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Dengue virus infection in a traveller returning from Croatia to Germany

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Dengue virus (DENV) is endemic in south-east Asia and Central to South America. In August 2010, a DENV infection was diagnosed in a German traveller returning from a trip to Croatia in south-east Europe. The patient presented with fever and other typical symptoms of DENV-infection. Virological investigation revealed the presence of DENV-specific IgM, a rise in DENV-specific IgG and the presence of DENV NS1 antigen in the patient's blood.

Dengue virus (DENV) is an arthropod-borne RNA virus of the Flaviviridae family causing dengue fever in humans. Since 2001 dengue fever has been mandatorily reported to the German public health authorities, in accordance with the Federal Protection against Infection Act [1]. According to German notification data, between 60 and 387 imported DENV infections are reported annually (Table).

The DENV infections in imported cases are mainly acquired in south-east Asia as well as South and Central America. Very recently, autochthonous DENV infections were reported in southern France, diagnosed for the first time ever in Europe [2]. Here we report on a case of DENV infection that was apparently acquired in Croatia and imported to Germany by a traveller.

Table

Imported cases of dengue fever per year, Germany, 2001–2010[a]

Year	Number of recorded cases
2001	60
2002	213
2003	131
2004	121
2005	144
2006	175
2007	264
2008	273
2009	298
2010	387

Source: Robert Koch Institute, SurvStat (http://www3.rki.de/SurvStat).

[a] As of 4 October 2010.

Case report

A 72-year-old man from Germany visited Croatia in August 2010: he left on 1 August and returned on 15 August. He was accompanied by seven family members, including grandchildren. The family travelled by car from Germany via Austria and Slovenia to Croatia without overnight stops. The group stayed the entire time around Podobuce close to Orebić on the Peljesac peninsula and on the isle of Korčula in the south of Croatia. Podobuce and Korčula are located approximately 100 km north-west of the city of Dubrovnik, which was also visited. Temperatures were reported to be very high (approximately 30 °C at night). After returning to Germany, on 16 August, the patient developed a febrile illness with a temperature of up to 39 °C, chills, arthralgia, headache, and retro-orbital pain. Following a short period of improvement, his temperature rose again to 39 °C on 21 August, and he continued to have arthralgia, myalgia, weakness and dyspnoea. Among several other diseases, dengue fever was suspected by the general practitioner, because of the clinical picture.

Laboratory results

Serum samples were taken from the patient for virological investigation on 23 and 30 August and on 2 September. The sample from 23 August was positive for DENV-specific IgM, but negative for IgG in an enzyme-linked immunosorbent assay (ELISA). On 30 August, DENV-specific IgM and IgG was positive with a titre of 1:2,560 (cut-off 1:20) and 1:80 (cut-off 1:20), respectively, in an indirect immunofluorescence assay based on DENV-infected cells. In addition, the serum sample tested positive for DENV NS1 antigen (Dengue Early ELISA, Panbio). Real-time reverse transcription-polymerase chain reaction (RT-PCR) for DENV [3] was negative. The detection of DENV NS1 antigen and the simultaneous absence of DENV RNA during this

phase of dengue fever are in line with previous studies demonstrating an acute DENV infection [4]. The sample taken on 2 September showed an increase in the DENV-specific IgG titre (1:1,280), while the IgM titre remained unchanged and the ELISA for NS1 antigen was negative. In this sample, immunofluorescence assay titres for related flaviviruses were lower than for DENV: West Nile virus (IgM negative, IgG 1:160) and tick-borne encephalitis virus (IgM 1:80, IgG 1:80). The patient did not report vaccination against tick-born encephalitis or yellow fever. A temporary thrombocytopenia with a minimal platelet count of 97,000/µl (norm: 150,000–440,000/µl) on the eighth day of the illness resolved without complications and the patient recovered within two weeks after disease onset.

Conclusions

The clinical suspicion of dengue fever was confirmed by the laboratory tests. As the incubation period for dengue fever ranges from three to 14 days , the infection was probably acquired in southern Croatia and not en route. The Croatian authorities were given all available information about the case, enabling them to investigate this further at local level. To our knowledge, this is the second report on an autochthonous DENV transmission in Europe after France. Antibodies against DENV have been previously detected in Croatian individuals in the context of international travel; however, the specificity of the assay is questionable [5]. The presence of *Aedes albopictus* as a potential DENV vector in Croatia [6] and the importation of confirmed dengue fever cases from endemic areas into Croatia [7,8] allow autochthonous DENV transmission within this country. The mosquito season in parts of the northern Mediterranean coast may last from May to November. Therefore, dengue fever should be considered in patients with fever of unknown origin and relevant clinical symptoms who stayed in areas in Europe where *Ae. albopictus* occurs.

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Spotlight on measles 2010: An ongoing measles outbreak in the district of Neamt, Romania, August – September 2010

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We report an outbreak of measles that has been ongoing in the district of Neamt, Romania, since 22 August 2010. As of 21 September, 17 of 21 suspected cases have been laboratory-confirmed and there was one measles-related fatality.

Introduction

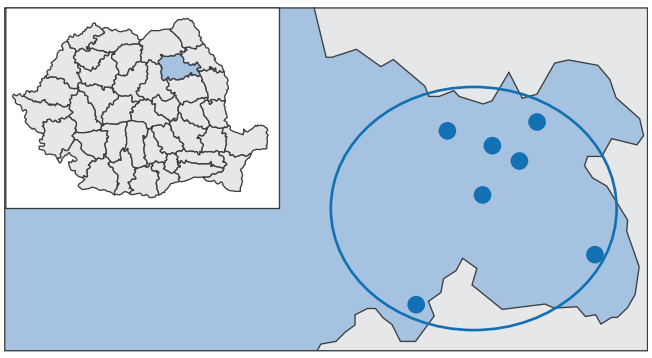
An outbreak of measles was detected in late August 2010 in the Romanian north-eastern district of Neamt with an estimated population of 566,940 (2009) (Figure 1). Earlier in the year, between 1 January and 3 August, 15 cases of measles had been notified from different parts of the country. These included two family clusters among members of the Roma ethnic minority. The first cluster involving five family members occurred between late February and mid-March (weeks 7 and 11). The index case had a history of travel to France. The second cluster involved three cases in Neamt and occurred in mid-June (week 24). We report on the outbreak that emerged in Neamt by analysing preliminary data from late August to late September (weeks 33 to 38).

Measles is a statutorily notifiable disease since 1978, obliging medical practitioners to immediately report suspected measles cases to the local Public Health Authorities. Notifications of measles cases are collected and analysed nationally at the National Centre for Communicable Diseases Surveillance and Control in Bucharest. National case-based notification was initiated in 1999 and the European Union (EU) case definition and case classification have been adopted since 2005 [1].

The measles vaccine was introduced in 1979 into the Romanian national immunisation programme for children 9-11 months of age. In 1994, the second measles vaccine dose was introduced for children six to seven years of age (first school grade). The combined measles-mumps-rubella (MMR) vaccine replaced the monovalent measles vaccine in 2004 and was recommended as a first dose for children at 12-15 months of age. The second MMR vaccine dose has been recommended since October 2005 for children at six to seven years of age. In the period form 2000 to 2008, the national measles vaccination coverage for children aged 18-24 months with the first dose of measles-containing vaccine was estimated at 97-98%. For children aged seven years, the measles vaccination coverage with the second dose was estimated at 96-98% [2].

Figure 1

Measles cases in the district of Neamt, Romania, August-September 2010



The blue spots indicate areas in Neamt affected where cases occurred. The blue circle indicates the containment area chosen for the vaccination campaign.

Outbreak description

Between 22 August and 21 September 2010, a total of 21 suspected cases were notified. In one case, the infection was fatal. The first measles cases of this outbreak were reported in two children and an infant. The close dates of onset of disease of these first cases suggest previous contact with an unreported case of measles.

Serum samples from all suspected cases were available for laboratory testing. Measles was confirmed in 17 of them (Figure 2), which corresponds to a crude incidence of three per 100,000 inhabitants in the district (95% confidence interval (CI): 1.9–4.9). Of the remaining four cases, three cases had a negative test result and were discarded and in one case the result is still pending.

Laboratory confirmation was performed by detecting measles IgM antibodies in serum samples. RT-PCR to detect measles virus nucleic acid was also used to confirm the first five cases. The National Reference Laboratory for Measles and Rubella 'Cantacuzino' identified measles virus genotype D4 in clinical specimens from these five cases.

The outbreak investigators reported that the laboratory-confirmed cases involved both the general population (n=11) and members of the Roma ethnic community (n=6). The median age was 11 months (range: four months to nine years). Ten of the 17 cases were infants (under the age of one year), six were one to four years-old and one was in the age group from five to nine years. The status of measles vaccination was known in all notified cases. Fifteen cases were unvaccinated (Table). These included 10 infants who were not eligible for vaccination because of their age and five cases who were eligible but for whom the indicated reason for non-vaccination was contraindications including underweight, hydrocephalus and Down syndrome. The remaining two cases had been vaccinated with one dose of MMR.

