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Increased incidence of *Mycoplasma pneumoniae* infections detected by laboratory-based surveillance in Denmark in 2010

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In Denmark recurrent epidemics of *Mycoplasma pneumoniae* infections have been described since the 1950s at intervals of approximately four to six years. The latest epidemic occurred in 2004/05 followed by two years of high incidence and more than three years of low incidence. Due to a recent increase in diagnosed cases since late summer 2010, we conducted a survey of positive *M. pneumoniae* PCR tests performed by clinical microbiology departments in Denmark, which indicated that a new epidemic may be underway.

Introduction

Mycoplasma pneumoniae is a common cause of upper and especially lower respiratory tract infections such as bronchitis and pneumonia. In addition, *M. pneumoniae* causes neurological symptoms and sequelae in a high proportion of cases [1,2]. The highest prevalence is seen in children and younger adults. Cases occur throughout the year, but the incidence is highest during autumn and winter. In Denmark, regular epidemics have been described since 1949/50. With the exception of a nine-year endemic period from 1978 to 1987 [3], these epidemics usually begin during summer, culminate in late autumn/early winter and fade out during winter. In some instances the epidemics span two winters: this was seen in 1962 to 1964 and 1971 to 1973 [3]. The latest epidemic in 2004/05 [4,5] was followed by two years of high incidence, but since 2007 the incidence has been very low judging by the low rate of on average approximately 3% positive samples seen in this period (Figure 1).

From 1946 until the late 1990s the central national laboratory at Statens Serum Institut (SSI) received samples from the whole country for the diagnosis of *M. pneumoniae* infections [3]. In the last decades the local clinical microbiology departments have taken over a large part of the laboratory tests for *M. pneumoniae*. The diagnosis had previously been based on serology but since the beginning of the 1990s PCR has been introduced as a routine diagnostic test at SSI for rapid and early diagnosis of *M. pneumoniae* infection [6], and in more recent years, most of the local departments have also adopted PCR. The countrywide use of PCR for diagnosis and surveillance of *M. pneumoniae* infections is probably unique for Denmark.

Although SSI is now predominantly receiving samples from the eastern part of the country only, the institute is the one laboratory in Denmark performing most tests for *M. pneumoniae* overall, and thus results obtained at SSI may be seen as indicative of the *M. pneumoniae* activity in Denmark as a whole. Each week the rate of positive samples is calculated, and a rise from approximately 5% to 15% or more positive samples within approximately six weeks are considered as indicative of an *M. pneumoniae* epidemic [4].

At SSI we saw an increase in the number of positive samples above the threshold in the beginning of October 2010. This prompted us to investigate whether this was the beginning of an epidemic of *M. pneumoniae* infections in Denmark in the autumn of 2010.

Methods

Because PCR is found superior to serology for the diagnosis of *M. pneumoniae* infection during the early phases of infection [7], we included in our investigation only those records that were diagnosed by a PCR-based method. The departments use a range of different PCR

assays, of which some are published [6,8,9] or commercial kits, but most are unpublished but validated *in-house* assays.

A survey was conducted collecting data from all clinical microbiology departments in Denmark performing PCR testing for *M. pneumoniae* for general practitioners and hospitals. In addition to SSI, there are 12 such departments in the country that perform this analysis and we received data from 11 of them. They represented all five regions in Denmark (Figure 2): Capital Region of Denmark (data from three of four departments), Region Zealand (data from the sole department), Region of Southern Denmark (data from three of three departments), Central Denmark Region (data from two of two departments) and North Denmark Region (data from the sole department).

From the local departments we obtained data on the total number of PCR analyses performed and the number of analyses positive for *M. pneumoniae* for week 1 in 2009 to week 41 in 2010. Only data for weeks 34 to 41 in 2009 and 2010 are compared in the analysis presented here. From SSI we obtained data from week 1 in 2004 to week 41 in 2010 (October 16). We present the number of positive tests and the weekly proportion of positive tests among all tests performed. Since the catchment areas of the departments are not well defined, i.e. the general practitioner can send the specimen to any department, it was not possible to calculate the regional incidences. However, the total population of Denmark is 5.5 million and we used this to calculate an estimated incidence of PCR-diagnosed *M. pneumoniae*.

Results

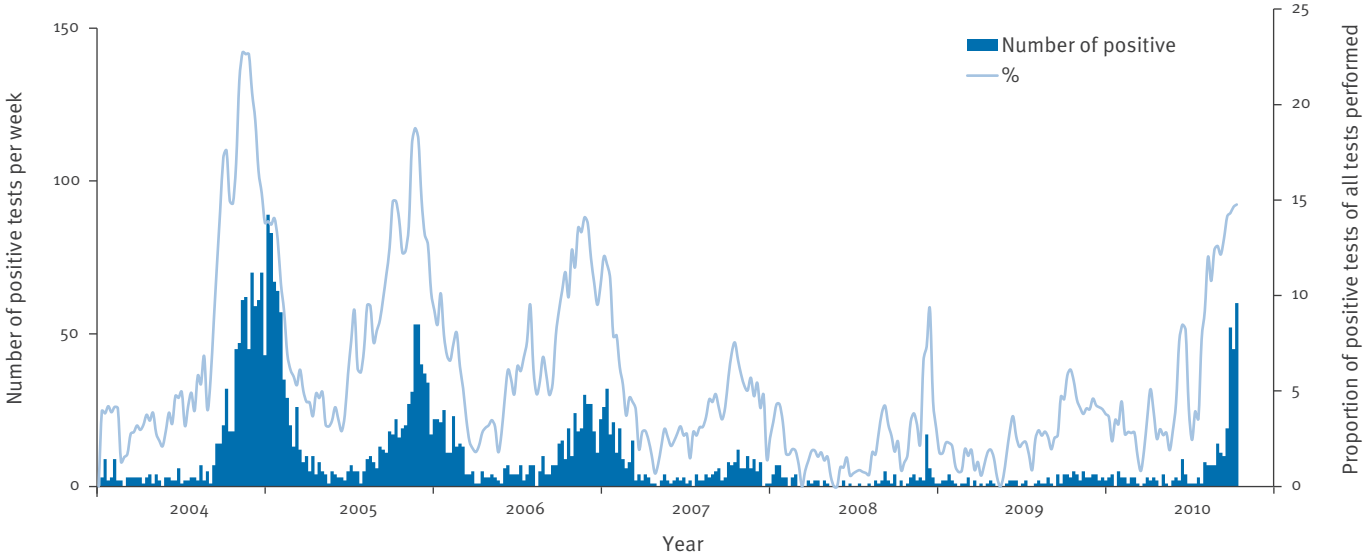
Figure 1 shows the *M. pneumoniae* tests performed at SSI from week 1 in 2004 to week 41 in 2010. From 2007 to 2010 the average positivity rate of *M. pneumoniae* infection in Denmark remained very low, at approximately 3% positive samples (Figure 1). Apart from a short peak in the number of positive tests observed in week 50 in 2008, the first increase in the positivity rate since 2007 was observed in late August 2010 (weeks 33–35) when it rose to approximately 10%. The rate increased further in the following weeks and reached approximately 15% in late September/early October (weeks 39–40) despite a three- to fourfold increase in the number of samples received for PCR in this period (Table). This increase in the rate of positive *M. pneumoniae* tests occurred in all regions, but was seen a little later in the regions than at SSI (Table). The estimated national incidence of PCR-diagnosed *M. pneumoniae* infections in 2010 rose from 0.4 per 100.000 in week 34 to 3 per 100.000 in week 41.

