



Impact
factor **4.659**

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 20 | Weekly issue 7 | 19 February 2015

RAPID COMMUNICATIONS

- Two cases of human cutaneous anthrax in Bosnia and Herzegovina, September 2014** 2
by J Arapović, S Skočibušić, B Jelavić, HB Ivanković, M Jurić, D Mamić, S Grgić, J Lesko, M Leventić,
I Soldo, J Ravlija, J Nikolić

SURVEILLANCE AND OUTBREAK REPORTS

- The impact of a national routine immunisation programme initiated in 1999
on Hepatitis A incidence in Israel, 1993 to 2012** 5
by H Levine, E Kopel, E Anis, N Givon-Lavi, R Dagan

RESEARCH ARTICLES

- Spatial distribution and cluster analysis of a leishmaniasis outbreak in the
south-western Madrid region, Spain, September 2009 to April 2013** 11
by D Gomez-Barroso, Z Herrador, JV San Martín, A Gherasim, M Aguado, A Romero-Maté, L Molina, P
Aparicio, A Benito
- Comparative safety evaluation of 7-valent and 13-valent pneumococcal vaccines in
routine paediatric vaccinations in four Italian regions, 2009 to 2011** 21
by F Trotta, C Rizzo, C Santuccio, A Bella, the Pharmacovigilance Study Group on Pneumococcal
Vaccination in Children

REVIEW ARTICLES

- Assessment of human influenza pandemic scenarios in Europe** 29
by C Napoli, M Fabiani, C Rizzo, M Barral, J Oxford, JM Cohen, L Niddam, P Goryński, A Pistol, C Lionis, S
Briand, A Nicoll, P Penttinen, C Gauci, A Bounekkar, S Bonnevey, A Beresniak

Two cases of human cutaneous anthrax in Bosnia and Herzegovina, September 2014

J Arapović (jarap@sve-mo.ba)^{1,2}, S Skočibušić^{1,2}, B Jelavić^{2,3}, H B Ivanković^{1,2}, M Jurić^{2,3}, D Mamić⁴, S Grgić^{1,2}, J Lesko^{2,3}, M Leventić^{2,3}, I Soldo^{2,5}, J Ravlija^{4,6}, J Nikolić^{1,2}

1. Department of Infectious Diseases, University Hospital Mostar, Mostar, Bosnia and Herzegovina

2. School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

3. Department of Otorhinolaryngology and Maxillofacial Surgery, University Hospital Mostar, Mostar, Bosnia and Herzegovina

4. Department of Epidemiology and Public Health, Public Health Institute of Livno, Livno, Bosnia and Herzegovina

5. Department of Radiology, University Hospital Mostar, Mostar, Bosnia and Herzegovina

6. Department of Epidemiology, Public Health Institute of Federation of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina

Citation style for this article:

Arapović J, Skočibušić S, Jelavić B, Ivanković HB, Jurić M, Mamić D, Grgić S, Lesko J, Leventić M, Soldo I, Ravlija J, Nikolić J. Two cases of human cutaneous anthrax in Bosnia and Herzegovina, September 2014. Euro Surveill. 2015;20(7):pii=21039. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21039>

Article submitted on 25 January 2015 / published on 19 February 2015

Two cases of human cutaneous anthrax were reported in September 2014 in south-western Bosnia and Herzegovina. The two men were involved in slaughtering a cow and handled its *Bacillus anthracis*-infected meat. Anthrax has been sporadically observed in livestock in Bosnia and Herzegovina, but no confirmed human cases had been reported in the country in the last two decades. Clinicians in the country should be aware that anthrax may occur in humans, arising from exposure to infected animals.

Case report

Two men from a rural community in south-western Bosnia and Herzegovina presented with cutaneous anthrax in September 2014 after slaughtering a cow and handling its meat, which was later found to be infected by *Bacillus anthracis*, the causative agent of anthrax.

Case 1

The man, in his early thirties, had been involved in the cow's slaughter and handling of its meat at the end of August 2014. During the meat handling, his right infraorbital region was struck by a bone of the cow, which resulted in a small superficial skin lesion and crusting. Due to swelling and redness of the right orbital and infraorbital region, he was examined by an otorhinolaryngologist and ophthalmologist at the regional general hospital. Despite being prescribed oral azithromycin 500 mg daily and corticosteroids, there was a progression of periorbital cellulitis and he was admitted to the same hospital in early September 2014. His treatment included azithromycin, local antibiotic and corticosteroid therapy for his eye and antihistamines. The day following admission, due to suspicion of rhinosinusitis complications, he was admitted to the Department of Otorhinolaryngology and Maxillofacial Surgery at the University Hospital in Mostar. He felt no

pain in his right eye and had no fever. Serum concentration of C-reactive protein (CRP) was increased (68.3 mg/L; norm: 0–5 mg/L), while the erythrocyte sedimentation rate, and levels of leukocytes, erythrocytes, haemoglobin, platelets, liver transaminases were all within the normal ranges. The results of urine analysis were also normal. Computed tomography scans showed healthy maxillary and ethmoid sinuses, and diffuse soft tissue oedema of the right-hand side of his face.

In the following days, due to the extent and spread of the swelling to the left orbital region, the patient reported intense pain. In order to decrease skin tension and facial pain, an incision in the right upper buccal vestibule was made and the pain was relieved successfully. Swab cultures of the wound and right eye tested negative for the presence of aerobic bacteria. However, a characteristic malignant pustule (dark crust) appeared in the affected infraorbital region.

Given the presence of the pustule, cutaneous anthrax was suspected and the patient was admitted to the Department of Infectious Diseases at the University Hospital in Mostar, and the local Department of Epidemiology and Public Health was informed about the case. Epidemiological investigation and analysis of frozen meat from the cow (by bacteriological cultivation) confirmed the presence of *B. anthracis* on 21 September 2014.