The death was reported in a seven month-old, unvaccinated infant who was admitted to a paediatric ward with gastrointestinal symptoms, anaemia and pharyngitis. The infant later developed a rash and acute pneumonia which was the ultimate cause of death. Of the 17 notified cases, 14 were hospitalised as in-patients in a paediatric ward. Six of those were probably infected with measles through nosocomial transmission on the ward.

Control measures

Several control measures have been implemented by local health authorities. A supplementary MMR vaccination campaign was started on 6 September in the defined containment area including all affected communities and neighbourhoods in Neamt (Figure 1). It targeted all children from seven months to seven years of age who did not have documented evidence of vaccination. The MMR vaccine was supplied by the Ministry of Health and offered free of charge through the routine immunisation services (family doctors) and special outreach teams in the community. Of 1,345 eligible individuals, 956 (71%) have been vaccinated by 19 September 2010. In addition, existing MMR vaccination campaigns were reinforced in the border areas of four neighbouring districts close to the affected areas in Neamt.

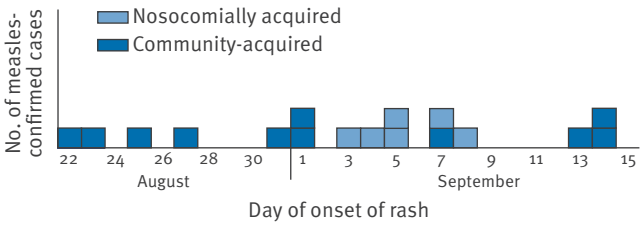
Moreover, active case finding by general practitioners has been instigated in the areas where cases were living, as well as tracing contacts of cases in hospitals and in the community. All children with fever and rash were referred to the infectious disease ward and were investigated. MMR vaccination was given to all contacts between seven months and seven years of age who did not have documented evidence of vaccination. To date, 29 close contacts of the hospitalised patients were identified, nine of whom acquired measles (secondary attack rate: 31%). Additional activities to increase awareness of the ongoing outbreak included sending medical bulletins with information to all physicians in the district and all public health authorities in the country.

Discussion

The source of this outbreak has not yet been identified. The earlier occurrence of measles in the same district in mid-June 2010, suggests that transmission may have continued unnoticed in the meantime. The local health

Figure 2

Number of measles cases by day of onset of rash, Neamt district, Romania, August-September 2010 (n=17)



Table

Number of measles cases by age group and vaccination status, Neamt district, Romania, August-September 2010 (n=17)

Age group	No. of measles cases	Vaccination status
<1 year	10	Not eligible for vaccination
1-4 years	6	1 had received one MMR dose, 5 had contraindications for vaccination
5-9 years	1	1 had received one MMR dose
Total	**17**	

MMR: measles, mumps, rubella vaccine.

authorities may not have been notified or the cases may not have sought medical attention. Nevertheless, any link between the earlier cluster and this outbreak remains speculative.

Despite the high national vaccination coverage with MMR vaccine, this outbreak highlights the presence of pockets of individuals vulnerable to measles, in the general population and among members of the Roma community. The vulnerability of Roma communities to acquire measles is well documented, most recently with the outbreak that occurred in Bulgaria [3]. In areas and communities where vaccination coverage remains sub-optimal, cohorts of susceptible individuals accumulate and represent a potential for outbreaks to occur. The current ongoing outbreak involves a large proportion of infants too young to be vaccinated according to the national childhood vaccination programme, which is indicative of widely circulating measles virus. A similar situation had been observed earlier in Romania in 2006 [4].

The five children with measles who were not vaccinated due to perceived contraindications may have been prevented. All of these children could have been vaccinated unless they also had a serious allergy to any of the ingredients of the MMR vaccine, an acute severe illness or severe immunodeficiency. Inadequate knowledge of the contraindications for MMR vaccination by general practitioners is a recognised problem that needs to be addressed.

In 2008 and 2009 the measles situation in Romania had improved dramatically compared with previous years, with reported incidences of less than 0.1 per 100,000 inhabitants [5,6]. However, the emergence of this outbreak highlights the need for urgent preventive and control measures to be taken once again. For the goal of measles elimination to be reached, awareness of the disease and a commitment by public health authorities in Romania are essential to strengthen vaccination programmes. The World Health Organization's strategic plan for the elimination of measles from the European region stipulates that vaccination programmes should achieve and sustain a minimum of 95% coverage with two doses of vaccine and should target susceptible individuals in the general population [7] as well as in vulnerable groups. Moreover, constant vigilance is needed to ensure that suspected measles cases are promptly investigated to identify outbreaks and instigate the control measures to curtail them.

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Seroprevalence of 2009 pandemic influenza A(H1N1) virus in Australian blood donors, October – December 2009

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Assessment of the severity of disease due to the 2009 pandemic influenza A(H1N1) in Australian states and territories has been hampered by the absence of denominator data on population exposure. We compared antibody reactivity to the pandemic virus using haemagglutination inhibition assays performed on plasma specimens taken from healthy adult blood donors (older than 16 years) before and after the influenza pandemic that occurred during the southern hemisphere winter. Pre-influenza season samples (April – May 2009, n=496) were taken from donation collection centres in North Queensland (in Cairns and Townsville); post-outbreak specimens (October – November 2009, n=779) were from donors at seven centres in five states. Using a threshold antibody titre of 40 as a marker of recent infection, we observed an increase in the influenza-seropositive proportion of donors from 12% to 22%, not dissimilar to recent reports of influenza A(H1N1)-specific immunity in adults from the United Kingdom. No significant differences in seroprevalence were observed between Australian states, although the ability to detect minor variations was limited by the sample size. On the basis of these figures and national reporting data, we estimate that approximately 0.23% of all individuals in Australia exposed to the pandemic virus required hospitalisation and 0.01% died. The low seroprevalence reported here suggests that some degree of prior immunity to the virus, perhaps mediated by broadly reactive T-cell responses to conserved influenza viral antigens, limited transmission among adults and thus constrained the pandemic in Australia.

Introduction

The global spread of a novel strain of influenza A(H1N1), which emerged in North America in March 2009, led the World Health Organization (WHO) to declare on 11 June 2009 a phase 6 pandemic alert – the first time in more than 40 years that a pandemic had been declared [1]. Australia's first case of imported active infection with the pandemic virus was reported on 20 May 2009, with confirmation of established community transmission in the state of Victoria only two days later [2]. This timing coincided with the usual onset of seasonal influenza activity, which peaks during the southern hemisphere winter months (June to August) [3]. The epidemic peaked in late July 2009, with most cases reported over an 18-week period until late September, slightly earlier and shorter than a typical influenza season [4]. Influenza-like illness incidence, reported through a variety of sources, appeared similar to that observed in the relatively severe seasonal influenza outbreak of 2007 in Australia [4]. While the majority of reported cases were mild, an excess of hospitalisations and intensive care unit admissions was reported, most markedly in adults aged 20–60 years [4].

Consistent with early observations from other countries [5,6], the pandemic virus appeared particularly transmissible in schools. In Victoria, almost 80% of cases reported during the first two weeks of the outbreak occurred in individuals aged less than 20 years (median: 15 years) [2]. The effective reproduction number (the number of secondary cases per case) only exceeded unity in this younger age group – an effective reproduction number of more than one is a requirement for sustained epidemic growth [2]. Subsequent spread of the virus around the country occurred in a staggered fashion [7], reflecting the large distances between Australian state and territory capital cities, which are mainly dispersed around the coast (Figure 1). Case-reporting rates *per capita* varied over time and by jurisdiction, probably reflecting variable intensity of case-finding efforts by pandemic phase, which was further influenced by local laboratory practices and capacity [7]. While reported hospitalisation and

death rates appeared more similar around the country [7], the absence of a consistent case denominator from which to infer exposure made assessment of severity difficult.

This study aimed to establish a representative collection of plasma samples from healthy adult blood donors in selected Australian jurisdictions following the 2009 influenza A(H1N1) pandemic in the winter. Samples were tested for immunity to the pandemic virus, as a proxy measure of recent virus exposure, to aid assessment of disease severity by age and location. Measurement of the proportion of influenza-seropositive donors would also inform estimates of residual susceptibility to infection in the population, to aid decision-making regarding optimal timing and coverage of proposed population immunisation campaigns.