Discussion and conclusion

Recurrent epidemics of *M. pneumoniae* infection are also well known in other countries [10,11] and a few reports indicate simultaneous epidemics in more than one country [12,13]. *M. pneumoniae* epidemics have a high impact on the community, and a laboratory-based system for the surveillance of this disease is recommendable. According to our knowledge Denmark is the only country with a PCR-based surveillance system for *M. pneumoniae*. A rapid increase in macrolide-resistant *M. pneumoniae* has been reported from Asia in the recent years, but macrolide resistance it is also seen in Europe and in the United States [14]. In Denmark SSI did a survey after the epidemic in 2004 and found 1-2% of macrolide resistance. This is in accordance with a recent German study [15] indicating a limited but not negligible level of resistance in Europe. If an epidemic is recognised it is possible to guide the hospitals and general practitioners in the diagnosis and antibiotic treatment of the disease. Only a focused use of

Figure 1

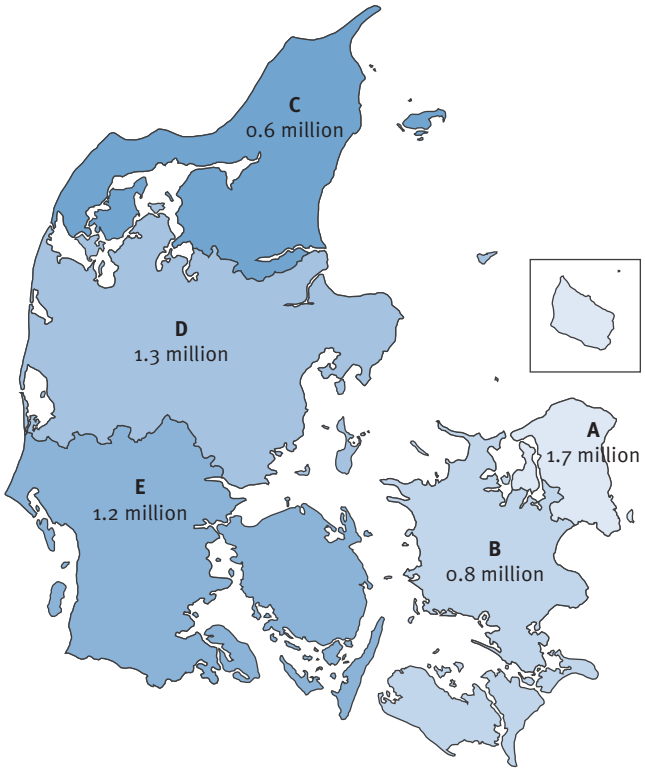
Mycoplasma pneumoniae PCR tests done at Statens Serum Institut, Denmark, week 1 2004 – week 41, 2010*



The percentages are the floating average of three weeks.

Figure 2

The five administrative regions of Denmark and population numbers



A: Capital Region of Denmark; B: Region Zealand; C: North Denmark Region; D: Central Denmark Region; E: Region of Southern Denmark

Population 1 July 2010. Source Statistics Denmark (http://www.dst.dk/HomeUK.aspx).

macrolide antibiotics in diagnosed cases can diminish the risk of spreading resistant bacteria.

In conclusion, we have seen an increase in the number of positive tests and also in the positivity rate of submitted samples since late summer 2010, indicating increased transmission of *M. pneumoniae*. The findings suggest that Denmark may be in the early phase of an epidemic. Other European countries, if data are available, should assess if they are in a similar situation.

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***Authors' correction:**
On request of the authors, Figure 1 was exchanged on 18 November 2010.

Table

Number and proportion of positive tests for *Mycoplasma pneumoniae* performed by Statens Serum Institut and the clinical microbiology departments in the regions, Denmark, 2009 and 2010

Region and year	Number of positive test and number of all tests performed (%)							
	Week 34	Week 35	Week 36	Week 37	Week 38	Week 39	Week 40	Week 41
SSI[a]								
2009	1 of 55 (1.8)	3 of 69 (4.3)	1 of 70 (1.4)	0 of 66 (0)	4 of 61 (6.6)	1 of 60 (1.7)	4 of 71 (5.6)	4 of 66 (6.1)
2010	7 of 68 (10.3)	14 of 91 (15.4)	11 of 96 (11.5)	10 of 101 (9.9)	20 of 112 (17.9)	52 of 362 (14.4)	45 of 338 (13.3)	60 of 374 (16.0)
Capital								
2009	0 of 30 (0)	1 of 34 (2.9)	5 of 29 (17.2)	0 of 37 (0)	1 of 37 (2.7)	1 of 49 (2.0)	1 of 47 (2.1)	0 of 39 (0)
2010	6 of 53 (11.3)	5 of 74 (6.8)	4 of 84 (4.8)	6 of 59 (10.2)	3 of 75 (4.0)	16 of 233 (6.9)	25 of 218 (11.5)	24 of 224 (10.7)
Zealand								
2009	0 of 5 (0)	0 of 12 (0)	0 of 14 (0)	0 of 15 (0)	1 of 17 (5.9)	0 of 13 (0)	0 of 11 (0)	0 of 11 (0)
2010	2 of 10 (20.0)	1 of 11 (9.1)	3 of 13 (23.1)	2 of 30 (6.7)	3 of 20 (15.0)	15 of 86 (17.4)	7 of 61 (11.5)	20 of 85 (23.5)
Southern Denmark								
2009	2 of 45 (4.4)	1 of 37 (2.7)	1 of 51 (2.0)	0 of 68 (0)	1 of 60 (1.7)	1 of 57 (1.8)	1 of 62 (1.6)	1 of 61 (1.6)
2010	2 of 41 (4.9)	1 of 40 (2.5)	3 of 43 (7.0)	2 of 82 (2.4)	2 of 81 (2.5)	10 of 137 (7.3)	20 of 165 (12.1)	22 of 189 (11.6)
Central Denmark								
2009	0 of 8 (0)	0 of 16 (0)	1 of 17 (5.9)	0 of 25 (0)	0 of 16 (0)	0 of 13 (0)	1 of 16 (6.3)	1 of 26 (3.8)
2010	4 of 25 (16.0)	1 of 18 (5.6)	2 of 23 (8.7)	4 of 23 (17.4)	1 of 22 (4.5)	7 of 53 (13.2)	11 of 60 (18.3)	10 of 65 (15.4)
North Denmark								
2009	0 of 6 (0)	0 of 3 (0)	0 of 8 (0)	0 of 10 (0)	0 of 15 (0)	0 of 11 (0)	1 of 12 (8.3)	1 of 8 (12.5)
2010	0 of 17 (0)	3 of 17 (17.6)	1 of 15 (6.7)	1 of 14 (7.1)	2 of 19 (10.5)	4 of 44 (9.1)	7 of 55 (12.7)	28 of 165 (17.0)
Total								
2009	3 of 149 (2.0)	5 of 171 (2.9)	8 of 189 (4.2)	0 of 221 (0)	7 of 206 (3.4)	3 of 203 (1.5)	8 of 219 (3.7)	7 of 211 (3.3)
2010	21 of 214 (9.8)	25 of 251 (10.0)	24 of 274 (8.8)	25 of 309 (8.1)	31 of 329 (9.4)	104 of 915 (11.4)	115 of 897 (12.8)	164 of 1102 (14.9)

[a] Statens Serums Institut (SSI) receives samples not only from the capital region but also from the rest of the country and is therefore presented separately.

References

1. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. Clin Microbiol Rev. 2004;17(4):697-728.
2. Tsiodras S, Kelesidis I, Kelesidis T, Stamboulis E, Giamarellou H. Central nervous system manifestations of Mycoplasma pneumoniae infections. J Infect. 2005;51(5):343-54
3. Lind K, Benzon MW, Jensen JS , Clyde WA Jr. A seroepidemiological study of Mycoplasma pneumoniae infections in Denmark over the 50-year period 1946-1995. Eur J Epidemiol. 1997;13(5):581-6
4. Uldum SA, Jensen JS, Nielsen SS. Increase in M. pneumoniae. EPI-NEWS 2004 (42/43). Available from: http://www.ssi.dk/English/News/EPI-NEWS/~/media/Indhold/EN%20-%20engelsk/EPI-NEWS/2004/pdf/EPI-NEWS%20-%202004%20-%20No%2043.ashx
5. Uldum SA. Epidemic of Mycoplasma pneumoniae. EPI-NEWS 2004 (51). Available from: http://www.ssi.dk/English/News/EPI-NEWS/~/media/Indhold/EN%20-%20engelsk/EPI-NEWS/2004/pdf/EPI-NEWS%20-%202004%20-%20No%2051.ashx
6. Jensen JS, Søndergård-Andersen J, Uldum SA, Lind K. Detection of Mycoplasma pneumoniae in simulated clinical samples by polymerase chain reaction. APMIS. 1989;11(11):1046-8
7. Nilsson AC, Björkman P, Persson K. Polymerase chain reaction is superior to serology for the diagnosis of acute Mycoplasma pneumoniae infection and reveals a high rate of persistent infection. BMC Microbiol. 2008,8:93.
8. Pitcher D, Chalker VJ, Sheppard C, George RC, Harrison TG. Real-time detection of Mycoplasma pneumoniae in respiratory samples with an internal processing control. J Med Microbiol. 2006;55(Pt 2):149-55
9. Welti M, Jaton K, Altwegg M, Sahli R, Wenger A, Bille J. Development of a multiplex real-time quantitative PCR assay to detect Chlamydia pneumoniae, Legionella pneumoniae and Mycoplasma pneumoniae in respiratory tract secretions. Diagn Microbiol Infect Dis. 2003;45(2):85-95.
10. Rastawicki W, Kaluzewski S, Jagielski M, Gierczyski R. Epidemiology of Mycoplasma pneumoniae infections in Poland: 28 years of surveillance in Warsaw 1970-1997. Euro Surveill. 1998;3(10):pii=95. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=95
11. Ghosh K, Clements GB. Surveillance of Mycoplasma pneumoniae in Scotland 1986-1991. J Infect .1992;13(2):221-7.
12. Pönkä. Occurrence of serological verified Mycoplasma pneumoniae infections in Finland and in Scandinavia in 1970--1977. Scand J Infect Dis. 1980;12(1):27-31.
13. Akehurst C. Mycoplasma epidemics. Euro Surveill. 1998;2(50):pii=1112. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1112
14. Li X, Atkinson TP, Hagood J, Makris C, Duffy LB, Waites KB. Emerging macrolide resistance in Mycoplasma pneumoniae in Children: detection and characterization of resistant isolates. Pediatr Infect Dis J. 2009;28(8):693-6.
15. Dumke R, von Baum H, Lück PC, Jacobs E. Occurrence of macrolide-resistant Mycoplasma pneumoniae strains in Germany. Clin Microbiol Infect. 2010;16(6):613-6.