The patient was initially treated with an empirically combined antimicrobial therapy (ceftriaxone, 2 g once daily, and metronidazole, 500 mg three times daily, intravenously) for periorbital and facial cellulitis. While still in the Department of Infectious Diseases, the antimicrobial therapy was modified, with administration of penicillin G, 4 million international units (IU) four times

intravenously daily for the next 10 days. The patient also received local ophthalmic antimicrobial therapy (tobramycin solution). Clinical recovery was followed by regression of local swelling and peeling of the dark crust and normalisation of CRP levels.

Case 2

A man in his early fifties, a relative of Case 1, who had also been involved in slaughtering the same cow and handling its meat, presented to a primary care practice in September 2014 with a wound on the volar side of his right forearm. The wound was about 3 cm in diameter and covered with a black crust. The area was slightly oedematous. At the regional general hospital, he was diagnosed as having allergic contact dermatitis and was initially treated with corticosteroids, then with amoxicillin/clavulanate potassium (800/200 mg) orally two times daily for seven days, and finally with corticosteroids and cefuroxime axetil 500 mg orally two times daily. As there had been no clinical improvement, and as it was known he had also been involved in the slaughter of the infected cow, he was admitted to the Department of Infectious Diseases, University Hospital in Mostar, towards the end of September 2014, shortly after *B. anthracis* had been detected in the cow's meat. Cutaneous anthrax was suspected, given his symptoms and following an epidemiological investigation. He was successfully treated with ciprofloxacin 500 mg orally two times daily for 14 days.

Background

B. anthracis is a sporulating Gram-positive bacterium. The main routes of human exposure to spores are by inhalation, ingestion, contact with skin and injection of contaminated drugs [1,2]. In most patients (95%), the disease manifests as cutaneous anthrax, whereas the remaining 5% of cases present with inhalational or gastrointestinal syndromes [3]. Of these two forms, the most severe is inhalational anthrax, which is usually fatal if left untreated. In such cases, often acts of bioterrorism are suspected [1-4]. Cutaneous anthrax on the other hand generally develops after direct contact with infected animals or animal products. Symptoms typically appear up to 17 days after contact [5], but in an experimental model, the incubation time can be up to 58 days [6]. Thus, cases of cutaneous anthrax are reported as an occupational disease, which mostly occurs in farmers, butchers, dealers of hides and animal hair, wool sorters and veterinarians [4]. Characteristic of cutaneous anthrax is the appearance of a malignant pustule surrounded by oedema at the infection site [7]. Due to the use of antibiotics, the mortality rate is typically under 1%, but if left untreated the fatality rate can reach 20% [2,7].

Anthrax has been used in bioterrorism. The only documented terrorist attack with anthrax spores was in 1993 by the Aum Shinrikyo cult in Japan; there were no cases [8]. During deliberate release of weaponised anthrax spores in 2001 in the United States, 28 people tested

positive for *B. anthracis* in nasal swabs, 22 became ill and five people died [1,6].

Sporadic anthrax in livestock was last reported in Bosnia and Herzegovina in 2010 [9-11], but no confirmed cases of cutaneous anthrax in humans had been reported in this country in the last two decades. In 2011, sporadic human cutaneous anthrax cases due to contact with infected livestock were reported in Serbia [12], which borders with Bosnia and Herzegovina, and in Romania [13]. Sporadic livestock anthrax cases have also been reported in the past 25 years in areas of Croatia bordering Bosnia and Herzegovina [9,14,15].

The two cases reported here should be considered as an alert to the medical community in Bosnia and Herzegovina to the possible appearance of anthrax in humans arising from exposure to infected animals.

Discussion

The last reported case of anthrax in humans in Bosnia and Herzegovina was in 1992, a woman from the rural south-western part of the country, who was hospitalised and treated for cutaneous anthrax in Split, Croatia [16]. A possible human anthrax case in Bosnia and Herzegovina – a person from neighbouring Serbia – was reported in 2009 in Bosnia and Herzegovina's epidemiological bulletin [17], but the infection was not confirmed by laboratory analysis or epidemiological investigation (unpublished data).

Floods in Bosnia and Herzegovina and neighbouring countries in May 2014 could wash and mobilise *B. anthracis* spores from deeper layers in the soil. In addition to classical infection by contact with bacteria or spores, *B. anthracis* might also be transmitted by insects [16,18], which are especially active in warmer (summer) days, although the epidemiological impact of biting and/or non-biting insects in anthrax infection is not yet fully understood.

The structure of the epidemiological and veterinary services in Bosnia and Herzegovina should also be borne in mind regarding anthrax control. One of the two political entities in the country is the Federation of Bosnia and Herzegovina, which is divided into 10 independently structured counties. Every county has its own department of public health and veterinary department, leading to challenging control, coordination and prevention of infectious diseases through appropriate vaccination of livestock.

As described by Kracalik et al. for Georgia [19,20], vaccination of livestock and education of cattle workers could be the basis of an effective strategy for the prevention of anthrax also in Bosnia and Herzegovina and thus for the avoidance of anthrax spread. Thus, a long-term vaccination programme in livestock coordinated by the government would be helpful as an important measure to prevent the spread of anthrax in Bosnia and Herzegovina.

The human cases reported here should serve as a reminder to healthcare personnel in Bosnia and Herzegovina to be vigilant.

Acknowledgements

The authors are grateful to all doctors and nurses at the University Hospital Mostar who helped in the handling and treatment of the cutaneous anthrax patients. Also, we thank Dr Maja Arapović and Dr Luka Traven for critical reading of the manuscript.

Conflicts of interest

None declared.

Authors' contributions

JA coordinated all correspondence, designed and wrote the first version of the manuscript. SS and BJ contributed to the conception and writing of the first version of manuscript. JA, SS, JN, HBI, SG, MJ, BJ, JL, IS, JR and ML contributed to clinical analysis. HBI contributed to the data interpretation and handled patients' approval for publication of personal data. JL, BJ and IS contributed in performing clinical data and interpretation of data. JR, JN and DM conducted all epidemiological measures on the spot of the anthrax outbreak. DM provided the analysis of anthrax. All authors contributed in the writing of the final version of manuscript.