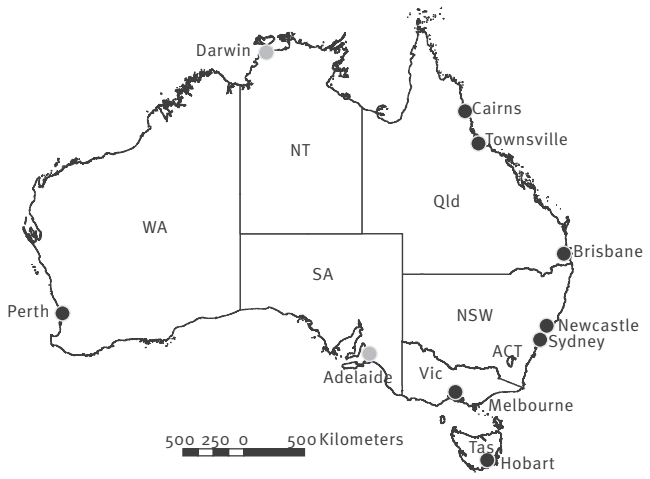
Methods

Pre-pandemic study population

Approximately 500 pre-pandemic plasma samples were randomly selected from anonymised specimens collected in late April – early May 2009 that had been stored by the Australian Red Cross Blood Service (the Blood Service) for dengue fever surveillance studies. The samples were drawn at random from samples stored in a freezer, as were the post-pandemic samples. The sampling time frame was chosen to predate circulation of the pandemic virus in Australia for assessment of baseline immunity. As dengue fever is confined to the tropical north of Australia, such specimens were only available from donor collection centres in Cairns and Townsville, jointly administered through the Townsville site.

Figure 1

Geographical distribution of blood donation collection centres, Australia, April– May and October – December 2009 (n=8)



NSW: New South Wales; NT: Northern Territory; Qld: Queensland; SA: South Australia; Tas: Tasmania; Vic: Victoria; WA: Western Australia.

Blood donation collection centres are marked in black.

Non-participating state and territory capital cities are shown in grey.

Post-pandemic study population

Discarded plasma samples, which had been routinely taken from healthy Blood Service donors for serological testing, were prospectively collected in Brisbane, Hobart, Melbourne, Newcastle, Perth, Sydney and Townsville (Figure 1) from late October to early December 2009 following the first wave of pandemic influenza in Australia [4]. Approximately 120 anonymised specimens were selected per site, with up to 20 randomly selected in each of the following age strata: 16–24, 25–34, 35–44, 45–54, 55–64 and ≥65 years. Accompanying information included age (years) and sex of the donor. Status of prior influenza A(H1N1) disease or vaccination was not routinely obtained at all sites, but most specimens were collected immediately following introduction of the pandemic vaccine, when anecdotally reported uptake was low. Vaccination fields were double checked for participants with high-titre antibodies (>640) to the pandemic virus.

In accordance with the provisions of the National Health and Medical Research Council's National Statement on Ethical Conduct in Human Research, individual consent was not required for use of these specimens, given the granting of institutional approval by the Blood Service Ethics Committee.

Laboratory assays

The tests were performed at the WHO Collaborating Centre for Reference and Research on Influenza, in Melbourne.

Reactivity of plasma against 2009 pandemic influenza A(H1N1) virus was measured using haemagglutination inhibition (HI) assays [8]. Egg-grown A/California/7/2009 virus was purified by sucrose gradient, concentrated and inactivated with β-propiolactone, to create an influenza zonal pool preparation (a gift from CSL Limited). Plasma samples were pretreated with receptor destroying enzyme II (Denka Seiken Co. Ltd), 1:5 (volume/volume) and tested as previously described [9]. Following a one-hour incubation, 25 µl 1% (volume/volume) turkey or human red blood cells (RBC) was added to each well. HI was read after 30 minutes for turkey RBC or 60 minutes for human RBC. Any samples that bound to the RBC in the absence of virus were adsorbed with RBC for one hour and re-assayed. Titres were expressed as the reciprocal of the highest dilution of plasma where haemagglutination was prevented.

The haemagglutinating ability of influenza A viruses can vary depending on the influenza subtype and the species of the RBC used. The ratio of α-2,3- to α-2,6-linked sialic acid residues differs between the RBC of various species [10] and this is reflected in the agglutination patterns of the viral haemagglutinin subtypes [11]. Recent influenza A(H3N2) viruses

typically agglutinate guinea pig RBC better [12], while A(H5N1) viruses agglutinate horse RBC better [13]. The 2009 pandemic influenza A(H1N1) virus agglutinates chicken, human, guinea pig and turkey RBC equally to date (data not shown). Turkey RBC have typically been used in HI assays for 2009 pandemic vaccine serological studies [9,14-16] and are routinely used in the WHO Collaborating Centre in Melbourne and in the other WHO collaborating centres for influenza. Human O-negative RBC are readily obtained and thus routinely used in many research and diagnostic laboratories. For these reasons, we performed HI assays using both turkey and human O-negative RBC.

A subset of samples was also tested by a modified microneutralisation assay [17]. Briefly, undiluted plasma was inactivated at 56 °C for 30 minutes. Heat-treated plasma (two-fold dilutions from 1:10 to 1:1,280) and A/Auckland/1/2009 virus (200 times the 50% tissue culture infective dose $(TCID)_{50}$) were incubated at 35 °C for one hour, then added to washed Madin-Darby canine kidney cells in 96-well flat-bottomed plates, as for $TCID_{50}$ assay, as previously described [18]. Titres were expressed as the reciprocal of the highest dilution of plasma where haemagglutination was prevented.

A panel of control sera and plasma samples was included in all assays. It comprised paired ferret sera pre- and post-infection with the pandemic virus or seasonal influenza A(H1N1), A(H3N2) or influenza B viruses and paired human plasma and sera collected from donors before April 2009 or after known infection with the pandemic virus or after immunisation with the Australian monovalent pandemic 2009 vaccine.

Data on hospitalisations and death

Rates of hospitalisation and death per 100,000 population by Australian jurisdiction were taken from the NetEpi database maintained by the Office of Health Protection, Australian Government Department of Health and Ageing. Permission to use these data was granted by the relevant states and territories that provided the information. The reporting period was from 1 May to 2 October 2009.

Statistical analysis and sample size

Immunity to the pandemic virus was reported as the proportion of donors (by age group or donor site) with HI antibody titres at or above the putative protective threshold of 40 observed to correlate with 50% protection against experimental influenza infection in challenge studies with seasonal influenza viruses, with 95% confidence intervals (CIs) of the estimate [19]. Proportions with HI titres ≥80 and ≥160 were also reported, as well as geometric mean titres (GMT) with 95% CIs. Univariate and multivariate logistic regression models were used to assess the relationship of sex and age of donor with seropositive status (HI titre ≥40) at baseline. In the post-pandemic study population the influence of donor location was also evaluated.

A minimum of 15 individuals within each age stratum was selected as a target sample size to allow estimation of a true seropositive proportion as low as 10%, with 95% confidence intervals excluding 0 and 90% power. A similar protocol involving between 100 and 120 donors of the Red Cross Blood Bank at the Royal Melbourne Hospital was conducted over 14 weeks in 1957. Rising seroprevalence of HI antibodies to the 'Asian' influenza A(H2N2) virus was observed, from 0% to a peak mean value of around 42% by the eighth week of study [20].

Results

Study population

Characteristics of the donor populations from whom samples were collected at each site are shown in Table 1, together with the period of specimen collection. A total of 32 samples were excluded from the analysis due to high haemagglutination titres in the absence of virus and more than two-fold difference between turkey RBC and human RBC. Samples with background HI titres that could not be eliminated by

Table 1

Blood donor characteristics, by collection site and age group, Australia, April – May and October – December 2009 (n=1,307)

Collection site (collection dates in 2009)	Total number of plasma specimens	Blood donor age group (years)						Male (%)
		16–24	25–34	35–44	45–54	55–64	≥65	
Baseline (pre-pandemic)								
Cairns and Townsville, 20 April – 9 May	501	88	59	64	129	132	29	54
Post-pandemic								
Brisbane, 22–30 Oct	107	20	21	16	20	20	10	65
Hobart, 16 Nov – 1 Dec	114	20	21	20	20	19	14	44
Melbourne, 16 Nov	113	20	20	20	20	20	13	54
Newcastle, 24–26 Nov	120	20	20	20	20	20	20	59
Perth, 17–18 Nov	120	20	20	20	20	20	20	50
Sydney, 19–20 Nov	120	20	20	20	20	20	20	65
Townsville, 13–27 Oct	112	19	21	20	20	21	11	63

RBC adsorption were distributed throughout the various locations and age groups. The remaining 1,275 samples were included in the final analysis: 496 from April – May and 779 from October – November 2009.

Assay results

Results are presented for HI assays using turkey RBC (Table 2 and Table 3). A slightly higher rate of background reactivity was seen in the human RBC assays (data not shown), suggesting that a higher threshold titre was required to indicate recent exposure. Despite this difference, there was 90.5% correlation between assay results, with a generally linear relationship ($p<0.001$).

Table 4 reports the findings of a multivariate logistic regression model examining the influence of sex, age and location on post-pandemic seropositivity (HI titre ≥40), although interactions between all factors were not explored due to the limited sample size. At baseline, neither sex nor age had a significant effect on serostatus (data not shown).