Increasing case numbers of adenovirus conjunctivitis in Germany, 2010

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In 2010 (as of 13 October 2010), the number of adenovirus conjunctivitis cases reported to the Robert Koch Institute in Berlin, Germany, has increased by more than 250% compared with same period in the previous two years. An investigation was initiated to identify spatial or temporal clusters, possible sources of infection and potential connections to cases abroad. The analysis did not show a disproportionately affected sex or age group, but many infections were preceded by exposure to ophthalmological facilities, communal facilities or public places.

Background

Several reports have recently been posted in ProMED of viral conjunctivitis in various parts of the world [1-7]. Since the beginning of 2010, there has been a rise in the number of notified cases of adenovirus conjunctivitis in several German Laender. The Robert Koch Institute (RKI) in Berlin initiated an investigation of all notified cases in the country in order to identify spatial or temporal clusters of cases, any potential connection to cases abroad and possible sources of infection, so that appropriate public health measures could be recommended.

Adenoviruses are non-enveloped double-stranded DNA-viruses, which are resistant to various treatments (e.g. extremes of pH) and are therefore difficult to inactivate. They can be transmitted via contaminated hands or objects, often in healthcare settings. Depending on the serotype, clinical manifestations are related to different organ systems, such as the eye and the respiratory or gastrointestinal tract, resulting in diverse clinical pictures. The most frequently detected human adenoviruses (HAdV) found in conjunctivitis are of serotypes 8, 19 and 37 [8].

Adenovirus conjunctivitis is characterised by sudden onset of symptoms and can be diagnosed clinically by keratitis with coin-shaped infiltrations in the cornea. It is usually self-limiting. The incubation period generally lasts from five to 12 days and the person remains infective for up to three weeks. Diagnosis can be laboratory confirmed by direct detection of the pathogen (by polymerase chain reaction (PCR), immunoassays or cell culture). As only the symptoms can be treated, good hygiene and disinfection management are therefore important for prevention and control of the disease [9,10].

Case notification

In Germany, notification to local health authorities is mandatory for laboratories that detect adenoviruses from conjunctival swabs (direct detection of antigen or DNA) [11]. Case data, including data on epidemiological links, is then reported to Robert Koch Institute according to the national case definition. The German notification system has been described previously [12].

Notified cases in 2010 (up to 13 October 2010) in Germany were analysed and compared with the two previous years regarding temporal and spatial distribution. Clusters were defined as epidemiologically linked cases with a minimum of two persons, whereas sporadic cases had no documented link to other cases.

We asked all cases notified from weeks 34 to 40 (i.e. over the previous six weeks) from six Laender whether they would be prepared to be interviewed, using an exploratory questionnaire, in order to generate a hypothesis of common infection modes or sources. Selecting weeks 34 to 40 allowed us to obtain a sufficiently large sample size and given the cases were recent, recall bias was minimised.

Analysis of notified cases

As of 13 October 2010, 370 persons with adenovirus conjunctivitis with onset of symptoms from 1 January 2010 were notified to local health authorities and reported to the Robert Koch Institute. The date of symptom onset was recorded for 303, while in a further 67 cases it was assumed to be in 2010 due to the time

of notification. During the same time period in 2008 and 2009, 141 and 134 cases respectively were notified (the total number of notified cases was 180 in 2008 and 169 in 2009). In those years, the infections were distributed widely, affecting urban and rural districts, with the highest number of cases occurring in August.

In 2010, the median and mean age of cases was 39 years (range: 0–90 years). A total of 186 (50%) were men (in 2008: 47%; in 2009: 51%). Cases were notified in all Laender, from laboratories to local health authorities in 121 rural and urban districts (30% of all districts). Cases were mainly clustered in northern Germany (whereas in 2009, they were more evenly distributed). The highest incidence was found in Mecklenburg-West-Pomerania (in the north-east of the country) with five cases per 100,000 population (Figure 1, Table 1). The highest number of cases was reported in week 13 (the week before Easter) (Figure 2). The appearance of cases in the same region within a four-week period suggests a common source of infection or related cause.

Country of infection was reported in 301 (81%) cases in 2010: Germany was most frequently mentioned (n=292), followed by Thailand (n=3), Egypt (n=1), India (n=1), Portugal (n=1), Romania (n=1), Russia (n=1) and Switzerland (n=1). Serotypes of 15 samples were available: HAdV-8 (n=7), HAdV-37 (n=4), HAdV-19 (n=3) and one case of HAdV-3, a serotype usually associated with respiratory infections [13].

Cluster cases

In 2010, 120 (32%) of the 370 cases were linked to 22 clusters (in 2008, there were 46 cases (33%) in 10 clusters; in 2009, there were 25 cases (15%) in nine clusters). The 2010 clusters were located across 16 rural and urban districts in eight Laender. The proportion of cases linked to clusters decreased around week 19 (Figure 2). The median age of cases in clusters was 32 years (mean: 33 years, range: 0–90).

Sporadic cases

There were 250 (67.6%) sporadic cases, of whom 133 were men. The median age was 43 years (mean: 42 years, range: 0–89). Approximately 40% of all sporadic cases appeared in locations close to clustered cases, which points to a possible connection. However, some sporadic cases were geographically isolated, in regions far from the clusters (Figure 1).

Most clusters were notified as being associated with ophthalmological facilities or hospitals. Some were related to outbreaks in child day-care centres or kindergartens, which lowered the median age of cases in clusters significantly (t-test p=0.004) in comparison with sporadic cases.

Analysis of interviewed cases

Detailed investigation of 27 cases who were interviewed revealed that the median duration of illness was 18 days (range: 4–84 days); 13 judged the severity of the disease as high or very high (Table 2).

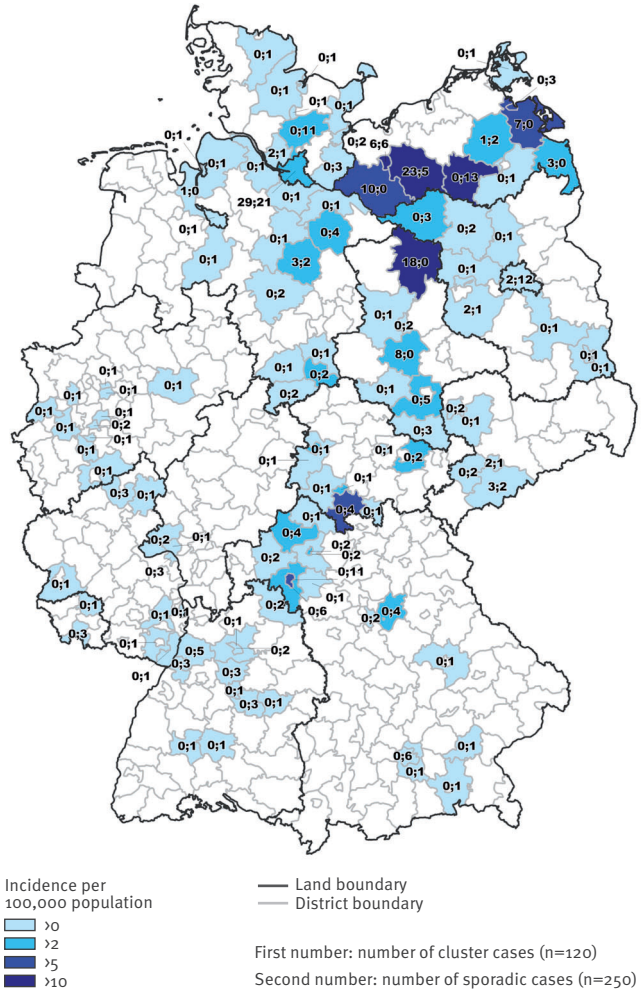
A majority (n=16) of the 27 interviewed cases wore either glasses or contact lenses. Among the interviewees, 21 were not aware of any contact with other affected persons. In the two weeks before onset of symptoms, excursions to public places were reported in 13 cases, while 12 stayed in or visited a medical facility (e.g. an ophthalmologist or eye clinics), where mostly eye drops were administered. A total of 11 cases had used communal facilities associated with sport or leisure activities (e.g. in a swimming pool, at the seaside or communal showers) (Table 2).