References

- Swartz MN. Recognition and management of anthrax--an update. *N Engl J Med*. 2001;345(22):1621-6. <http://dx.doi.org/10.1056/NEJMra012892> PMID:11704686
- Sweeney DA, Hicks CW, Cui X, Li Y, Eichacker PQ. Anthrax infection. *Am J Respir Crit Care Med*. 2011;184(12):1333-41. <http://dx.doi.org/10.1164/rccm.201102-0209CI> PMID:21852539
- Dixon TC, Meselson M, Guillemin J, Hanna PC. Anthrax. *N Engl J Med*. 1999;341(11):815-26. <http://dx.doi.org/10.1056/NEJM199909093411107> PMID:10477781
- Schmid G, Kaufmann A. Anthrax in Europe: its epidemiology, clinical characteristics, and role in bioterrorism. *Clin Microbiol Infect*. 2002;8(8):479-88. <http://dx.doi.org/10.1046/j.1469-0691.2002.00500.x>
- Doganay M, Metan G, Alp E. A review of cutaneous anthrax and its outcome. *J Infect Public Health*. 2010;3(3):98-105. <http://dx.doi.org/10.1016/j.jiph.2010.07.004> PMID:20869669
- Wright JG, Quinn CP, Shadomy S, Messonnier N; Centers for Disease Control and Prevention (CDC). Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep*. 2010;59(RR-6):1-30. PMID: 20651644
- Wenner KA, Kenner JR. Anthrax. *Dermatol Clin*. 2004;22(3):247-56, v. <http://dx.doi.org/10.1016/j.det.2004.03.001> PMID: 15207306
- Keim P, Smith KL, Keys C, Takahashi H, Kurata T, Kaufmann A. Molecular investigation of the Aum Shinrikyo anthrax release in Kameido, Japan. *J Clin Microbiol*. 2001;39(12):4566-7.
- Steiner I, Račić I, Spičić S, Habrun B. Genotyping of *Bacillus anthracis* isolated from Croatia and Bosnia and Herzegovina. *Zoonoses Public Health*. 2013;60(3):202-8. <http://dx.doi.org/10.1111/j.1863-2378.2012.01513.x> PMID:22726272
- Hukic M, Numanovic F, Sisirak M, Moro A, Dervovic E, Jakovec S, et al. Surveillance of wildlife zoonotic diseases in the Balkans Region. *Med Glas (Zenica)*. 2010;7(2):96-105.
- FluTrackers.com. Bosnia and Herzegovina - Again anthrax confirmed in cows. Winter Park, FL: FluTrackers.com, Inc. Posted 26 Aug 2010. [Accessed 12 Feb 2015]. Available from: <https://flutrackers.com/forum/forum/emerging-diseases-other-health-threats-alphabetical-a-thru-h/anthrax-aa/g2288-bosnia-and-herzegovina-again-anthrax-confirmed-in-cows>

- Durić P, Cosić G, Rajčević S, Petrovic V, Tomković M, Subić Z, et al. Three probable cases of cutaneous anthrax in autonomous province of Vojvodina, Serbia, June 2011. *Euro Surveill*. 2012;17(1). pii: 20050. 20050. PMID: 22264812
- Popescu R, Pistol A, Miltaru L, Caplan D, Cucuiu R, Popovici F. Two cases of infection with *Bacillus anthracis*, Romania, October 2011. *Euro Surveill*. 2011;16(45). pii: 20008. PMID: 22114977
- Markotić A, Krajinović LC, Margaletić J, Turk N, Miletić-Medved M, Zmak L, et al. Zoonoses and vector-borne diseases in Croatia - a multidisciplinary approach. *Vet Ital*. 2009;45(1):55-66. PMID:20391390
- Habrun B, Račić I, Kompes G, Spičić S, BeniĆ M, Mihaljević Z, et al. The antimicrobial susceptibility and virulence factors of *Bacillus anthracis* strains isolated in Croatia. *Vet Med (Praha)*. 2011;56(1):22-7. <http://vri.cz/docs/vetmed/56-1-22.pdf>
- Bradarić N, Punda-Polić V. Cutaneous anthrax due to penicillin-resistant *Bacillus anthracis* transmitted by an insect bite. *Lancet*. 1992;340(8814):306-7. [http://dx.doi.org/10.1016/0140-6736\(92\)92395-V](http://dx.doi.org/10.1016/0140-6736(92)92395-V) PMID:1353216
- Epidemiološka situacija na području Federacije Bosne i Hercegovine U 2010. [The epidemiological situation in the Federation of Bosnia and Herzegovina in 2010]. *Epidemiological bulletin* 18(29). Sarajevo/Mostar: Institute of Public Health FB&H; 2011. Croatian. p. 22,34-5. Available from: <http://www.zzzfbih.ba/wp-content/uploads/2010/02/Epidemioloski-bilten-29-god-2010.pdf>
- Bradarić N, Vilić N. (Epidemiologic and clinical characteristics of anthrax in patients at the University Hospital in Split 1956-1987]. *Lijec Vjesn*. 1992;114(5-8):122-6. Croatian. PMID:1343041
- Kracalik I, Malania L, Tsertsvadze N, Manvelyan J, Bakanidze L, Imnadze P, et al. Human cutaneous anthrax, Georgia 2010-2012. *Emerg Infect Dis*. 2014;20(2):261-4. <http://dx.doi.org/10.3201/eid2002.130522> PMID:24447721
- Kracalik IT, Malania L, Tsertsvadze N, Manvelyan J, Bakanidze L, Imnadze P, et al. Evidence of local persistence of human anthrax in the country of Georgia associated with environmental and anthropogenic factors. *PLoS Negl Trop Dis*. 2013;7(9):e2388. <http://dx.doi.org/10.1371/journal.pntd.0002388> PMID:24040426