Table 2

Haemagglutination inhibition assays of plasma samples from blood donors, by collection site, Australia, April – May and October – December 2009 (n=1,275)

Collection site	Number of samples	GMT (95% CI)	Percentage with HI titre ≥40 (95% CI)	Percentage with HI titre ≥80 (95% CI)	Percentage with HI titre ≥160 (95% CI)
Baseline collection (Apr – May 2009)					
Cairns and Townsville	496	8.40 (7.72–9.14)	12 (9.1–14.9)	6 (3.9–8.1)	3 (1.5–4.5)
Post-pandemic collection (Oct –Nov 2009)					
Brisbane	102	9.03 (7.27–11.2)	18 (10.5–25.5)	9 (3.4–14.6)	6 (1.4–10.6)
Hobart	108	14.6 (10.9–19.5)	31 (22.3–39.7)	23 (15.1–30.9)	12 (5.9–18.1)
Melbourne	107	10.8 (8.40–13.9)	22 (14.2–29.8)	13 (6.6–19.4)	9 (3.5–14.4)
Newcastle	120	10.9 (8.37–14.2)	23 (15.5–30.5)	16 (9.4–22.6)	10 (4.6–15.4)
Perth	117	12.4 (9.63–15.9)	24 (16.3–31.7)	15 (8.5–21.5)	8 (3.1–12.9)
Sydney	116	12.1 (9.53–15.4)	22 (14.5–29.5)	17 (10.2–23.8)	7 (2.4–11.6)
Townsville	109	8.98 (7.25, 11.1)	19 (11.6–26.4)	9 (3.6–14.4)	4 (0.3–7.7)
Total (Oct–Nov)	779	11.1 (10.2–12.2)	22 (19.1–24.9)	15 (12.5–17.5)	8 (6.0–9.9)

CI: confidence interval; GMT: geometric mean titres; HI: haemagglutination inhibition.

In the shaded cells, the post-pandemic HI antibody titres to the 2009 pandemic influenza A(H1N1) virus are significantly higher than those measured in the baseline samples, on the basis of non-overlapping 95% CIs.

Table 3

Haemagglutination inhibition assay results of plasma samples from blood donors, by age, Australia, April – May and October – December 2009 (n=1,275)

Donor age group in years	Number of samples	GMT (95% CI)	Percentage with HI titre ≥40 (95% CI)	Percentage with HI titre ≥80 (95% CI)	Percentage with HI titre ≥160 (95% CI)
Baseline collection (Apr – May 2009)					
16–24	88	9.77 (7.61 to 12.5)	16 (8.3 to 23.7)	9 (3.0 to 15.0)	6 (1.0 to 11.0)
25–34	59	7.72 (6.05 to 9.86)	7 (0.4 to 13.5)	5 (-0.5 to 10.6)	3 (–1.4 to 7.4)
35–44	64	9.07 (7.00 to 11.8)	13 (4.8 to 21.2)	6 (0.2 to 11.8)	5 (–0.3 to 10.3)
45–54	129	7.39 (6.31 to 8.66)	9 (4.1 to 14.0)	6 (1.9 to 10.1)	2 (–0.4 to 4.4)
55–64	129	8.26 (7.10 to 9.61)	13 (7.2 to 18.8)	3 (0.05 to 5.9)	0 (0 to 0)
≥65	27	11.8 (7.64 to 18.2)	19 (4.2 to 33.8)	11 (–0.8 to 22.8)	4 (–3.4 to 11.4)
Post-pandemic collection (Oct – Nov 2009)					
16–24	138	17.1 (13.0 to 22.5)	37 (28.9 to 45,1)	24 (16.9 to 31.1)	16 (9.9 to 22.1)
25–34	139	10.7 (8.75 to 13.0)	22 (15.1 to 28.9)	14 (8.2 to 19.8)	5 (1.4 to 8.6)
35–44	131	9.19 (7.34 to 11.5)	15 (8.9 to 21.1)	13 (7.2 to 18.8)	7 (2.6 to 11.4)
45–54	138	8.56 (7.03 to 10.4)	16 (9.9 to 22.1)	11 (5.8 to 16.2)	5 (1.4 to 8.6)
55–64	131	11.2 (8.86 to 14.1)	20 (13.2 to 26.8)	13 (7.2 to 18.8)	9 (4.1 to 13.9)
≥65	102	12.1 (9.60 to 15.2)	25 (16.6 to 33.4)	11 (4.9 to 17.1)	5 (0.8 to 9.2)

CI: confidence interval; GMT: geometric mean titres; HI: haemagglutination inhibition.

In the shaded cells, the post-pandemic HI antibody titres to the 2009 pandemic influenza A(H1N1) virus are significantly higher than those measured in the baseline samples, on the basis of non-overlapping 95% CIs.

No significant difference in GMT was observed between post-pandemic collection sites, although there was a trend towards a higher seropositive proportion in Hobart than in the other sites (Table 4). Three centres had significantly higher GMTs, demonstrated by non-overlapping 95% CIs, than were measured in the baseline plasma collection (Table 2). Seropositive proportions that differed significantly from baseline are also indicated (shaded cells). The difference between the two collection periods (baseline and post-pandemic) is further demonstrated in the reverse cumulative distribution plots in Figure 2, in which data from October to November specimens have been pooled for all sites, in three age strata.

While titres appeared to rise over time in several age cohorts, the only age group in which GMTs increased significantly from baseline, demonstrated by non-overlapping 95% CIs, was 16–24 years (Table 3 and Figure 2A). The proportion seropositive (HI titre ≥40) rose significantly in both the 16–24 and 25–34 year strata over the course of the outbreak (Table 3), and was significantly higher in the youngest age group than among individuals aged 25–64 years (Table 4, Figure 2B). Figure 2C further demonstrates the higher seropositive proportion in the elderly population at baseline, with very little evidence of exposure resulting in seroconversion during the pandemic. No donors with high-titre antibodies (HI titre >640) had a record of prior immunisation with the pandemic vaccine.

Table 4

Multivariate logistic regression analysis: influence of factors on post-pandemic seropositivity (HI titre ≥40) in blood donors, Australia, October – December 2009 (n=779)

Variable	Odds ratio	95% confidence interval	P value
Sex (reference group: female)			
Male	0.75	0.54–1.05	0.09
Age group in years (reference group: <25 years)			
25–34	0.54	0.32–0.91	0.02
35–44	0.39	0.22–0.68	0.001
45–54	0.36	0.21–0.64	<0.001
55–64	0.58	0.34–0.98	0.04
≥65	0.71	0.41–1.23	0.2
Location (reference site: Brisbane)			
Hobart	1.83	0.99–3.4	0.06
Melbourne	1.23	0.65–2.3	0.5
Newcastle	1.04	0.55–2.0	0.9
Perth	1.22	0.65–2.3	0.5
Sydney	1.16	0.62–2.2	0.6
Townsville	1.01	0.52–1.9	1.0

HI: haemagglutination inhibition.

Correlation between HI and microneutralisation titres, by assay type

A randomly selected subset of 63 samples were further tested by microneutralisation assay for analysis of concordance between the two assay types. For both the turkey and human RBC assays, measured values were higher than observed in the microneutralisation assay. The correlation between assay results was 70.5% ($p<0.001$) with a generally linear relationship observed.

Interpretation of severity, in relation to serosurvey findings

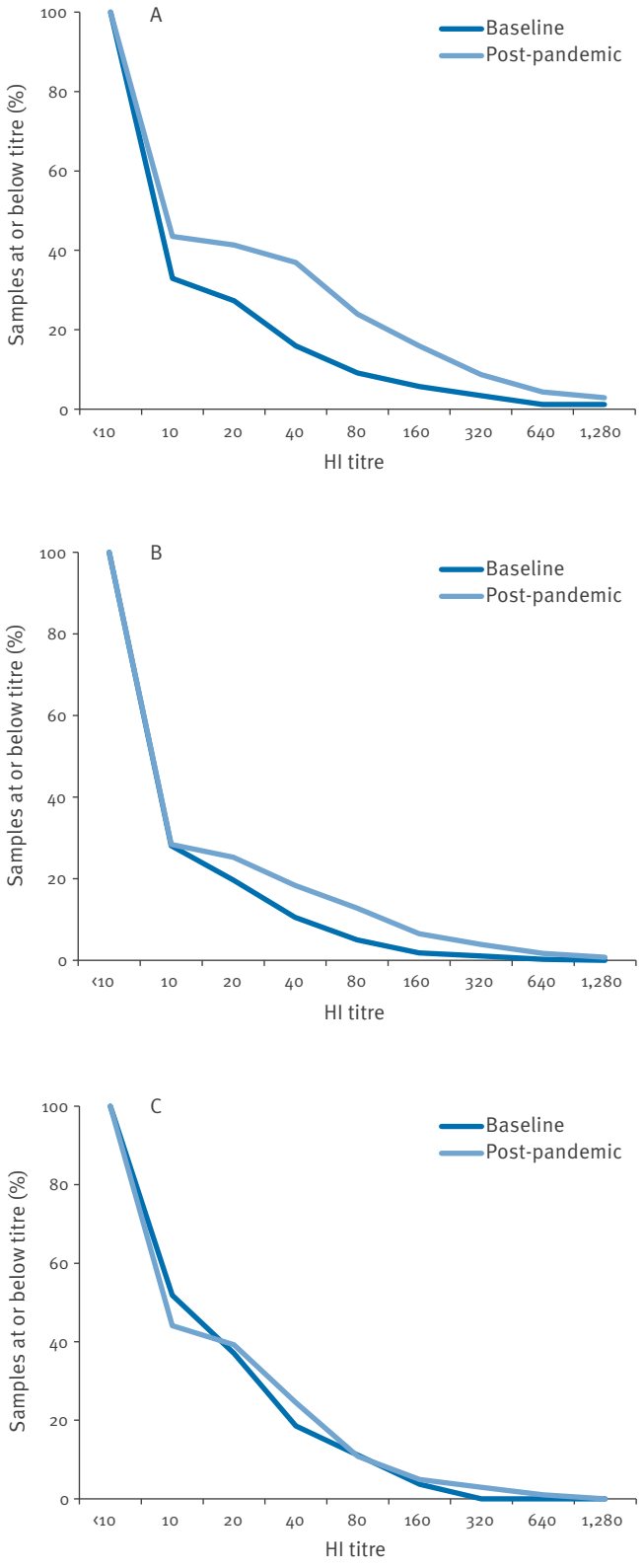
The average reported rate of hospitalisation due to pandemic influenza for the Australian population during the 2009 winter was 23 per 100,000 population [4], ranging from eight per 100,000 population (Victoria) to 40 per 100,000 population (Western Australia). The Northern Territory was a marked outlier, with a rate of 167 per 100,000 population reflecting the heightened susceptibility of indigenous Australians, who make up a greater population proportion in that jurisdiction than elsewhere. Similarly, rates of death due to pandemic influenza in the Northern Territory significantly exceeded those in the rest of the country at 2.7 per 100,000 population, compared with a national average of 0.9 per 100,000 population (range: 0.5 per 100,000 population in Victoria to 1.6 per 100,000 population in South Australia). Assessment of seropositivity in this vulnerable group was not possible as blood donor records do not include indigenous status. In the absence of convincing differences in exposure rates between jurisdictions, the rate of infection exposure sufficient for seroconversion from this serosurvey overall was approximately 10,000 per 100,000 population. On the basis of this figure, we estimate that 0.23% of exposed individuals required hospitalisation and 0.01% died.