Discussion

Compared with the previous two years, an increase of notified adenovirus conjunctivitis cases of more than 250% was seen in 2010. Given the delay in reporting, it is likely that case numbers will continue to rise [14]. As

Figure 1

Geographical distribution of notified adenovirus conjunctivitis cases[a], Germany, 2010[b] (n=370)



[a] Laboratory notifications reported to the Robert Koch Institute, Berlin, Germany.

[b] Data as of 13 October 2010. Cases with onset of symptoms on or after 1 January 2010 or unknown.

notification is confined to laboratory-confirmed cases – thus excluding cases that are only clinically diagnosed – it can be assumed that not all cases of adenovirus conjunctivitis are captured and a bias towards severe cases is possible. Although it was not feasible to interview all notified cases, the interviewed cases represented a similar sample in terms of regional, sex and age distribution.

Our findings – showing that the majority of cases who were interviewed was exposed through use of community facilities, excursions to public places within Germany or ophthalmological treatment – are in line with other published investigations [15,16]. No direct connection with recently described outbreaks of conjunctivitis in various countries [1-7] could be established due to the few records of German cases with known relevant travel history abroad.

Table 1

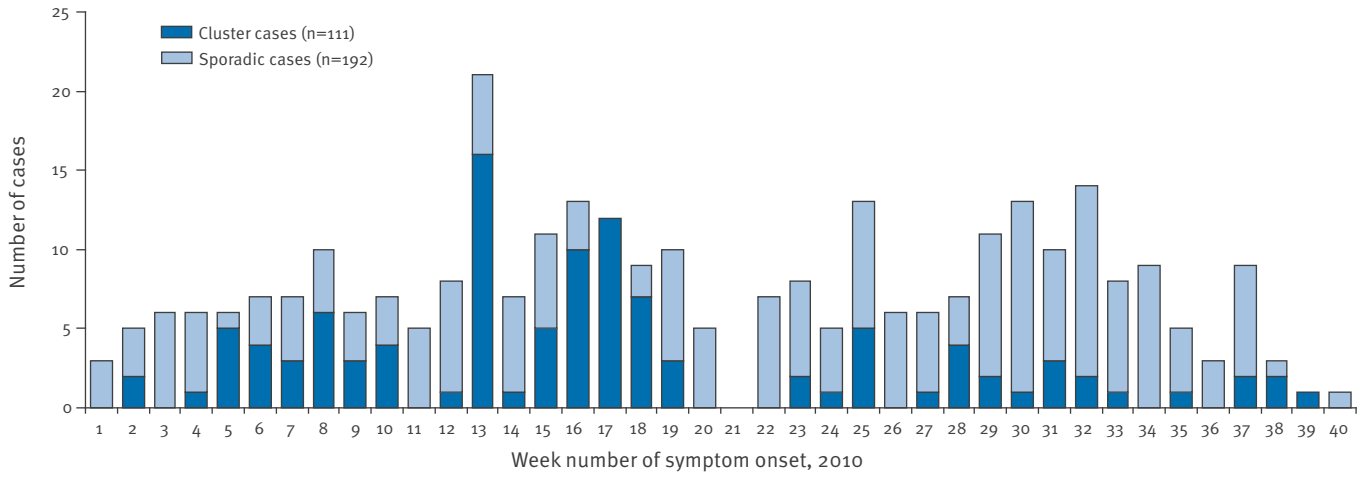
Notified adenovirus conjunctivitis cases[a], Germany, by Land, 2010[b] (n=370)

Land	All cases		Cluster cases		Sporadic cases	
	Number	Incidence[c]	Number	Incidence[c]	Number	Incidence[c]
Baden-Wuerttemberg	23	0.2	0	0.0	23	0.2
Bavaria	45	0.4	0	0.0	45	0.4
Berlin	14	0.4	2	0.1	12	0.4
Brandenburg	13	0.5	2	0.1	11	0.4
Bremen	2	0.3	0	0.0	2	0.3
Hamburg	50	2.8	29	1.6	21	1.2
Hesse	3	0.1	0	0.0	3	0.1
Lower Saxony	24	0.3	4	0.1	20	0.3
Mecklenburg-West-Pomerania	81	4.9	50	3.0	31	1.9
North Rhine-Westphalia	12	0.1	0	0.0	12	0.1
Rhineland-Palatinate	12	0.3	0	0.0	12	0.3
Saarland	4	0.4	0	0.0	4	0.4
Saxony	13	0.3	5	0.1	8	0.2
Saxony-Anhalt	38	1.6	26	1.1	12	0.5
Schleswig-Holstein	24	0.9	2	0.1	22	0.8
Thuringia	12	0.5	0	0.0	12	0.5
Total	**370**	**0.5**	**120**	**0.2**	**250**	**0.3**

[a] Laboratory notifications reported to the Robert Koch Institute, Berlin, Germany.
[b] Data as of 13 October 2010. Cases with onset of symptoms on or after 1 January 2010 or unknown.
[c] Per 100,000 population.

Figure 2

Notified adenovirus conjunctivitis cases[a], by week of symptom onset, Germany, 2010[b] (n=303)



The number of cases in weeks 38–40 was underreported due to reporting delay.
[a] Laboratory notifications reported to the Robert Koch Institute, Berlin, Germany.
[b] Data as of 13 October 2010. Date of symptom onset on or after 1 January 2010 was recorded for 303 cases.

In conclusion, the analysis did not show a disproportionally affected sex or age group, except for outbreaks in child day-care centres and kindergartens [17]. The cause of the increase of notified cases in the country is still unclear, although the majority of infections in clusters was nosocomial. Information about the recent epidemiological developments regarding adenovirus conjunctivitis was shared with German ophthalmologists, with a request to submit conjunctival samples from patients with acute conjunctivitis to the national reference laboratory for serotyping. Improved hygiene measures are recommended, especially in ophthalmological centres [8,9]. It would be interesting to know if the increase seen in Germany in the number and severity of cases of adenovirus conjunctivitis is also seen in other European countries.

Table 2

Description of interviewed adenovirus conjunctivitis cases, Germany, 2010[a] (n=27)

Description	Number of cases[b]
Male / female / not recorded	10 / 9 / 8
Median age (range)	51 years (2–79 years)
Optical aid used[c]	16 (15 with glasses, 2 with contact lenses)
Self-rating of severity of disease	
Light	6
Medium	6
High	5
Very high	8
Eye affected	
Right	7
Left	6
Both	12
Visit to or stay in a medical facility[c,d]	12
Ophthalmologist	7
Eye clinic	4 (3 outpatients, 1 hospitalised)
Hospital visit	1
Optician or other medical professional or facility	0
Excursion[d] (musical, adventure park, campsite, lake, city trip, cruise)	13
Overnight stay	10
Day trip	10
Other	
Contact with other people with similar symptoms[d]	6
Visit to communal facility[d] (e.g. swimming pool or pond, communal showers, sea, fitness club, sauna)	11
Use of eye cosmetics or medical products for eyes[d]	7
Use of optical instruments (e.g. binoculars, camera, 3D-glasses)[d]	6
Sharing of facecloths or towels with others[d]	5

[a] Data as of 13 October 2010. Cases with onset of symptoms on or after 1 January 2010 or unknown.

[b] Unless otherwise stated.

[c] Multiple answers allowed.

[d] In the two weeks before symptom onset.

