore, it is possible that *Leptospira* may have so far unknown similar chronicity and sequelae as seen in other infections with spirochaetes such as *Borrelia* and *Treponema*.

The non-specific symptoms make the disease likely to be underdiagnosed. In addition, the incidence could increase in the future due to predicted extreme weather conditions and the increase in adventure travels which can include water sports in exotic destinations. However, there is also potential for prevention. To prevent leptospirosis in Denmark, it is recommended to raise awareness among specific groups, such as fish farmers and travellers to Asia, about the risks and prevention of exposure. In addition, awareness should be raised among clinicians about the risk of leptospirosis exposure among these groups.

Acknowledgments

We would like to thank Kirsten Søndergaard from the Danish Ministry of the Environment for providing the data on the rat sightings, Laura Espenhain for her help with the geographical imaging, Charlotte Sværke Jørgensen, SSI and the laboratory staff of the serology lab for technical assistance and Aftab Jasir from ECDC for critical reading of the manuscript. Contains data from the Danish Geodata Agency, County and Region Map SHAPE_UTM32-EUREF89, January 2015.

Conflict of interest

None declared.

Authors' contributions

LBvA, AK and CK have collected the leptospirosis data. LBvA, TC, JK, SE and KAK have analysed the data. LBvA wrote the first draft of the manuscript. All other authors have contributed to further versions of the manuscript and approved the final version before submission.

References

1. Vijayachari P, Sugunan AP, Shriram AN. Leptospirosis: an emerging global public health problem. *J Biosci.* 2008;33(4):557-69. Available from: <http://dx.doi.org/10.1007/s12038-008-0074-z> PMID:19208981
2. Krøjgaard LH, Villumsen S, Markussen MD, Jensen JS, Leirs H, Heiberg AC. High prevalence of *Leptospira* spp. in sewer rats (*Rattus norvegicus*). *Epidemiol Infect.* 2009;137(11):1586-92. Available from: <http://dx.doi.org/10.1017/S0950268809002647> PMID:19393116