Discussion and conclusions

Exposure to the 2009 pandemic influenza A(H1N1) virus appears to have been relatively uncommon among the healthy adult blood donor population during the Australian 2009 winter outbreak. The difference in seropositivity assessed by HI assay compared with baseline was in the order of 5–10% overall, depending on the threshold titre employed for comparison. No significant difference in GMTs was observed between jurisdictions, and only half of the sites surveyed in October – November 2009 had group GMTs higher than in the baseline collection. This finding differs from observations in England and Wales [21] and Scotland [22], suggesting a greater degree of heterogeneity of population mixing in those countries than in Australia. Despite vast geographical distances, the majority of the largely urbanised Australian population is concentrated in a handful of state capital cities, which act as important hubs for each jurisdiction [23]. The only age group in which GMTs were significantly higher (on the basis of non-overlapping 95% CIs) after the winter outbreak was the 16–24-year-old cohort, consistent with trends observed in the United Kingdom (UK) studies [21,22].

Figure 2

Reverse cumulative distribution plot of haemagglutination inhibition titres of plasma samples from blood donors collected Apr – May 2009 (baseline) and Oct – Nov 2009 (post-pandemic), by age group: (A) <25 years, (B) 25–64 years, (C) ≥65 years



HI: haemagglutination inhibition.

This study has a number of limitations that must be taken into account when interpreting its findings. Time and budgetary constraints necessarily limited the number and frequency of specimen collections. As plasma samples for serological testing are routinely discarded after several days in most centres, stored specimens for baseline antibody assessment were only available from Cairns and Townsville, where collections are maintained for research purposes. Given the connectedness of the Australian population, we do not believe that this is likely to have biased baseline assessment of immunity, but a nationwide survey would have been preferable. Also, the collections were cross-sectional in nature and did not allow measurement of evolving immune status within an individual over time, which would provide a more accurate assessment of seroconversion. Moreover, specimens used for this study were recovered plasma samples from blood donors taken after completion of mandatory viral nucleic acid testing. Stringent procedures are in place to ensure that blood donors are healthy at the time of donation, but their past illness experience is unrecorded. Donors might differ from the general population in relation to illness avoidance behaviours as well as in the prevalence of risk factors for infection and severe disease. They were also, by definition, at least 16 years of age, precluding inclusion of paediatric samples.

Higher rates of background reactivity are observed in plasma than in serum; the laboratory methods employed in this study were therefore designed to minimise this additional 'noise'. Plasma samples have previously been used in related studies as part of dengue blood donor surveillance studies for less stable viral RNA markers [24].

HI assay results vary significantly between laboratories, despite best efforts at standardisation [25]. HI titres in this study tended to be higher than microneutralisation titres, as observed in baseline samples assayed in a recent pandemic vaccine trial [15]. Post-immunisation measures of immunity by either assay were more closely concordant [15]. Interpretation of population susceptibility to disease based on these results is made more difficult by the absence of definitive correlates of exposure to or protection against influenza infection. The HI threshold titre of 40 required for seasonal vaccine licensure is based on historical demonstration of 50% protection against experimental infection with partially attenuated challenge strains [26,19]. Household cohort studies suggest that an HI titre of 80 substantially reduces the risk of naturally acquired influenza A(H3N2) infection, with lower titres associated with a modified disease course [27]. Paired serum samples from a limited number of patients (n=10) with known 2009 pandemic influenza showed at least a four-fold rise in HI titres to the pandemic virus between collection times in nine of the patients. Further, all patients who seroconverted had an HI titre of >40 for their second bleed (unpublished

data, K.L. Laurie). Protection from subsequent infection in patients known to have been infected will be of interest as there are no definitive correlates of recent exposure to a novel influenza viral strain.

Baseline reactivity to the pandemic virus was somewhat higher across the age spectrum in our study than previously observed, but similarly low in people aged over 65 years in estimates from a recently published United States (US) study of vaccine trial participants [28], as well as a UK serosurvey [21]. While the influenza-seropositive proportion was higher in a study of elderly people (aged >65 years) in Finland [29], only 5% of the blood donors we studied exceeded 66 years of age; the eldest participant was aged 78 years. Overall rates of pandemic virus seropositivity in the October–November 2009 samples assessed by GMT or defined by an HI threshold titre of 40 were further similar to those observed in a pandemic vaccine study in adults [15]. That trial was conducted in 240 healthy adult participants aged between 18 and 64 years without prior evidence of pandemic virus infection in Adelaide recruited between 22 and 26 July 2009, around the time of the peak of the pandemic in South Australia [15]. Other estimates of disease severity have reported hospitalisation and death rates in relation to inferred symptomatic case presentations, rather than seroconversions, making direct comparisons difficult [30,31]. While reported influenza hospitalisation rates *per capita* were higher in Australia than the US [30], severity in relation to all estimated infections appeared less because of a greater 'exposure' denominator, perhaps suggesting that a large proportion of cases inferred from our study were asymptomatic.

The apparently low rate of population exposure suggested by this serosurvey might be an underestimate of the true attack rate if first exposure to the pandemic virus was poorly immunogenic, resulting in low and/or rapidly declining antibody responses. Poor immunogenicity of the novel virus seems implausible, however, given the robust immune responses to the pandemic virus as a vaccine antigen after only a single 15 g dose in adults [15] and the data from infected patients discussed above. Furthermore, the figure is very similar to that estimated in New Zealand where pandemic influenza had similar characteristics [32]. Alternatively, the effective reproduction number of the virus in adults may have been substantially lower than that observed in children or overall. Such inference was drawn from observations during the intensive case-finding and management phase during the initial weeks of the pandemic response in Victoria, during which time approximately 80% of reported cases were among children [2]. Over this period, the number of secondary cases per case only exceeded one (an essential requirement for epidemic growth) for transmissions between individuals under the age of 20 years, suggesting significant constraint of infectiousness between adults [2]. These findings were consistent with modelling evaluation of the initial outbreak of pandemic influenza A(H1N1) respiratory infection described in La Gloria, Mexico, where children were estimated to be both substantially more susceptible to and infectious with the pandemic virus than adults [5]. Further, a recent analysis of data from the US on within-household transmission of the pandemic virus has demonstrated that children are twice as likely as adults to be infected by an index case in the family [33].

If the true exposure rate in the population was less than 10%, can we explain how the 2009 pandemic influenza stopped? Given the limited application of social distancing measures, restricted to the early 'contain' response [4], and minimal use of antiviral prophylaxis, one possible explanation is to infer partial protection of the population through antecedent exposure to seasonal viruses [34]. T-cell epitopes in the pandemic virus are highly conserved in relation to recently circulating seasonal influenza viruses [35]. There is strong suggestive evidence of a role for broadly cross-reactive cellular immune responses in reducing morbidity and mortality from seasonal and pandemic influenza infection in humans [36]. Accordingly, one study has demonstrated an inverse correlation in humans between the presence of inducible cytotoxic T-cell responses and virus shedding following experimental influenza infection [37], with likely but untested implications for infectiousness. Even partial reduction of infectiousness among adults by these means would have a substantial impact on transmission at a population level, reducing the effective reproduction number below unity and halting an outbreak more rapidly than may be anticipated from measurement of the proportion seropositive by HI assay alone.