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References

1. ProMED-mail. Conjunctivitis, internally displaced persons - Pakistan (Sindh). Archive Number 20100923.3439. 23 Sep 2010. Available from: http://www.promedmail.org
2. ProMED-mail. Conjunctivitis - Mexico (03): (Tamaulipas). Archive Number 20100922.3427. 22 Sep 2010. Available from: http://www.promedmail.org
3. ProMED-mail. Conjunctivitis, Viral - China: (Fujian). Archive Number 20100921.3406. 21 Sep 2010. Available from: http://www.promedmail.org
4. ProMED-mail. Conjunctivitis - Mexico (02): (Guerrero). Archive Number 20100919.3387. 19 Sep 2010. Available from: http://www.promedmail.org
5. ProMED-mail. Conjunctivitis - Mexico: (Colima). Archive Number 20100903.3155. 3 Sep 2010. Available from: http://www.promedmail.org
6. ProMED-mail. Conjunctivitis - Uganda And Southern Sudan, Turkey. Archive Number 20100819.2890. 19 Aug 2010. Available from: http://www.promedmail.org
7. ProMED-mail. Conjunctivitis - Bhutan. Archive Number 20100723.2471. 23 Jul 2010. Available from: http://www.promedmail.org
8. Robert Koch Institute (RKI). [Epidemic keratoconjunctivitis and other conjunctivitis caused by adenovirus - RKI Manual on infectious diseases - Manual for physicians (Edition March 2010)]. 2010. Berlin: RKI; 2010. German.
9. Dart JK, El-Amir AN, Maddison T, Desai P, Verma S, Hughes A, et al. Identification and control of nosocomial adenovirus keratoconjunctivitis in an ophthalmic department. Br J Ophthalmol. 2009;93(1):18-20.
10. Reed K. Epidemic viral keratoconjunctivitis diagnosis and management. J Am Optom Assoc. 1983;54(2):141-4.
11. Robert Koch Institute (RKI). [Case definitions by the Robert Koch Institute for the reporting of illness, death, and confirmation of pathogens - 2004 edition]. Berlin: RKI: 2003. German,
12. Faensen D, Claus H, Benzler J, Ammon A, Pfoch T, Breuer T, et al. SurvNet@RKI--a multistate electronic reporting system for communicable diseases. Euro Surveill. 2006;11(4):100. pii: 614. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=614
13. Lebeck MG, McCarthy TA, Capuano AW, Schnurr DP, Landry ML, Setterquist SF, et al. Emergent US adenovirus 3 strains associated with an epidemic and serious disease. J Clin Virol. 2009;46(4):331-6.
14. Krause G, Altmann D, Faensen D, Porten K, Benzler J, Pfoch T, et al. SurvNet electronic surveillance system for infectious disease outbreaks, Germany. Emerg Infect Dis. 2007;13(10):1548-55.
15. Artieda J, Pineiro L, Gonzalez M, Munoz M, Basterrechea M, Iturzaeta A, et al. A swimming pool-related outbreak of pharyngoconjunctival fever in children due to adenovirus type 4, Gipuzkoa, Spain, 2008. Euro Surveill. 2009 Feb 26;14(8). pii: 19125. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19125
16. Schrauder A, Altmann D, Laude G, Claus H, Wegner K, Kohler R, et al. Epidemic conjunctivitis in Germany, 2004. Euro Surveill. 2006 Jul;11(7). pii: 637. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=637
17. Robert Koch Institute (RKI).[Analysis of nationally reported notification data on adenovirus conjunctivitis]. Epidemiologisches Bulletin. 2010;37. German.

Introduction and control of three invasive mosquito species in the Netherlands, July-October 2010

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In July 2010, during routine mosquito surveillance inspections at companies that import used tires, three invasive species were found at five locations in the Netherlands: the yellow fever mosquito (*Aedes aegypti*), the Asian tiger mosquito (*Ae. albopictus*), and the American rock-pool mosquito (*Ae. atropalpus*). This is the first time that *Ae. aegypti* is reported from the Netherlands. Mosquito control was initiated one week after the first invasive mosquito was found, using adulticides and larvicides. The available data suggest that the implemented control measures have been effective for this season.

Introduction

Following the discovery of *Aedes albopictus* in the Netherlands in 2005 related to companies that import Lucky bamboo [1], continuous surveillance at these companies was started in 2006. Gradually, other national surveillance activities for this mosquito species were initiated, including passive surveillance (since 2007), active surveillance at parking lots along main highways entering the country from the south and east (since 2008), and at companies that import used tires (since 2009). In 2009, during routine surveillance activities, the exotic mosquito species *Ae. atropalpus*, a North American species that had been encountered several times in Europe [2], but had never established here, was found for the first time in the Netherlands [3].

These surveillance activities are meant to identify as early as possible the presence of exotic mosquito species with the aim to prevent the establishment of invasive exotic mosquito species, especially those that are known to be vectors of pathogens of public health importance such as dengue- and chikungunya virus. Here we report the finding and the successive control of three invasive mosquito species, *Ae. aegypti*, *Ae. albopictus* and *Ae. atropalpus* in the Netherlands.

Methods

A total of 34 companies that import used tires into the Netherlands were included in the invasive mosquito survey. Routine inspections were carried out from April to the last week of October [2]. A qualitative risk assessment on the introduction of invasive mosquito species was performed to determine the frequency of inspection of a company. Parameters in the risk assessment were (i) the type of tires that are imported, (ii) the countries from which tires are imported, and (iii) whether the tire storage is in- or outdoors. Collected larvae and adult mosquitoes were diagnosed either morphologically by using the diagnostic keys from Schaffner *et al.* [4], or molecularly by PCR sequencing the mitochondrial cytochrome oxidase subunit 1 (CO1) gene [5]. A week after the first finding, infested locations were treated by spraying *Bacillus thuringiensis israelensis* (*B.t.i.*) serotype H14 or *Bacillus sphaericus* (*B.s.*) against larvae and/or deltamethrin (aqua K-Othrine, Bayer Environmental Sciences) against adult mosquitoes. Larval control of the surrounding area (predefined perimeter of 500 m) consisted of removal of potential larval habitats for container-breeding Aedes spp. when possible, or treatment with either *B.t.i.* space spray (VectoBac WG, Valent BioSciences), or with *B.t.i./Bacillus sphaericus* (*B.s.*) granules (Vectomax, Valent BioSciences). It was decided to perform larvicidal treatment once every two to three weeks, until the first week of November.

Following the discovery of an exotic species at a location, surveillance was intensified to assess the potential spread of the invasive species and the effectiveness of the control activities by placing traps for adult mosquitoes (BG-sentinel, Biogents) and oviposi-

tion traps [6] in the 500 m perimeter surrounding the company site.

Results

Three exotic mosquito species (*Ae. aegypti*, *Ae. albopictus*, and *Ae. atropalpus*) were found in five locations in the Netherlands. The first two mosquito larvae, *Ae. atropalpus*, were found on 21 July 2010, during a routine inspection at Location 1 (Heijningen) (Figure, Table 1).

On the next day, during an intensified inspection, one adult *Ae. albopictus* and one adult *Ae. aegypti* were collected, in addition to the two initial *Ae. atropalpus* larvae. The infestation level for *Ae. atropalpus* (in terms of percentage of infested tires and total number of larvae) at this company was relatively high, but less so for *Ae. albopictus* and *Ae. aegypti*, of which no larvae and/or pupae were found. Results of intensified inspection suggest that *Ae. atropalpus* and *Ae. albopictus* (but not *Ae. aegypti*) had spread to the surrounding areas of Location 1. On 3 September 2010, the last exotic species was collected from Location 1 and its surroundings (Table 2).

At Location 2 (Oosterhout), several male *Ae. aegypti* specimens were collected starting with 26 July. The last invasive species were found at this location on 6 August, when two adult *Ae. atropalpus* were collected. Despite intensive surveillance, no immature forms of invasive species were found at the company's premises or in the surrounding areas.

Figure

Locations of tire companies that were positive (n=5) and negative (n=29) for at least one of the invasive mosquito species, the Netherlands, 2010

On 26 July, three adult specimens (but no larvae) of *Ae. atropalpus* were collected from Location 3 (Oss). In addition, another three adult *Ae. atropalpus* were found on 5 August at this location. In the surrounding area, one *Ae. albopictus* was collected in a BG-sentinel trap placed approximately 50 m from the tire platform on 9 August, but no larvae of exotic species were found in the surrounding area. The last specimen (larva) was found at this location on 23 August.

On 24 August, the first larvae (six specimens) of *Ae. atropalpus* were collected from Location 4 (Weert). On 13 September, high numbers of this species (larvae and adults) were found at this location and several larvae were found in the surrounding area, including at the premises of a neighbouring tire-importing company. The two companies are considered as one location (Location 4). In addition, one *Ae. albopictus* specimen was collected from the tire platform on 13 September and one *Ae. albopictus* specimen was found in a BG sentinel trap at approximately 25 m from the infested companies, one week later. The last specimen was found on 27 September.