The impact of a national routine immunisation programme initiated in 1999 on Hepatitis A incidence in Israel, 1993 to 2012

H Levine (hlevine@hadassah.org.il)¹, E Kopel², E Anis^{1,2}, N Givon-Lavi³, R Dagan³

1. Braun School of Public Health and Community Medicine, Hebrew University-Hadassah, Jerusalem, Israel

2. Division of Epidemiology, Ministry of Health, Jerusalem, Israel

3. Pediatric Infectious Disease Unit, Soroka University Medical Center and the Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Citation style for this article:

Levine H, Kopel E, Anis E, Givon-Lavi N, Dagan R. The impact of a national routine immunisation programme initiated in 1999 on Hepatitis A incidence in Israel, 1993 to 2012. *Euro Surveill.* 2015;20(7):pii=21040. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21040>

Article submitted on 04 March 2014 / published on 19 February 2015

Data on long-term impact of universal national vaccination programmes against hepatitis A are lacking. We aimed at evaluating the impact on hepatitis A incidence of the Israeli toddlers-only universal routine two-dose vaccination programme against hepatitis A initiated in 1999. All hepatitis A episodes reported to the national surveillance system from 1993 to 2012 were analysed in relation to the vaccination programme and coverage. Mean vaccine coverage in Israel between 2003 and 2010 was 92% for the first dose, given at 18 months of age, and 88% for the second dose, given at 24 months. The annual hepatitis A incidence declined from a mean of 50.4 per 100,000 in the period between 1993 and 1998 to a mean of 1.0, during the period from 2008 to 2012, representing a reduction of 98%. The decline was evident in all ages and ethnicity groups, including unvaccinated populations. Of the 1,247 cases reported nationwide between 2002 and 2012, the vaccination status could be ascertained in 1,108 (89%). Among them, only 20 (2%) were reported be vaccinated with one dose and three (1%) received two doses. The sustained results of this long-term impact study suggest that a toddlers-only universal routine two-dose vaccination programme is highly effective and practical. These findings underscore the importance of sustainability in both the surveillance systems and vaccination programmes and will aid to determine vaccination policies.

Introduction

In many low-income countries, hepatitis A virus infects more than 80% of the population by late adolescence [1]. The infection is also common in middle and high-income countries [1].

The disease caused by hepatitis A virus is usually more severe with increasing age. Hepatitis A virus is mostly transmitted from person to person by the faecal–oral route, however, common source outbreaks related to contaminated water or food occur. High risk groups

include persons with chronic liver disease, men who have sex with men, people who inject drugs, travellers to countries where hepatitis A is endemic and children in communities with consistently elevated rates of hepatitis A infections [2].

The long-term impact after the introduction of new immunisation programmes is dependent not only on the vaccine itself, but on vaccination coverage and its sustainability as well. Hepatitis A vaccine is highly effective when given in two doses, six to 12 months apart [3]. Furthermore, in Argentina, the recent introduction of a single dose of hepatitis A vaccine in the universal immunisation plan to 12 months-old children resulted in a profound impact on disease in all ages with a magnitude comparable to that of a two-dose schedule within a short time [4]. No data on long-term population impact of hepatitis A national immunisation plan (NIP) exist, not enabling long-term definitive predictions [5]. The 2012 World Health Organization (WHO) position paper on hepatitis A vaccines stated that following its introduction, the assessment of hepatitis A vaccine impact is important, using information on morbidity generated by surveillance and study data [6].

Until 1999, Israel was considered a country with intermediate hepatitis A endemicity [7]. Between 1993 and 1998 incidence rates were 30 to 70 per 100,000 population, with higher rates in the non-Jewish population [8]. The first hepatitis A vaccine licensed in Israel was in 1996 [8]. Initially, vaccines were used sporadically, except for targeted vaccination in the Israeli military that started in 1997 [9]. In July 1999 however, Israel was the first country to introduce hepatitis A vaccine to its NIP as a two-dose schedule, at ages 18 and 24 months, with no catch-up campaign. We previously reported the early impact of the programme, five and a half years after its introduction [8]. In brief, the annual incidence declined by 95% or more when comparing the period from 2002 to 2004 to that between 1993 and 1998.

The decline was most prominent in the vaccinated age group (1–4 years), but was remarkable in all age groups, demonstrating herd protection. Other studies, based on records of a large health maintenance organisation in Israel, showed a reduction of 88% in hepatitis A incidence from 1998 to 2004 and of 95% from 1998 to 2007 [10,11].

We report here on the long-term (14 years) impact of the toddlers-only universal hepatitis A two-dose vaccination programme initiated in 1999 on hepatitis A incidence in all ages in Israel.