This study suggests that exposure to the pandemic virus during the 2009 winter season was relatively uncommon among the healthy Australian adult population, at around 10%. Further evaluation of specimens from children is required in order to assess ongoing susceptibility to the virus in that more vulnerable age group, in whom transmission potential has been clearly demonstrated. Additional plasma collections prior to and following the 2010 influenza season are envisaged, to aid interpretation of relative exposure and severity of H1N1 infections.

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Characteristics of reoffending accommodation sites in Europe with clusters of Legionnaires' disease, 2003–2007

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Between 2003 and 2007, 21% (n=100/477) of accommodation sites linked to clusters of two or more cases of Legionnaires' disease that were investigated by the European Surveillance Scheme for Travel-Associated Legionnaires' Disease (EWGLINET) went on to be associated with at least one further case, despite reporting that satisfactory control measures had been implemented at the time the cluster was first detected. This paper examines these sites (termed reoffenders) in order to determine whether they share any characteristics that may have contributed to the reoffence. All investigations conducted at cluster sites between 2003 and 2007 were included in the analysis, giving a total of 615 investigations conducted at 477 sites. Every country that investigated more than three cluster sites had to deal with at least one reoffence, and one site reoffended five times. The cases involved in the cluster that stayed elsewhere during their incubation periods could be used to help assess the probability of exposure, and therefore the risk, posed by particular cluster sites. A more extensive investigation and control regime may be needed in some instances to better control the risk of Legionnaires' disease at an accommodation site.

Introduction

Legionnaires' disease is an atypical pneumonic illness caused by inhalation of aerosolised water droplets containing *Legionella* spp. bacteria. The disease has an incubation period of two to 10 days and a case fatality rate of approximately 12% [1]. The bacteria live naturally in the aquatic environment, and can cause outbreaks of disease if water systems become colonised. Stagnation, warm temperatures and the presence of nutrients can all lead to increased bacterial growth and replication. Hotels and other public accommodation sites are particularly associated with the risk of Legionnaires' disease because their water systems often include a large number of outlets (such as showers and washbasins). These outlets should all be flushed through at regular intervals to ensure there is no build-up of bacteria in the pipework. However, if a room is left unoccupied the flushing will depend upon routine control and maintenance procedures at the accommodation site, and there is therefore a risk that the water in the system may be allowed to stagnate [2]. In addition, there may often be long lengths of pipework and it can be difficult to ensure that water temperatures are maintained at a high enough level throughout the building to control bacterial numbers.

The European Legionnaires' Disease Surveillance Network (ELDSNet), formerly known as the European Surveillance Scheme for Travel-Associated Legionnaires' Disease (EWGLINET), collects information on cases of Legionnaires' disease in European residents who have stayed at a public accommodation site in the two to 10 days before the onset of symptoms [3]. If two or more cases of Legionnaires' disease are associated with the same accommodation site within two years, a cluster is formed. In response to each cluster, the country of infection is required to conduct an environmental investigation that meets with the standards required by European guidelines for the control and prevention of travel-associated Legionnaires' disease [4]. A risk assessment must be conducted and control measures initiated within two weeks, resulting in a so-called Form A report. Within a further four weeks (six weeks in total) these control measures should have been completed and environmental sampling for *Legionella* spp. carried out, resulting in a second report, a Form B report. If either of these reports is not submitted on time or if the investigations are inadequate, there are sanctions that can be applied; the name of the accommodation site is published on the ELDSNet website (formerly the EWGLI website), often resulting in the withdrawal of tour-operators.

In 2003, 632 travel-associated cases were notified to EWGLINET, and a total of 89 new clusters were identified [5-6]. In comparison, 946 travel-associated cases

were notified to EWGLINET in 2007, and a total of 112 new clusters were identified [7].

Some sites that have been investigated to the standards required in the European guidelines are later associated with further cases. This paper examines these 'reoffending' sites in order to determine whether they share any characteristics that may have contributed to the reoffence.

The European guidelines for the control and prevention of travel-associated Legionnaires' disease were introduced in July 2002 [4]. As the first six months were considered to be an acclimatisation period, this paper addresses accommodation sites with clusters of cases with symptom onset from 2003 to 2007.

Methods

All investigations conducted in accordance with the European guidelines at accommodation sites with clusters of cases between 2003 and 2007 were included in the analysis. Some sites appeared more than once in the dataset, representing either reoffences or the onset of new clusters at the site (if there is a period of more than two years between a cluster and a subsequent case, the case is classified as a single case and the site reverts to a non-cluster status).

Following each investigation, a Form B containing summary information is returned by the country to the ELDSNET coordinating centre in Stockholm, Sweden (formerly the EWGLINET coordinating centre in London, United Kingdom). This form includes information on the sampling results at the accommodation site and the control measures applied. The number of rooms available at each of the sites was found using Internet search engines.

Between 2003 and 2007, 615 investigations were conducted at 477 sites. The dataset of all the sites was linked with that of all the investigations to obtain data on mean length of stay, cluster size and whether travel to other sites occurred. The covariates of interest were the country of the site, year of cluster and any reoffence, type of accommodation and number of rooms at the accommodation site, time between previous investigation and reoffence, length of time the case stayed at the site, results of environmental sampling, and the likelihood of the site being the source of infection (whether the cases involved in the cluster used other sites as well). Variables were considered for inclusion as covariates in a logistic regression model if either the chi-square p value or Fisher's exact test p value (as applicable) was less than 0.10.

Results

A total of 477 accommodation sites in Europe with clusters of cases of Legionnaires' disease were investigated during 2003 to 2007. Of these, 377 (79%) did not reoffend, leaving 100 sites that were associated with subsequent cases within two years of the first investigation. Of the reoffenders, 75 sites reoffended once, 16 reoffended twice (in France (n=3), Greece (n=3), Italy (n=5), Malta (n=2), Turkey (n=5)), six sites reoffended three times (in France (n=3), Italy (n=3), Poland (n=1), Turkey (n=1)), two sites reoffended four times (in Bulgaria and Turkey), and one site reoffended five times (in Turkey). This involved 238 investigations that were conducted at these reoffending sites (100 original investigations and 138 reoffence investigations), giving a total of 615 investigations (Figure).

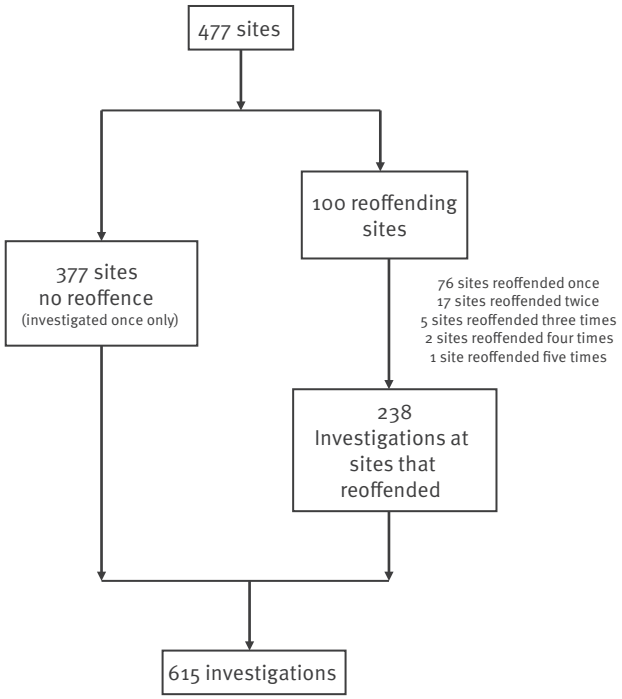
The countries associated with the cluster sites and investigations included in this analysis are shown in Table 1. While Italy conducted the most investigations (n=194), only 24% of these (n=47) were initiated as a response to reoffences. In contrast, 34 of Turkey's 102 investigations (33%) were as a result of reoffences.

The overall percentage of investigations associated with reoffending sites increased over time from 10% in 2003 to 28% in 2007 (Table 2). The proportion of Italian sites reoffending increased in 2006 and 2007, the proportion of French sites reoffending dropped markedly in 2007, while the proportion of Spanish sites reoffending in 2007 rose dramatically. The proportion of Turkish sites reoffending fell in 2006 and 2007, but remained high.

The most common type of accommodation site in the study were hotels (n=393, 88%), however holiday apartments were slightly more likely to reoffend (24% of apartments reoffended compared with 22% of hotels). None of the more unusual types of accommodation

Figure

Flow chart showing number of sites and investigations included in dataset



(classified as 'other', such as ships, university halls of residence and truck stops) reoffended during the study period (Table 3). The accommodation sites most likely to be associated with reoffences were those with 200–299 rooms: 33% (n=18) of these sites reoffended at least once during 2003 to 2007 (Table 3). None of these results were statistically significant.