On 28 September, two *Ae. albopictus* larvae were collected from Location 5 (Montfoort). The third (and last) specimen was collected in an adult trap on the tire platform on 5 October. No specimens were found in the surrounding area.

All infested companies described here belong to the 'high risk'-category for importing exotic mosquito species, based on the type, origin and storage of the tires that are imported, and are therefore inspected every two weeks. No invasive mosquito species were found at any of the other companies that were included in the survey.

Discussion and conclusion

The discovery of *Ae. aegypti* in the Netherlands was unexpected, mostly because, unlike *Ae. albopictus* [3], *Ae. aegypti* is not directly associated with the international trade in used tires [7]. Even without control measures, the tropical *Ae. aegypti* will probably not survive the winter in temperate areas such as the Netherlands and consequently does not pose a direct health risk for the country. This is in contrast with the public health risks related to re-introduction of *Ae. aegypti* into southern Europe [8,9].

In addition, this report describes the discovery of an *Ae. albopictus* for the first time in the outdoor environment in the Netherlands. Although the species is still regularly found in glasshouses as hitchhikers in importation of Lucky bamboo [10], preventive and curative indoor control measures in these glasshouses appear to be effective to prevent indoor or outdoor establishment, since a location never stays positive for *Ae. albopictus* longer than 1,5 month (Scholte, unpublished data).

Back-tracing data of the company at Location 1 suggests introduction of *Ae. albopictus* and *Ae. aegypti* by a shipment of used airplane tires at the end of May 2010, originating from southern Florida, an area inhabited by both species. On 24 July, part of the same shipment was transported to Location 2 (belonging to the same company), and on 4 August to Location 3. Back-tracing information of the companies at Location 4 showed recent tire import from Italy. *Ae. albopictus* from Italy [11] and the United States [12] are considered to display diapause and potentially to survive temperate climates [13,14]. *Ae. atropalpus* had already been found at two sites in the Netherlands in 2009 [2] which indicates that the first introduction of *Ae. atropalpus* was in or before 2009, although more recent introductions are not excluded either. This species had a relatively large population at Locations 1 and 4, and colonised larval habitats in the surrounding areas, other than tires.

The fact that relatively few adults and no other life-stages of *Ae. aegypti* and *Ae. atropalpus* were found at Location 2, indicates a low level of infestation.

The available data for this season (Table 2) suggest that the implemented control measures have been effective, although it is too early at this moment in time to assess if eradication has been achieved. Per location, it took between one and three treatments and a maximum time span of seven weeks between the first treatment and the day when the last exotic species was found. It will be crucial in the years to come to monitor the locations (including the surrounding areas) that had been infested with one or more of the exotic species in 2009 and 2010, in order to restart mosquito control as early as possible.

Having witnessed these introductions of exotic invasive mosquito species that pose a potential threat to public health in Europe, international collaboration and action of medical entomologists, public health experts, policy makers, and the tire-business industry is critical to address this.

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Table 1

Summary of the results of the invasive mosquito survey at used tire companies by location, the Netherlands, July-October 2010

Location	Adults collected				Larvae collected			
	Ae. aegypti	*Ae. albopictus*	*Ae. atropalpus*	Total	*Ae. aegypti*	*Ae. albopictus*	*Ae. atropalpus*	Total
1	5	11	68	84	0	0	80	80
2	8	0	2	10	0	0	0	0
3	0	1	6	7	0	0	1	1
4	0	2	45	47	0	6	122	128
5	0	1	0	1	0	2	0	2
Total	13	15	121	149	0	8	203	211

Table 2

Inspections, mosquito control, and findings of at least one of the three exotic mosquito species for each location per week, the Netherlands, July-October 2010

Location	Week (2010)																											
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
1																X	X		X			X			X		X	X	
2																X			X			X			X			X	
3																		X			X			X			X	X	
4																								X	X	X			X
5																										X			X

No inspection

No exotic species found (negative)

Larvae and/or adults found of one of the three exotic mosquito species

X Control measures

References

1. Scholte EJ, Jacobs F, Linton YM, Dijkstra E, Fransen J, Takken W. 2007. First record of Aedes (Stegomyia) albopictus in the Netherlands. Euro Mosq Bull. 2007;22:5-9.
2. Schaffner F, Van Bortel W. Current status of invasive mosquitoes in Europe. European Centre for Disease Prevention and Control; 31 Jan 2010. Available from: http://www.ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f-77d4-4ad8-b6d6-bfof23083f30&ID=758&RootFolder=/en/activities/sciadvice/Lists/ECDC%20Reviews&MasterPage=1
3. Scholte EJ, Den Hartog W, Braks M, Reusken C, Dik M, Hessels A. First report of a North American invasive mosquito species Ochlerotatus atropalpus (Coquillett) in the Netherlands, 2009. Euro Surveill. 2009;14(45):pii=19400. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19400
4. Schaffner F, Angel G, Geoffrey B, Hervy J-P, Rhaiem A, Brunhes J. The mosquitoes of Europe. CD-ROM. Montpellier: Institut de Recherche pour le Développement/Entente interdépartementale pour la démoustication du littoral (EID) Méditerrannée ; 2001.
5. Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann Entomol Soc America. 1994;87(6):651-701.
6. Fay RW, Eliason DA. A preferred oviposition site as a surveillance method for Aedes aegypti. Mosq News. 1966;26:531-5.
7. Schaffner F. Mosquitoes in used tyres in Europe: species list and larval key. Eur Mosq Bull. 2003;16:7-12.
8. Theiler M, Casals J, Moutousses C. Etiology of the 1927-28 epidemic of dengue in Greece. Proc Soc Exp Biol Med. 1960;103:244-6.
9. Reiter P. Yellow fever and dengue: a threat to Europe?. Euro Surveill. 2010;15(10):pii=19509. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19509
10. Scholte EJ, Dijkstra E, Blok H, De Vries A, Takken W, Hofhuis A, et al. Accidental importation of the mosquito Aedes albopictus into the Netherlands: a survey of mosquito distribution and the presence of dengue virus. Med Vet Entomol. 2008;22(4):352-8.
11. Romi R, Severini F, Toma L. Cold acclimation and overwintering of female Aedes albopictus in Roma. J Am Mosq Control Assoc. 2006;22(1):149-51.
12. Lounibos LP, Escher RL, Lourenço-de-Oliveira R. Asymmetric evolution of photoperiodic diapause in temperate and tropical invasive populations of Aedes albopictus (Diptera: Culicidae). Ann Entomol Soc Am. 96(4):512-18.
13. European Centre for Disease Prevention and Control (ECDC). Development of Aedes albopictus risk maps. Technical report. Stockholm:ECDC. 2009. Available from: http://ecdc.europa.eu/en/publications/Publications/0905_TER_Development_of_Aedes_Albopictus_Risk_Maps.pdf
14. Takumi K, Scholte EJ, Braks M, Reusken C, Avenell D, Medlock JM. Introduction, scenarios for establishment and seasonal activity of Aedes albopictus in the Netherlands. Vector Borne Zoonotic Dis. 2009;9(2):191-6.

Outbreak of 2009 pandemic influenza A(H1N1) in a Finnish garrison - a serological survey

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In September 2009, an outbreak of 2009 pandemic influenza A(H1N1) took place in a Finnish garrison. In November 2009, we performed a serological survey among 984 recruits undergoing their military service at the garrison and related the results to self-reported upper respiratory tract infection (URTI) with or without fever. Of 346 volunteers who donated a blood sample, 169 (49%) had pandemic influenza A(H1N1) virus-specific antibodies. Of those, 84 (50%) reported no recent history of URTI, suggesting that a major part of those infected with pandemic influenza A(H1N1) virus may be asymptomatic.

Outbreak description

In September 2009, one of the earliest outbreaks of 2009 pandemic influenza A(H1N1) in Finland took place in a garrison of 984 military conscripts. Before this outbreak, most of the infections caused by the pandemic influenza A(H1N1) virus in Finland were sporadic and often related to prior travel abroad. The number of visits to the primary healthcare services (PHS) of the garrison due to upper respiratory tract infections (URTI) increased rapidly during week 36 starting on 31 August 2009.

During the preceding weekend leave, on 29-30 August, six conscripts had fallen ill with high fever and cough. Nasopharyngeal swabs were taken as part of a routine screening diagnostic test for respiratory viruses and tested positive for 2009 pandemic influenza A(H1N1) virus by polymerase chain reaction (PCR). In total, 335 conscripts (34% of the garrison population) visited the PHS due to URTI between 31 August and 30 September 2009. During the same time period nasopharyngeal swabs were collected from 52 of 335 (13%) of the conscripts and 28 of these 52 (54%) were pandemic influenza A(H1N1)-positive by PCR. The most common symptoms among the PCR-confirmed cases were fever (≥38°C) (27/28), lethargy (27/28), cough (22/28), sore throat (18/28) headache (15/28), rhinorrhoea (12/28 and myalgia or arthralgia (9/28). The mean duration of fever was three days (range, 0-5 days), and on average the conscripts returned to service after five days. None of them had severe complications or required intensive care. Seven conscripts with asthma were treated with antiviral agents during the outbreak, which waned by the end of September. In late October and in November new cases appeared when sustained community transmission of the pandemic began in Finland.