3. Levett PN. Leptospirosis. *Clin Microbiol Rev.* 2001;14(2):296-326.
4. Faine S, Adler B, Bolin C, Perolat P. *Leptospira* and *Leptospirosis*. 2nd ed. Melbourne: MediSci; 1999.
5. Lemcke A, Krogfelt KA. Leptospirosis 1982-2001. *Epi-News* 2002; 9. Available from: <http://www.ssi.dk/English/News/EPI-NEWS/~media/Indhold/EN%20-%20engelsk/EPI-NEWS/2002/pdf/EPI-NEWS%20-%202002%20-%20No%209.ashx>
6. Terpstra WJ. World Health Organization (WHO), International Leptospirosis Society. Human leptospirosis: guidance for diagnosis, surveillance and control. Geneva: WHO; 2003. Available from: http://www.who.int/csr/don/en/WHO_CDS_CSR_EPH_2002.23.pdf
7. Levett PN, Morey RE, Galloway RL, Turner DE, Steigerwalt AG, Mayer LW. Detection of pathogenic leptospires by real-time quantitative PCR. *J Med Microbiol.* 2005;54(Pt 1):45-9. Available from: <http://dx.doi.org/10.1099/jmm.0.45860-0> PMID:15591254
8. Villumsen S, Pedersen R, Krogfelt KA, Jensen JS. Expanding the diagnostic use of PCR in leptospirosis: improved method for DNA extraction from blood cultures. *PLoS ONE.* 2010;5(8):e12095. Available from: <http://dx.doi.org/10.1371/journal.pone.0012095> PMID:20711446
9. Jansen A, Schöneberg I, Frank C, Alpers K, Schneider T, Stark K. Leptospirosis in Germany, 1962-2003. *Emerg Infect Dis.* 2005;11(7):1048-54. Available from: <http://dx.doi.org/10.3201/eid1107.041172> PMID:16022779
10. Baranton G, Postic D. Trends in leptospirosis epidemiology in France. Sixty-six years of passive serological surveillance from 1920 to 2003. *Int J Infect Dis.* 2006;10(2):162-70. Available from: <http://dx.doi.org/10.1016/j.ijid.2005.02.010> PMID:16298537
11. Goris MG, Boer KR, Duarte TA, Kliffen SJ, Hartskeerl RA. Human leptospirosis trends, the Netherlands, 1925-2008. *Emerg Infect Dis.* 2013;19(3):371-8. Available from: <http://dx.doi.org/10.3201/eid1903.111260> PMID:23622144
12. Vieira ML, Gama-Simões MJ, Collares-Pereira M. Human leptospirosis in Portugal: A retrospective study of eighteen years. *Int J Infect Dis.* 2006;10(5):378-86. Available from: <http://dx.doi.org/10.1016/j.ijid.2005.07.006> PMID:16600656
13. Caldas EM, Sampaio MB, Costa E, Tishchenko LM. Patoc 1 and Rufino strains of *Leptospira biflexa*, as screening antigens in the diagnosis of leptospirosis. *Int J Zoonoses.* 1978;5(2):91-6. Available from: PMID:744706
14. Levett PN. Usefulness of serologic analysis as a predictor of the infecting serovar in patients with severe leptospirosis. *Clin Infect Dis.* 2003;36(4):447-52. Available from: <http://dx.doi.org/10.1086/346208> PMID:12567302
15. Villumsen S, Pedersen R, Borre MB, Ahrens P, Jensen JS, Krogfelt KA. Novel TaqMan® PCR for detection of *Leptospira* species in urine and blood: pit-falls of in silico validation. *J Microbiol Methods.* 2012;91(1):184-90. Available from: <http://dx.doi.org/10.1016/j.mimet.2012.06.009> PMID:22750039
16. Kort och matrikelstyrelsen. Dokumentation till KORT10 adressetema. Copenhagen: Geodatastyrelsen; 2005. Danish. Available from: ftp://ftp.kms.dk/KMS-MAR/mette/1031151_FMo_UTM32-EUREF89/DIVERSE/Dokumentation%20til%20KORT10%20Adressetema.pdf
17. World Health Organisation. Leptospirosis worldwide, 1999. *Wkly Epidemiol Rec.* 1999;74(29):237-42. Available from: PMID:10437435
18. Jansen A, Stark K, Schneider T, Schöneberg I. Sex differences in clinical leptospirosis in Germany: 1997-2005. *Clin Infect Dis.* 2007;44(9):e69-72. Available from: <http://dx.doi.org/10.1086/513431> PMID:17407027
19. Garvey P, Connell J, O'Flanagan D, McKeown P. Leptospirosis in Ireland: annual incidence and exposures associated with infection. *Epidemiol Infect.* 2014;142(4):847-55. <http://dx.doi.org/10.1017/S0950268813001775> PMID:23916046
20. Holk K, Nielsen SV, Rønne T. Human leptospirosis in Denmark 1970-1996: an epidemiological and clinical study. *Scand J Infect Dis.* 2000;32(5):533-8. Available from: <http://dx.doi.org/10.1080/003655400458839> PMID:11055660
21. Calvo-Cano A, Aldasoro E, Ramirez M, Martinez M, Requena-Mendez A, Gascon J. Author information: Two cases of laboratory-confirmed leptospirosis in travellers returning to Spain from Thailand, September 2013. *Euro Surveill.* 2014;19:4-6.
22. Bandara M, Ananda M, Wickramage K, Berger E, Agampodi S. Globalization of leptospirosis through travel and migration. *Global Health.* 2014;10(1):61. Available from: <http://dx.doi.org/10.1186/s12992-014-0061-0> PMID:25112368
23. Regnskabsstatistik for akvakultur 2012. [Accounts statistics for aquaculture 2012]. Copenhagen: Danmarks Statistik; 2013. ISSN 1901-8932. Available from: <http://www.dst.dk/pukora/epub/upload/18673/akva.pdf>
24. Borg-Petersen CL. (L. saxkoebing, a new serological Leptospirosis type). saxkoebing, ein neuer serologischer Leptospirentyp. *Acta Pathol Microbiol Scand.* 1944;21(1):165-79.
25. Sebek Z. A characterisation of leptospirosis foci on the territory of Czechoslovakia. In: Rosicky B, Heyberger K, eds. Theoretical questions on natural foci diseases. Prague: Publ House Czech Acad Sci; 1965.
26. Wójcik OP, Holt J, Kjerulf A, Müller L, Ethelberg S, Mølbak K. Personal protective equipment, hygiene behaviours and occupational risk of illness after July 2011 flood in Copenhagen, Denmark. *Epidemiol Infect.* 2013;141(8):1756-63. Available from: <http://dx.doi.org/10.1017/S0950268812002038> PMID: 22989427
27. Andersen M. Flere ekstreme regnskyl og storme. [More extreme rainshowers and storms]. Copenhagen: Danmarks Meteorologiske Institut. [Accessed: Jul 2014]. Danish. Available from: <http://www.dmi.dk/laer-om/tema/klimatemaer/fremtidens-ekstreme-vejr/>
28. Cann KF, Thomas DR, Salmon RL, Wyn-Jones AP, Kay D. Extreme water-related weather events and waterborne disease. *Epidemiol Infect.* 2013;141(4):671-86. Available from: <http://dx.doi.org/10.1017/S0950268812001653> PMID:22877498
29. Wasiński B, Dutkiewicz J. Leptospirosis--current risk factors connected with human activity and the environment. *Ann Agric Environ Med.* 2013;20(2):239-44. Available from: PMID:23772568
30. Petersen AM, Boye K, Blom J, Schlichting P, Krogfelt KA. First isolation of *Leptospira fairi* serovar Hurstbridge from two human patients with Weil's syndrome. *J Med Microbiol.* 2001;50(1):96-100.
31. Barkay S, Garzoni H. Leptospirosis and uveitis. *Ann Ophthalmol.* 1984;16(2):164-8. Available from: PMID:6538392