Of the 615 investigations analysed in this study, 355 (57.7%) gave water samples that tested positive for *Legionella* spp., 245 (39.8%) were negative and 15 had unknown results (2.4%). Most of the investigations with unknown results were conducted towards the beginning of the study, when Form Bs were not always completed as fully as they were towards the end of the study. Of those investigations that had a known sampling result, the proportion that were positive did not change over time (2003: 55.2%, 2004: 58.7%, 2005: 57.6%, 2006: 68.1%, 2007: 54.7%). Of the 355 positive sites, 69 (19.4%) went on to be associated with further cases. In comparison, 66 of the 245 (26.9%) negative sites reoffended, suggesting that sites with negative sampling results are more likely to reoffend than those with positive results (chi-square test value: 4.26, p=0.039). Three of the remaining sites also reoffended (from those that had unknown sampling results).

The median time to reoffence (the time between investigation and onset of a further case of Legionnaires' disease) by country ranged from 65 days (England and Wales) to 408 days (Germany). A total of 32 cases that initiated the reoffence stayed at the accommodation site in question only one night, 62 stayed between two and seven nights, 42 stayed between eight and 14 nights, and two cases stayed for longer.

The most recent sampling result at each site before the reoffence was extracted. (Six sites were excluded from

Table 1

European countries with accommodation sites, including reoffending sites, investigated following clusters of Legionnaires' disease cases, 2003–2007[a]

Country	Accommodation sites			Investigations conducted		
	Total number of sites investigated	Number of reoffending sites	Percentage of reoffenders	Total number of investigations	Number of repeat investigations	Percentage of repeat investigations
Austria	3	1	33	4	1	25
Belgium	1	0	0	1	0	0
Bulgaria	6	2	33	11	5	45
Croatia	1	0	0	1	0	0
Cyprus	2	0	0	2	0	0
Czech Republic	3	1	33	4	1	25
Denmark	1	0	0	1	0	0
England and Wales[b]	11	1	9	12	1	8
France	100	15	15	118	18	15
Germany	12	1	8	13	1	8
Greece	25	5	20	33	8	24
Italy	147	36	24	194	47	24
Latvia	1	1	100	2	1	50
Luxembourg	1	0	0	1	0	0
Malta	10	2	20	14	4	29
Netherlands	3	0	0	3	0	0
Poland	3	1	33	6	3	50
Portugal	7	1	14	8	1	13
Russia	2	0	0	2	0	0
Scotlandb	1	0	0	1	0	0
Spain	59	13	22	72	13	18
Sweden	2	0	0	2	0	0
Turkey	68	20	29	102	34	33
Total	**469**	**100**	**21**	**607**	**138**	**22**
Ship[c]	8	0	0	8	0	0
Total	**477**	**100**	**21**	**615**	**138**	**22**

[a] Accommodation sites in the European Surveillance Scheme for Travel-Associated Legionnaires' Disease (EWGLINET) scheme.

[b] England and Wales (together) and Scotland are independently responsible for the investigation of clusters in their respective countries. Scotland is therefore listed separately in this table.

[c] This category mostly comprises cruise ships, where individuals have slept in cabins onboard.

this portion of the analysis because the results were not available.) Of the 69 sites that previously tested positive, 41 remained positive following the reoffence, while 26 tested negative (two sites had unknown results). Of the 66 sites that previously tested negative, 35 were also negative during the reinvestigation while 30 tested positive (one had unknown results). Of the three sites with unknown results on previous sampling, one tested positive and two tested negative on reoffence (Table 4).

All cases involved in each cluster up to and including the reoffence were analysed to determine if they had also visited other accommodation sites during their 2-10 day incubation period. In 66 of the 138 reoffending sites (excluding six sites as above), the cases had not stayed elsewhere. Of these 66 sites, 52 identified *Legionella* spp. in the water system either during the original investigation or during the reinvestigation (or both). In 41 reoffending sites, the cases involved in the cluster were a mixture of those who had stayed at that site only and those who had also stayed elsewhere. *Legionella* spp. were identified in the water system of 31 of these 41 sites. For the remaining 25 reoffending sites, all of the cases involved had visited other sites during their incubation period. Only 14 of these sites returned positive sampling results (Table 4). These results were not statistically significant.

Discussion

Between 2003 and 2007, 21% (n=100/477) of accommodation sites investigated by EWGLINET and reported as having implemented satisfactory control measures went on to be associated with at least one further case of travel-associated Legionnaires' disease within two years. Every country that investigated more than three cluster sites had to deal with at least one reoffence. In contrast, none of the eight clusters located on ships led to reoffences. It is possible that the more unusual cluster sites may be investigated more thoroughly and therefore are less likely to reoffend.

Table 2

Investigations conducted into European accommodation sites, including reoffending sites, with clusters of Legionnaires' disease cases, by year, 2003–2007[a] (n=615)

Country	Number of investigations (percentage of reoffences)					
	2003	2004	2005	2006	2007	Total
Austria	0	2 (0)	0	1 (0)	1 (100)	4 (25)
Belgium	0	0	0	0	1 (0)	1 (0)
Bulgaria	4 (50)	2 (50)	0	3 (67)	2 (0)	11 (45)
Croatia	0	0	0	1 (0)	0	1 (0)
Cyprus	1 (0)	0	1 (0)	0	0	2 (0)
Czech Republic	0	0	1 (0)	1 (0)	2 (50)	4 (25)
Denmark	0	0	0	1 (0)	0	1 (0)
England and Wales[b]	4 (0)	1 (0)	2 (0)	4 (25)	1 (0)	12 (8)
France	22 (5)	24 (25)	20 (15)	29 (21)	23 (9)	118 (15)
Germany	1 (0)	2 (0)	2 (0)	5 (0)	3 (33)	13 (8)
Greece	7 (0)	4 (50)	10 (20)	8 (50)	4 (0)	33 (24)
Italy	21 (10)	22 (23)	38 (13)	46 (30)	67 (31)	194 (24)
Latvia	0	0	0	1 (0)	1 (100)	2 (50)
Luxembourg	0	0	0	1 (0)	0	1 (0)
Malta	3 (0)	6 (33)	1 (100)	2 (50)	2 (0)	14 (29)
Netherlands	0	1 (0)	0	2 (0)	0	3 (0)
Poland	0	1 (0)	2 (100)	3 (33)	0	6 (50)
Portugal	0	4 (0)	0	0	4 (25)	8 (13)
Russia	0	1 (0)	0	0	1 (0)	2 (0)
Scotlandb	0	0	0	0	1 (0)	1 (0)
Spain	12 (8)	9 (0)	11 (27)	25 (4)	15 (53)	72 (18)
Sweden	0	0	0	2 (0)	0	2 (0)
Turkey	26 (15)	15 (33)	29 (48)	12 (42)	20 (30)	102 (33)
Total	**101 (10)**	**94 (22)**	**117 (25)**	**147 (24)**	**148 (28)**	**607 (23)**
Ship[c]	3 (0)	1 (0)	1 (0)	0	3 (0)	8 (0)
Total	**104 (10)**	**95 (22)**	**118 (25)**	**147 (24)**	**151 (28)**	**615 (23)**

[a] Accommodation sites in the European Surveillance Scheme for Travel-Associated Legionnaires' Disease (EWGLINET) scheme.

[b] England and Wales (together) and Scotland are independently responsible for the investigation of clusters in their respective countries. They are therefore listed separately in this table.

[c] This category mostly comprises cruise ships, where individuals had slept in cabins onboard.

The overall proportion of reoffences increased from 10% in 2003 to 28% in 2007, however it should be noted that this does not necessarily reflect an increase in risk over the study period. The number of reoffenders occurring in the early years of the study may not be comparable to those occurring in the later years, since a site had to be investigated once under the European guidelines (introduced in July 2002) before a re-offence could occur. The number of reoffences occurring in the early years will therefore be artificially low.

There are several reasons why a site might reoffend. The control measures applied might have been inappropriate and/or inadequate, or there may have been a lack of long-term control measures and/or ongoing monitoring after the initial introduction of control measures. Cano et al. studied Spanish hotels and described the persistence of *Legionella* spp. in 29% of their reoffender accommodation sites. They concluded that there had most probably been failures in the action carried out by environmental inspectors at these sites [8]. Some countries do not have strong reference facilities for microbiological testing for *Legionella* spp. and may incorrectly determine that *Legionella* spp. cannot be detected in the water system, or the original sampling may not have been conducted properly. In these instances, negative sampling results may lead public health officials to be less stringent about control measures than they should be. There is some support for this hypothesis in the data: sites with negative sampling results were statistically more likely to reoffend than sites with positive sampling results.

Even when the initial set of control measures have been carried out correctly, the accommodation site may still reoffend if there is a change of staff and the new staff are not correctly trained in these procedures. This was one of the reasons identified for the ongoing problems experienced by a hotel in Turkey [9]. Alternatively, if an accommodation site closes over the winter period, control measures may not be reapplied as rigorously when it reopens. It is also possible that a site may reoffend despite the best efforts of public health teams, as *Legionella* spp. can be very difficult to eradicate from systems. It can become endemic and resist multiple rounds of chlorination and thermal disinfection, or there may be a change in the quality of the incoming water supply to an accommodation site that disrupts the system. Alternatively, the bacteria may hide in dead legs of pipework so that a site can test negative and still have *Legionella* spp. present in the system, which then reseeds the water system.