Methods

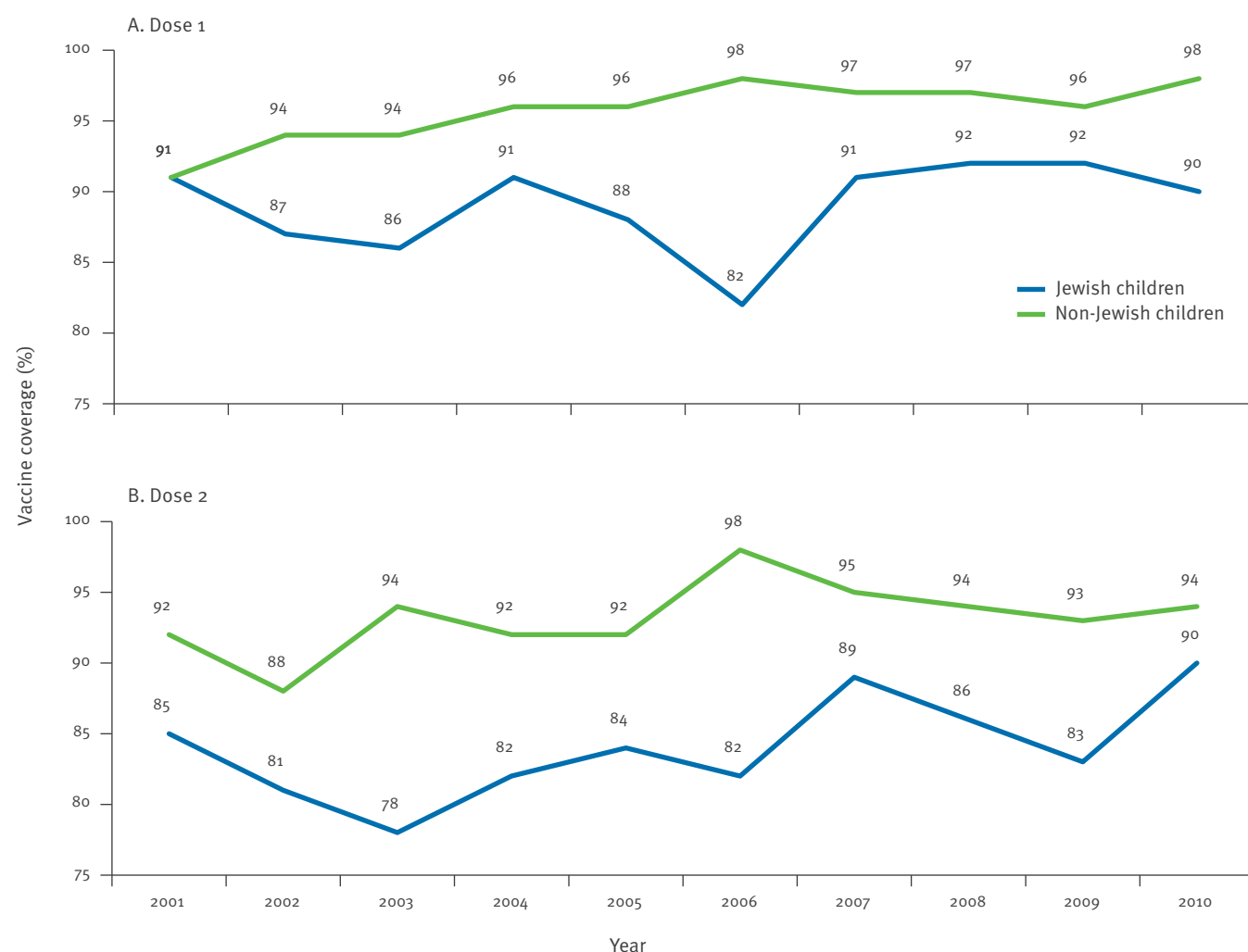
Acute infectious hepatitis has been notifiable by law since 1950 in Israel and hepatitis A cases have been reported separately from other infectious hepatitis cases since 1993 [8]. The Division of Epidemiology at the Ministry of Health collects and reviews reports

from all districts and collates data weekly and annually. While there is no official criteria for the diagnosis of hepatitis A disease, reports of cases will usually be discarded unless there is a positive laboratory test result for anti-hepatitis A virus IgM antibodies or epidemiologic linkage with a previous serologically-confirmed case [8]. The passive surveillance system and diagnosis methods remained unchanged during the time between 1993 and 2012. For the present study, reports of hepatitis A from 1 January 1993, through 31 December 2012 were reviewed.

Data on total population size, as well as age-specific and ethnicity-specific populations, were taken annually from the Israel Central Bureau of Statistics reports for the appropriate years. Age was divided into six groups (<1 year, 1–4, 5–9, 10–14, 15–44, ≥45). Due to differences by ethnicity in socio-demographics and in hepatitis A incidence dynamics before the introduction of the vaccine, ethnicity was classified based on being

FIGURE 1

Hepatitis A virus vaccine coverage among Jewish and non-Jewish populations, Israel, 2001–2010



A. First dose coverage, planned at 18 months of age. The data are from two year-old children.

B. Second dose coverage, planned at 24 months of age. The data are from three year-old children.

from Jewish (ca 80%) or non-Jewish populations [8]. The non-Jewish population includes mostly the Moslem Arab population. Overall and age/ethnicity specific annual incidence rates per 100,000 population were calculated.

Data on vaccination coverage of first and second doses by year, district and ethnicity were calculated based on reports provided to the Division of Epidemiology from all 15 public health districts in Israel, as previously reported [8]. In Israel, ca 95% of all routine immunisations are given free of charge in public sector mother-child health centres. Immunisation coverage rates are based on doses of specific vaccines given in these centres per number of newborns residing in each of the 15 public health districts. In eight districts with a large number of annual births, a systematic 16.7% sample of newborns (i.e. those born every sixth calendar day) is selected for calculation of coverage. The vaccination coverage for the first hepatitis A vaccine dose is calculated at the age of two years and for the second dose at the age of three years.

Results

Vaccine uptake

Among the Jewish population, the mean annual vaccine coverage between 2003 and 2010 for the first hepatitis A vaccine dose was 89% (range: 82–92%) and for the second dose, 84% (range: 78–90%) (Figure 1). The respective figures among the non-Jewish population were 97% (range: 94–98%) and 94% (range: 92–98%). The coverage of both first and second hepatitis A vaccine doses between 2003 and 2010 was comparable

to that in the period from 2001 to 2002 for the Jewish population and higher than that of 2001 to 2002, for the non-Jewish population. The overall mean annual vaccine coverage for 2003 to 2010 was 92% for the first dose and 88% for the second dose, compared with 90% and 85% in the period from 2001 to 2002. Vaccination coverage differed by districts and years, with pockets of low coverage, most notably in the Tel Aviv district. In this district, inhabited mainly by a Jewish population, vaccination coverage for the second dose ranged between 56% and 79% during 2003 to 2010.