A serological and epidemiological survey was conducted in order to study the prevalence of pandemic influenza A(H1N1) virus infections in this defined population after the outbreak.

Methods

Nasopharyngeal swabs

Initially, during 1-14 September, one to five nasopharyngeal samples were taken per day from conscripts who presented with URTI and fever at the PHS to confirm the diagnosis. After 15 September, sampling was performed randomly from URTI patients. The samples were analysed at the national influenza center of the Finnish National Institute for Health and Welfare (THL) by PCR for pandemic influenza A(H1N1) virus [1], seasonal influenza A, influenza B, parainfluenza 1, 2 and 3, adeno- and respiratory syncytial viruses. Nasopharyngeal samples taken during September were analysed by PCR also for rhino- and enteroviruses at the THL enterovirus laboratory.

Serological and epidemiological survey

All conscripts who had been serving in the garrison during the outbreak (n=984) were invited to participate in the study. The study was approved by the coordinating ethics committee of the hospital districts of Helsinki and Uusimaa, and the volunteers gave informed consent before enrolment in the study. Serum samples were taken from volunteers between 6 November and 3 December 2009.The participants were instructed to fill out a questionnaire at the time of sampling about possible symptoms of URTI, fever and/or diarrhoea experienced during the observation period of July to November 2009. Specific antibodies to the pandemic influenza A(H1N1) virus were analysed by haemagglutination inhibition (HI) according to standard methods [2]. The virus strain used as antigen was A/Finland/554/2009(H1N1v) [3]. Serum samples were

tested in two-fold serial dilutions starting at an initial dilution of 1:10. The highest serum dilution was 1:640. Antibody titres ≥10 were regarded positive.

Clinical records from the PHS were examined according to the international classification of diseases (ICD-10) codes specific for influenza (J10 and J11). SPSS version 16 and Microsoft Excel were used for statistical analyses.

Results

Nasopharyngeal swabs

Altogether, 79 nasopharyngeal swabs were taken between 1 September and 3 December; 44% (35/79) of them were positive for 2009 pandemic influenza A(H1N1) virus by PCR. From a total of 52 nasopharyngeal samples taken during the outbreak in September 54% (28/52) were positive for 2009 pandemic influenza A(H1N1) virus, whereas 37% (19/52) tested positive for rhinovirus. Five samples were negative for all tested viruses. Three conscripts had a simultaneous infection with rhinovirus and 2009 pandemic influenza A(H1N1) virus.

The pandemic influenza A(H1N1) virus was found more frequently during the first half of September, but later the rhinovirus predominated (Figure). Other viruses were rarely present in the nasopharyngeal samples: seasonal influenza A was detected in samples from two conscripts and parainfluenza type 2 in one.

Serological and epidemiological survey

A total of 346 (35%) conscripts volunteered to donate a blood sample and filled out the questionnaire; 99% were male (mean age 21 years; range 20–28). In addition, 139 (14%) conscripts only filled out the questionnaire, making the total who responded to the questionnaire survey 485 (49%).

Nearly half of those who volunteered to give a blood sample (49%, 169/346) had detectable antibodies (titres ≥10) against the 2009 pandemic influenza A(H1N1) virus. In approximately half of these seropositive individuals (46%, 77/169) antibody titres ≥40 were detected.

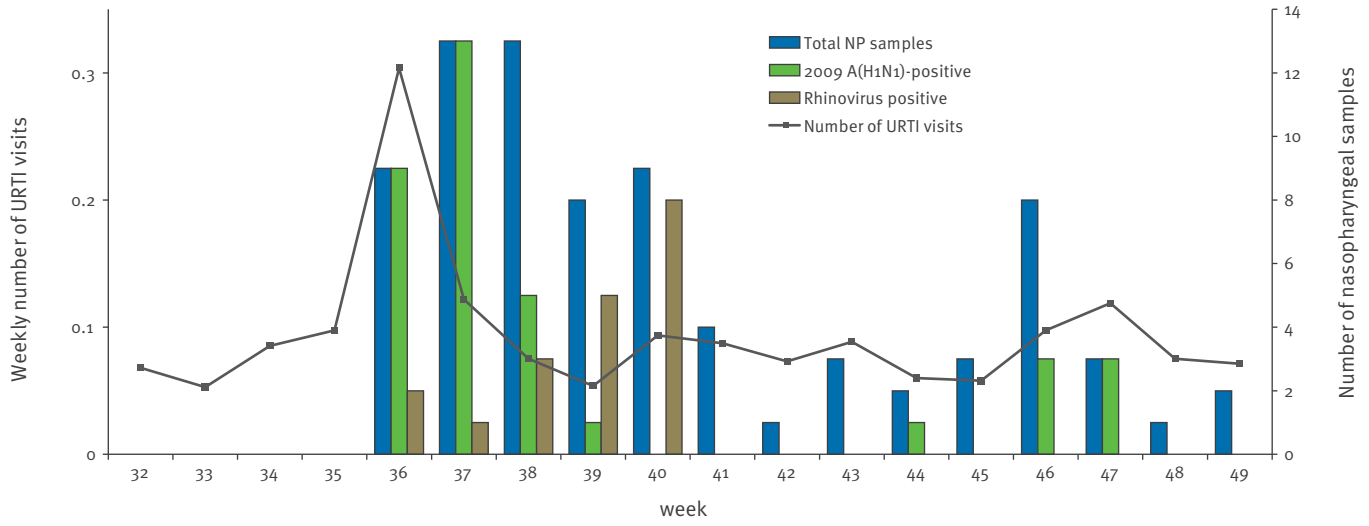
Eight of the participants had a preceding PCR-confirmed 2009 pandemic influenza A(H1N1) virus infection; seven of them were seropositive (median antibody titre 80, range 10-160) and in one individual no 2009 A(H1N1)-specific antibodies were detected two months after the PCR-confirmed 2009 pandemic influenza A(H1N1) virus infection.

Based on the responses to the questionnaire, 50% (84/169) of the seropositive participants did not report any history of URTI, 35% (59/169) reported having had a URTI with fever and 15% (26/169) a URTI without fever (Table). The proportion of seropositives among the participants without any history of URTI was 45% (84/186). The history of having had a URTI with fever was slightly more common among seropositive than among seronegative participants (35% versus 24%), but the difference was not statistically significant. Of the 139 conscripts who only filled out the questionnaire, 97 (70%) did not report any history of URTI, 25 (18%) reported having had a URTI without fever, and 17 (12%) a URTI with fever.

According to the PHS clinical records, a clinical diagnosis of influenza was made in 103 of the 984 conscripts (10%) between 31 August and 30 September, and in 123

FIGURE

Weekly number of URTI-associated visits of the conscript population (n=984), August – November 2009, Finland[a]



NP: nasopharyngeal; URTI: upper respiratory tract infection

[a] The number of NP samples collected during August-November 2009 and the samples positive for 2009 pandemic A(H1N1) virus and rhinovirus by PCR are presented as bars.

conscripts (13%) during the whole observation period covered by the questionnaire (July to November 2009). Of the 346 study participants who donated a blood sample the clinical diagnosis of influenza was made in 47 (14%), and 77% (36/47) of these were seropositive.

Discussion and conclusions

We combined PCR and serological laboratory results with clinical data from the PHS of a garrison as well as from questionnaires filled out by voluntary military recruits after the outbreak of 2009 pandemic influenza A(H1N1), which started before the sustained community transmission of the virus was ongoing in Finland. The epidemic in the general Finnish population began later, during weeks 41-42 in October 2009, and it peaked during weeks 43-45 and 45-48 in northern and southern Finland, respectively.