This analysis shows that, if the cases involved in the cluster have not stayed elsewhere during their incubation period, the likelihood of achieving at least one

Table 3

Characteristics of European accommodation sites, including reoffending sites, investigated following clusters of Legionnaires' disease cases, 2003–2007[a] (n=477)

Characteristic	Total number of sites[b]	Number of reoffenders	Percentage of sites reoffending
Number of rooms			
0–99	230	43	19
100–199	108	25	23
200–299	55	18	33
300–399	22	4	18
400–499	10	1	10
≥500	18	5	28
Not known	34	4	12
Total	**477**	**100**	**21**
Type			
Holiday apartment	25	6	24
Campsite	44	6	14
Hotel	393	88	22
Other[c]	15	0	0
Total	**477**	**100**	**21**

[a] Accommodation sites in the European Surveillance Scheme for Travel-Associated Legionnaires' Disease (EWGLINET) scheme.

[b] Every cluster site included in dataset. Two clusters at same site included twice.

[c] Includes ships, university halls of residence, truck stops, etc.

Table 4

Investigations conducted in reoffending European accommodation sites with clusters of Legionnaires' disease cases, by sampling results, 2003–2007[a] (n=132)

Sampling results (previous result/reoffence result)	All cases went to site only		Some cases went to other sites		All cases went to other sites		Total number of investigations
	n	%	n	%	n	%	
Positive/Positive	23	35	15	37	3	12	41
Positive/Negative	13	20	9	22	4	16	26
Negative/Positive	16	24	7	17	7	28	30
Negative/Negative	14	21	10	24	11	44	35
Total[b]	**66**	**100**	**41**	**100**	**25**	**100**	**132**

[a] Accommodation sites in the European Surveillance Scheme for Travel-Associated Legionnaires' Disease (EWGLINET) scheme,

[b] Does not include three sites with unknown results on previous sampling (one tested positive and two tested negative following reoffence) and three sites that were closed and have not yet been resampled.

positive water sample result from the accommodation site (the original investigation, the reinvestigation, or both) is higher than if all of the cases had also stayed at other sites (although the difference was not statistically significant). This could be a useful proxy for the probability of exposure at a particular cluster site, and could be used by investigators to identify cluster sites that pose a higher than normal risk.

Over 20% of sites reoffending is an unacceptably high proportion and it may be that a more extensive investigation and control regime is needed at reoffending sites. Programmes of continuous monitoring may also need to be introduced in order to better manage the risk associated with these sites.

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Benefit and risks of trivalent 2010 seasonal influenza vaccine in Australian children

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To the editor: In a recent issue of *Eurosurveillance*, Kelly *et al.* conclude that the risks associated with the 2010 trivalent influenza vaccine from CSL Biotherapies outweighed its benefits, based on a comparison of the risk of hospitalisation due to influenza compared with that arising from an adverse reaction [1]. However the analysis is misleading and the authors' conclusions must be considered unsound.

The authors fail to recognise the obvious and significant differences between being hospitalised for overnight observation following a febrile convulsion and being admitted to intensive care units in respiratory failure as a result of influenza infection. A study in the United States conducted in 2006 reported a 15% intensive care units admission rate in children in this age group hospitalised with influenza infection [2].

An Australian study demonstrated that for children hospitalised with influenza 12.3% had pneumonia, 7.4% required intensive care units admission for ventilatory support and 2.5% required inotropes [3]. In Australian children aged less than five years deaths from influenza have been reported at a rate of 0.2 per 100,000 children [4]. By contrast simple febrile seizures have not been shown to increase the risk of death, and although there is a small increase in risk associated with complex febrile seizures, this is very rare even in high-risk children [5,6]. A calculation of risks and benefits using the crude measure of rates of hospitalisation alone as applied by the authors fails to take into account the relevant mortality and morbidity rates associated with these admissions. In addition, it does not account for morbidity of influenza in the broader community.

Though not mentioned in the article, it might be relevant in any analysis of its conclusions to note that the authors were directly involved in the design and oversight of the population-based clinical trial that is the subject of the article (i.e. the administration of influenza vaccine to all children under the age of five years occurring only in Western Australia). The vast majority of adverse events referred to occurred in West Australian children participating in this trial in which influenza vaccination of young children was extended beyond the relevant national recommendations. The authors' observation that *'the benefit-risk profile would be improved if only children who were at increased risk of hospitalisation following influenza infection were targeted for vaccination'* in fact describes the recommendations of the Australian Government's National Immunisation Program, that the use of seasonal vaccine in this age group be limited to those at increased risk of influenza infection. The baseline risk benefit calculation in this population is clearly quite different to the population involved in the Western Australian trial.

Because of the authors' methodological approach conclusions have been made about the risks and benefits of vaccines without consideration of all the relevant factors. However, while the lack of consideration of relevant factors must call into question the validity of the conclusions, the analysis may well give rise to reconsideration within Western Australia of the population-based clinical trial.

Of even greater concern however is the unnecessary and damaging impact simplistic analyses can have on public confidence in childhood immunisation programmes in general and the consequences of immunisation recommendations being ignored out of fear and misinformation.

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Authors' reply: Benefit and risks of trivalent 2010 seasonal influenza vaccine in Australian children

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To the editor: Dr Lopert from Australia's regulatory body for therapeutic goods, the Therapeutics Goods Administration (TGA), raises a number of issues about our quantification of the risk-benefit ratio for seasonal trivalent influenza vaccines administered to children aged six months to four years in Australia in 2010 [1]. While we continue to believe the current data support vaccination of healthy children, it is important to conduct robust post-marketing surveillance, support an open scientific discussion of the observations, and ensure a rapid, comprehensive response to any potential adverse events following immunisation (AEFI).

The authors of the Cochrane review of influenza vaccine effectiveness in children have commented on the relative paucity in the public domain of good quality safety data on influenza vaccines for children aged less than five years [2]. Risk-benefit estimations, such as the approach we have explored, are also uncommon. However, one should not dismiss febrile convulsions as an adverse event. Febrile convulsions would be expected only rarely as demonstrated in a recent large population-based safety study which reported no significantly elevated risk for adverse events (including seizures in children) following administration of more than one million doses of trivalent influenza vaccine to children under the age of 18 years between 2005 and 2008 in the United States [3].

Our rapid communication to *Eurosurveillance* aimed to explore a method to quantify both risk and benefit [4] and was prompted by the TGA status report of 1 July 2010 that describes the investigation of an observed increase in febrile convulsions in young children following receipt of seasonal influenza vaccine in Australia [5] and contains a detailed analysis of risk by the vaccine manufacturer CSL Biotherapies. We did this after governments in New Zealand and Australia had recommended against using the CSL vaccines in children aged less than five years [4] and after the CSL vaccines had been licensed for use in the United States only for children nine years or older [6]. Our results support this decision. Moreover we indicated that our estimate of an unfavourable risk-benefit ratio applied only to one vaccine manufacturer in one year. Generalisation to wider vaccine programmes would have been inappropriate.

Three of the four authors of our article are involved in the Western Australian programme aimed at assessing the public health impact of providing seasonal influenza vaccines to children aged less than five years. We believe that our involvement in promoting a vaccination programme to protect children from influenza, while being prepared to examine both the risks and the benefits of this programme, does not constitute a conflict of interest.

We chose to examine hospital admission for a febrile convulsion within 24 hours of receipt of seasonal influenza vaccine because hospital admission (or prolongation of hospital admission) is one of the four *serious* AEFI identified by the World Health Organization. The other three are death, permanent disability and any event that is life-threatening [7]. We acknowledge that hospital admission for febrile convulsion may be of shorter duration than hospital admission for influenza and that associated morbidity may be different, but suggest it is important not to underestimate the impact of either cause of hospital admission. It is also important to compare outcomes in the current context

Since 2008, for reasons we outlined in our rapid communication, Western Australia has conducted a population-wide vaccination programme aimed at assessing the public health benefits of providing greater access to influenza vaccines for children under five years of age [8,9]. This is not a clinical trial, but a programme using influenza vaccines licensed for use pre-school aged children, evaluated by observational studies. It is consistent with recommendations in the Australian Immunisation Handbook which states: *'Annual influenza vaccination is recommended for any person › 6 months of age who wishes to reduce the likelihood of becoming ill with influenza'* [10]. Universal vaccination of healthy children in this age cohort has been recommended by the Advisory Committee on Immunization Practices in the United States since 2006 [11].

Moreover a study from South Australia supports the need to evaluate a policy of providing influenza vaccine to healthy children, as well as those with known underlying conditions. The study demonstrated that 81% of children aged less than five years admitted to hospital with influenza between 1996 and 2006 had no documented risk factor that increased their risk of a serious outcome following infection [12].

We believe assessing risk and benefit will ultimately improve confidence in vaccine programmes.

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