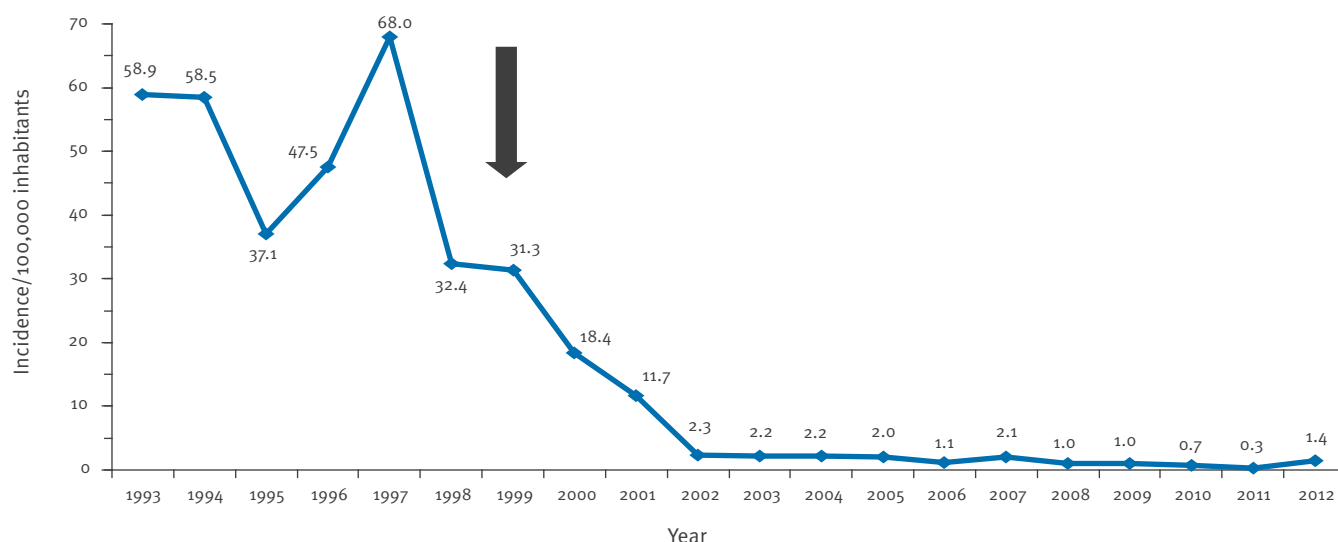
Hepatitis A incidence

The overall pre-vaccination hepatitis A incidence in the years from 1993 to 1998 was 50.4 per 100,000. Shortly after the initiation of the programme in July 1999, a sharp decline in incidence occurred within the following three years reaching 2.3 or less per 100,000 after 2002 [8] (Figure 2). From 2008 to 2012, the rates remained stable with a mean of <1.0 per 100,000. This represents a persistent reduction of >98% compared with the pre-vaccine period ($p < 0.001$; chi-squared test). The incidence decline in hepatitis A morbidity was evident in all age groups and in both Jewish and non-Jewish ethnic groups (Figure 3).

Furthermore, during the period from 2003 to 2011, a reduction in the occurrence of large outbreaks occurred. No outbreaks comprising five cases or more were reported during this entire period (data not shown). Between March 2012 and February 2013, one large outbreak was reported in the Tel Aviv district, with 80 cases in total, mainly young male adults. An urban cluster of people who inject drugs and homeless men,

FIGURE 2

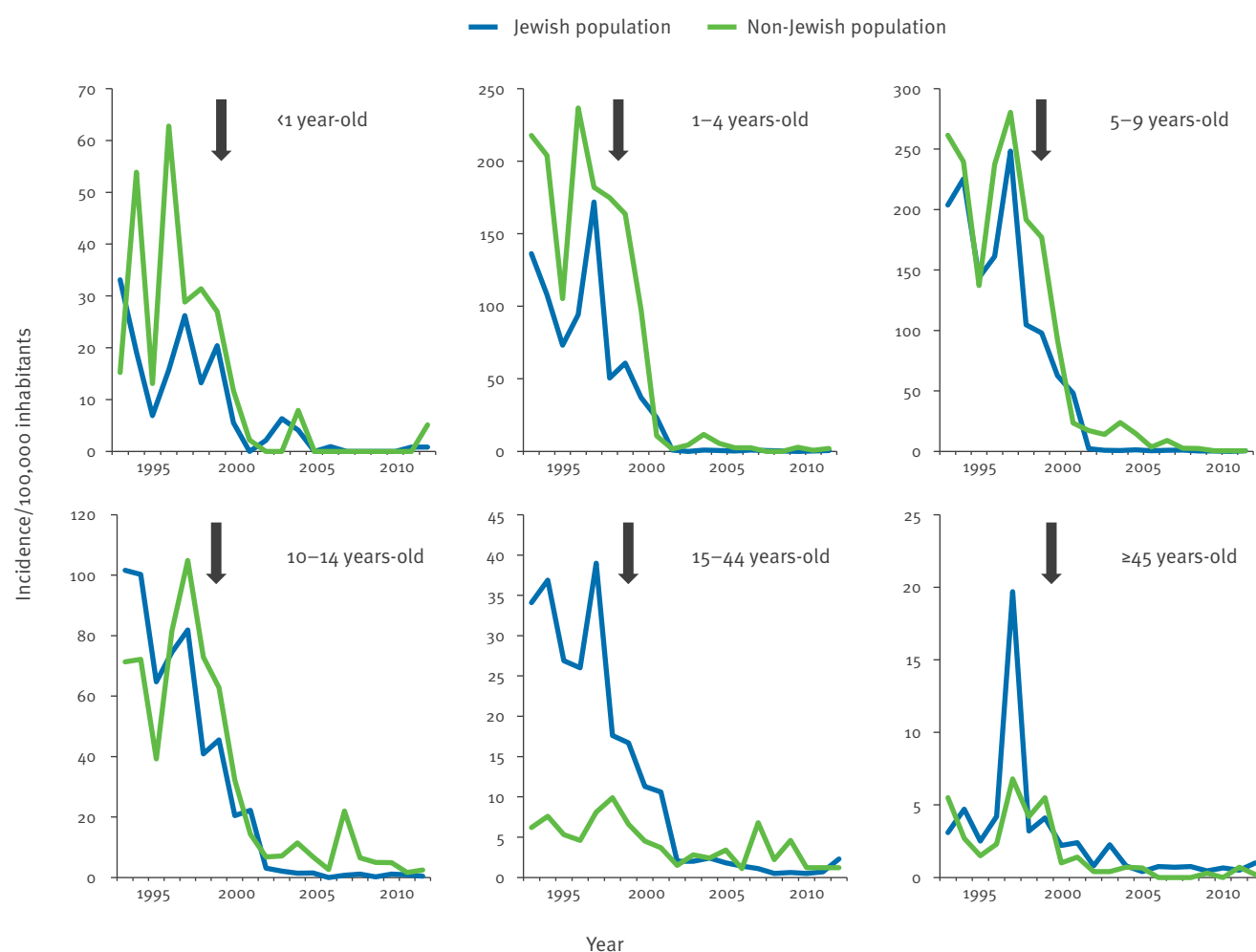
Annual incidence rates of hepatitis A per 100,000 population, Israel, 1993–2012