Military garrisons present a high risk environment for the spread of respiratory disease due to large numbers of conscripts living in close proximity [4]. This may partly explain the high proportion of infected subjects in our study population. A total of 346 conscripts (35%) donated a blood sample and nearly half (49%) of these individuals had antibodies against the 2009 pandemic influenza A(H1N1) virus. The true seroprevalence caused by the outbreak may have been lower than observed due to possible pre-existing cross-reactive immunity. Although some of the conscripts may have been infected during travel abroad or by contacts to travellers in spring and summer 2009 before entering the service in July, the majority of the recruit population was expected to lack immunity against the virus and the baseline prevalence of cross-reacting antibodies against the 2009 pandemic influenza A(H1N1) virus among the conscripts was assumed to be low. According to a recent serological study in Finland, the prevalence of cross-reacting antibodies (HI titre ≥1:10) against the 2009 A(H1N1) virus was less than 2.5% in the 20-39 age group.[3]. This study was based, however, on serum specimens collected during 2004 and 2005, and the seasonal A(H1N1) influenza epidemic in Europe during the winter 2007-8 may have elevated the prevalence of cross-reacting antibodies. In a recent cross-sectional serological study from England, a detectable level of cross-reacting antibodies (HI titre ≥1:8) was found as frequently as in 25% of serum samples obtained in 2008 from 15-24 year-olds [5].

Based on the questionnaire, a history of URTI was more common among the volunteers who donated a blood sample (46%) than among the conscripts who only filled out the questionnaire (30%). As the prevalence of antibodies specific to the 2009 pandemic influenza A(H1N1) virus was high (45%) also among the participants without symptoms of URTI, the proportion of infected individuals in the entire study population might be assumed to be approximately of the same magnitude. However, it is unlikely that this reflects high infection rates also in the general Finnish population. In fact, in England fewer infections were reported - one third of children were considered to have been infected during the 2009 pandemic wave in regions with a high incidence [5].

According to the responses to our questionnaire (n=485), half of the seropositive individuals did not have a history of a URTI or fever. Of the seropositives, 15% had experienced only URTI symptoms, and only one third of the seropositives reported to have had URTI with fever during the study period. Of note is that a proportion of the seropositive individuals may have had pre-existing cross-reactive immunity, and thus these subjects may have been misclassified as asymptomatic cases, which would tend to lower the true proportion of asymptomatic infections. On the other hand, the high frequency of symptoms reported by the seronegative participants was probably caused by the concurrent circulation of rhinovirus. Also, a proportion of the seropositives may have had URTI symptoms due to rhinovirus infection, but not because of A(H1N1) infection. Consequently, the proportion of asymptomatic 2009 A(H1N1) infections may have been higher than observed, after all.

Table

Serological status and history of upper respiratory tract infection with and without fever reported by 346 volunteers, July to November 2009, Finland

	Number of participants (%)						Total	
	Haemagglutination inhibition titre							
	Seronegative		Seropositive					
	< 10		10 - 20		≥40			
URTI with fever	42 (23.7)	(41.6)	32 (34.8)	(31.7)	27 (35.1)	(26.7)	101 (29.2)	(100)
URTI without fever	33 (18.6)	(55.9)	16 (17.4)	(27.1)	10 (13.0)	(16.9)	59 (17.1)	(100)
No reported symptoms of URTI	102 (57.6)	(54.8)	44 (47.8)	(23.7)	40 (51.9)	(21.5)	186 (53.8)	(100)
Total	177 (100)	(51.2)	92 (100)	(26.6)	77 (100)	(22.3)	346 (100)	(100)

URTI: upper respiratory tract infection

In this thoroughly monitored population of 984 conscripts, the clinical diagnosis of influenza was made by a healthcare professional in only 10% of these during the outbreak, suggesting at least five times higher than estimated incidence of infection as compared with clinical surveillance. Well in line with these findings, Miller *et al*. [5] suggested a ten times higher incidence of pandemic influenza A(H1N1) virus infection among English children than that based on the number of clinical diagnoses of influenza-like illness made in general practice. Furthermore, previously reported school outbreaks are similar to our findings of rapid and extensive spread of the infection [6]. The high proportion of asymptomatic and mild infections in our study may partly be explained by the characteristics of the study population, which consisted of young healthy adults. Remarkably, none of our subjects developed severe symptoms.

Timely implementation of strict control measures such as strict isolation and active case finding in other similar, perhaps even more closed, settings e.g. on board navy vessels, has been reported to be effective in limiting the spread of infection [7, 8]. In contrast to these earlier reports, extensive measures were not taken during our outbreak to reduce the spread of the disease in the garrison. The importance of hand hygiene was stressed and proper coughing and sneezing behaviour was advised and encouraged. Only patients with fever were kept hospitalised in the infirmary, and obviously this was not enough to prevent the spread of infection. Instead, strict isolation of the infected units might have limited or perhaps even stopped the transmission within the garrison. In order to reduce influenza morbidity in garrisons and the risk of transmission to the community, specific vaccinations could be the only effective preventive measure.

In conclusion, we report that approximately half of the military conscripts living in crowded garrison quarters may have been infected during an outbreak of pandemic influenza A(H1N1) virus in September 2009. Half of the infections may have been asymptomatic, only a minority of the infected individuals developed clinically typical influenza, and none severe illness. It has been suggested earlier that other respiratory virus infections may inhibit the spread of influenza [9] and *vice versa* [10]. Whether there was interference between rhinovirus and 2009 pandemic influenza A(H1N1) virus in our outbreak remains unsolved.

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References

1. Österlund P, Pirhonen J, Ikonen N, Rönkkö E, Strengell M, Mäkelä S, et al. Pandemic H1N1 2009 influenza A virus induces weak cytokine responses in human macrophages and dendritic cells and is highly sensitive to the antiviral actions of interferons. J Virol. 2010;84(3):1414-22.
2. Kendal AP, Pereira MS, Skehel JJ, editors. Concepts and procedures for laboratory-based influenza surveillance. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention; 1982.
3. Ikonen N, Strengell M, Kinnunen L, Österlund P, Pirhonen J, Broman M, et al. High frequency of cross-reacting antibodies against 2009 pandemic influenza A(H1N1) virus among the elderly in Finland. Euro Surveill. 2010;15(5):pii=19478. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19478
4. Vartti A-M, Mäkitie I, Aro A, Henriksson M, Jormanainen V, Nikkari S: Influenza: knowledge and risk perception among Finnish conscripts beginning military service Suom Laakaril. 2009;64:3303-10. Finnish. English summary available from: http://www.laakarilehti.fi/e/summary.html?type=4/news_id=7845/Influenza
5. Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. Lancet. Published Online 21 January 2010. DOI:10.1016/S0140-6736(09)62126-7.
6. Lessler J, Reich NG, Cummings DA. Outbreak of 2009 pandemic influenza A(H1N1) at a New York City school. N Engl J Med. 2009;361(27):2628-36.
7. Dill C, Favata A. Novel Influenza A(H1N1) Outbreak on board a US navy vessel. Disaster Med Public Health Prep. 2009;3 Suppl 2:117-120.
8. Vera DM, Gonzaga V, Hora RA, Ramos M, Loret de Mola C, Neyra JM, et al. Outbreak of 2009 pandemic A(H1N1) on a Peruvian navy ship –June-July 2009. MMWR Morb Mortal Wkly Rep. 2010;59(6):162-165.
9. Linde A, Rotzén-Östlund M, Zweyberg-Wirgart B, Rubinova S, Brytting M. Does viral interference affect spread of influenza?. Euro Surveill. 2009;14(40):pii=19354. Available from: http/www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19354
10. Casalegno JS, Ottmann M, Bouscambert-Duchamp M, Valette M, Morfin F, Lina B. Impact of the 2009 influenza A(H1N1) pandemic wave on the pattern of hibernal respiratory virus epidemics, France, 2009. Euro Surveill. 2010;15(6):pii=19485. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19485

The European Centre for Disease Prevention and Control launches call for external experts

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The European Centre for Disease Prevention and Control (ECDC) launched a call for external experts on 10 November 2010 inviting scientists to apply to sit on ECDC scientific panels and working groups and to assist the Centre in its activities.

Scientists with relevant expertise are invited to apply online. All experts who submit a complete application are included in the ECDC Candidate Expert Directory. At this stage, only limited information needs to be provided to ECDC. Only when the Centre selects a candidate for possible participation in a specific panel or for other scientific involvement, supplemental information will be requested.

ECDC uses external expertise for many of its tasks. In order to widen its roster of potential experts, ECDC has set up a 'Candidate Expert Directory' and welcomes applications from experts in all fields of its mandate. The activities of ECDC cover public health areas related to communicable diseases, clinical and public health microbiology, epidemiology, statistical analysis and modelling of communicable disease data, and many more.

More detailed information including questions and answers is available on the ECDC website [1].

References

1. European Centre for Disease Prevention and Control. Stockholm; 2010. Invitation to submit application. Available from: http://www.ecdc.europa.eu/en/aboutus/organisation/external_experts/Pages/invitation.aspx