The start of the routine vaccination programme is marked by an arrow.

FIGURE 3

Annual incidence rates of hepatitis A per 100,000 population, by specific age and ethnic groups, Israel, 1993–2012



In each panel, the start of the routine vaccination programme is marked by an arrow.

which comprised ca 15% of the cases including the index case, was identified (data not shown). Of the 1,247 cases reported nationwide between 2002 and 2012, vaccination status could be ascertained in 1,108 (88.9%). Of these, 1,085 (98%) were not vaccinated, and 20 (2%) had received one dose. Only three cases (<1%) were reported to be vaccinated with two doses before onset of illness. The latter three cases were adults, with questionable verification of vaccination status in two cases, and immunosuppression in the third one [12].

Discussion

We show sustained success of the Israeli NIP in almost complete elimination of hepatitis A morbidity and transmission. Our findings are of universal importance since Israel was the first country to include hepatitis A vaccine into the NIP in 1999. The differences in hepatitis A incidence by ethnicity prior to the NIP were eliminated. The sustained reduction was evident in all

ethnicity groups, thus showing the potential of a vaccination programme to reduce health disparities, as was shown in Arizona, United States [13].

Sporadic cases occurred, mostly in high-risk individuals and most commonly among travellers to endemic areas outside Israel [14].

Further support for this reported reduction stems from the elimination of hepatitis A outbreaks in school children during the study period, from a range of eight to 48 outbreaks in the southern district between 1993 and 1998 to zero outbreaks in the period from 2001 to 2005 [15]. Only one outbreak with more than five cases ($n=80$) occurred during the entire period, which occurred mainly among young adults (in the county with the lowest vaccination rate).

The vast majority of cases in our study had not received hepatitis A vaccinations in the past, while hepatitis A

cases among those vaccinated with one dose were rare, pointing to the high protection given by the vaccine on the individual level. Such cases highlight the need to ensure a full vaccination schedule among individuals susceptible to both hepatitis A exposure and vaccination failure [12]. Recent reports suggest memory and persistence of immunity even after one hepatitis A vaccine dose in adults [16-18] and high short-term effectiveness after one dose only when given to children at one year of age, as part of the NIP [4].

Additional support to the decline observed in this study, is a similar decline in hepatitis A incidence in Israel Defence Forces soldiers (individuals >18 years of age), based on the military surveillance system, as well as a decline in the proportion of hepatitis A IgG seropositive recruits with time [9]. Furthermore, the virtual elimination of hepatitis A IgG seropositivity rates in 18 month-old toddlers (pre-vaccination age) living in a previously hyperendemic area after introduction of hepatitis A NIP (from 16.2 to 19.6% in 1991 through 2000 to 0% in 2003 through 2007) suggests that the virus circulation in the community is close to being eliminated [19].

The rapid decline and sustained very low incidence following the introduction of toddlers-only hepatitis A NIP in Israel, strongly supports the claim that the decline is due to the vaccination programme. Our findings are in line with similar observations in other countries with diverse and heterogeneous epidemiology, including Argentina, Belarus, China, Italy and Spain, where the implementation of routine vaccination of children in one or more age cohorts in all or part of the country was followed by immediate and extensive overall declines in hepatitis A incidence [1].

Other factors, such as improved hygiene or socio-demographic changes, cyclic trends, and vaccination beyond the NIP, should be considered as possible additional explanations to the declining incidence. However, the immediate and rapid effect post hepatitis A vaccine introduction speaks for only a small, if at all, role of these factors in the events. Cyclic pattern of disease incidence with peaks every five to 10 years has been noted in some low-income countries with temperate climates [1]. In our case, the long-term 14 years follow-up, makes cyclic trend a very unlikely explanation for this decline. Some individuals were immunised beyond the NIP due to occupational, travel, medical or other reasons. However, the limited scope of this immunisation points against significant contribution to the decline.

The overall benefit to society of the near complete elimination following introduction and maintenance of NIP is extensive. Previous cost-benefit model predicting hepatitis A NIP in Israel during the 1997 to 2014 period showed a societal benefit:cost ratio of 2.54:1 [20]. However, the real benefit was higher than

predicted, due to herd protection effect in unvaccinated individuals.

Our study was limited by the passive surveillance system. However, as our surveillance system, reporting and diagnosis methods were mainly unchanged between 1993 and 2012, our data provide strong evidence for the continuous success of the vaccination programme. This was validated in the past with active surveillance [8]. Our vaccination coverage estimation is limited and might be somewhat biased. As the denominator for vaccination coverage estimation is based on births, a number of children residing in Israel and not reported to the Ministry of Health (such as children of immigrants), may have lower vaccination coverage, leading to biased over estimation of vaccination coverage. However, these represent a small minority. Furthermore, since medical service is universal and provided free of charge, we expect similar vaccination coverage in these populations.

A major strength of the current study lies in it being a long-term (20 years) prospective surveillance of hepatitis A incidence, including the six years before initiation of the NIP. An additional strength of the study is its nationwide population-based nature, covering the entire Israeli population, and including age and ethnicity sub-group analyses.

In conclusion, the results of this long-term impact study document that the toddlers-only universal routine two-dose vaccination programme is highly effective, and resulted in the sustained near elimination of hepatitis A in Israel.

Conflicts of interest

None declared.

Authors' contributions

All the authors have carefully read the manuscript, provided constructive remarks and approved the final version of the submitted manuscript. Hagai Levine: Manuscript concept and design; drafting of the manuscript; Eran Kopel: Vaccine coverage data; Emilia Anis: Supervised the data collection of cases and vaccination by the Ministry of Health; Noga Givon-Lavi: Epidemiology and statistics consultation; Ron Dagan: Led the hepatitis A surveillance project; manuscript concept and design; critical revision of the manuscript for important intellectual content.

References

1. Murphy TV, Feinstone SM, Bell BP. Hepatitis A vaccines. In: Vaccines. 6th edition. Elsevier; 2012: p. 183.
2. Heymann DL, editor. Viral hepatitis A. In: Control of communicable diseases manual. 19th edition. Washington DC: American Public Health Association; 2008: p. 278-284.
3. Furesz J, Scheifele DW, Palkonyay L. Safety and effectiveness of the new inactivated hepatitis A virus vaccine. CMAJ. 1995;152(3):343-8. PMID:7828098
4. Vizzotti C, González J, Gentile A, Rearte A, Ramonet M, Cañero-Velasco MC, et al. Impact of the single-dose immunization strategy against hepatitis A in Argentina.

- Pediatr Infect Dis J. 2014;33(1):84-8. <http://dx.doi.org/10.1097/INF.000000000000042> PMID:24352191
5. Fiore A, Feinstone S, Bell B. Hepatitis A vaccines. In: Plotkin SA, Orenstein WA, Offit P. Editors. Vaccines. 5th ed. Philadelphia: Elsevier Inc; 2008: p.177-203.
 6. WHO position paper on hepatitis A vaccines – June 2012. Wkly Epidemiol Rec. 2012;87(28/29):261-76. PMID:22905367
 7. Green MS, Aharonowitz G, Shohat T, Levine R, Anis E, Slater PE. The changing epidemiology of viral hepatitis A in Israel. Isr Med Assoc J. 2001;3(5):347-51. PMID:11411199
 8. Dagan R, Leventhal A, Anis E, Slater P, Ashur Y, Shouval D. Incidence of hepatitis A in Israel following universal immunization of toddlers. JAMA. 2005;294(2):202-10. <http://dx.doi.org/10.1001/jama.294.2.202> PMID:16014594
 9. Zahavi A, Levine H, Zelikovich Y, Hartal M. Epidemiology of Hepatitis A in the Israeli Defense Forces, 1970-2010. Journal of Israeli Military Medicine. 2012;2(26):5-10.
 10. Chodick G, Green MS, Heymann A, Rosenman L, Shalev V. The shifting epidemiology of hepatitis A following routine childhood immunisation programme in Israel. Prev Med. 2007;45(5):386-91. <http://dx.doi.org/10.1016/j.ypmed.2007.05.011> PMID: 17599401
 11. Chodick G, Heymann AD, Ashkenazi S, Kokia E, Shalev V. Long-term trends in hepatitis A incidence following the inclusion of Hepatitis A vaccine in the routine nationwide immunization program. J Viral Hepat. 2008;15(Suppl 2):62-5. <http://dx.doi.org/10.1111/j.1365-2893.2008.01032.x> PMID:18837837
 12. Mor Z, Lurie Y, Katchman E. A case of hepatitis A vaccination failure in an HIV-positive man who had sex with men in Israel. Int J STD AIDS. 2012;23(7):529-30. <http://dx.doi.org/10.1258/ijisa.2010.010269> PMID:22844014
 13. Erhart LM, Ernst KC. The changing epidemiology of hepatitis A in Arizona following intensive immunization programs (1988-2007). Vaccine. 2012;30(42):6103-10. <http://dx.doi.org/10.1016/j.vaccine.2012.07.029> PMID:22835739
 14. Mor Z, Srur S, Dagan R, Rishpon S. Hepatitis A disease following the implementation of universal vaccination: who is at risk? J Viral Hepat. 2010;17(4):293-7. <http://dx.doi.org/10.1111/j.1365-2893.2009.01176.x> PMID:19691457
 15. Belmaker I, Dukhan L, Yosef Y, Leventhal A, Dagan R. Elimination of hepatitis a infection outbreaks in day care and school settings in southern Israel after introduction of the national universal toddler hepatitis a immunization program. Pediatr Infect Dis J. 2007;26(1):36-40. <http://dx.doi.org/10.1097/01.inf.0000247105.45185.13> PMID:17195703
 16. Orr N, Klement E, Gillis D, Sela T, Kayouf R, Derazne E, et al. Long-term immunity in young adults after a single dose of inactivated Hepatitis A vaccines. Vaccine. 2006;24(20):4328-32. <http://dx.doi.org/10.1016/j.vaccine.2006.03.010> PMID:16581163
 17. Hatz C, van der Ploeg R, Beck BR, Frösner G, Hunt M, Herzog C. Successful memory response following a booster dose with a virosome-formulated hepatitis a vaccine delayed up to 11 years. Clin Vaccine Immunol. 2011;18(5):885-7. <http://dx.doi.org/10.1128/CVI.00358-10> PMID:21411599
 18. Iwarson S, Lindh M, Widerström L. Excellent booster response 4 to 8 years after a single primary dose of an inactivated hepatitis A vaccine. J Travel Med. 2004;11(2):120-1. <http://dx.doi.org/10.2310/7060.2004.17079> PMID:15109480
 19. Barkai G, Belmaker I, Givon-Lavi N, Dagan R. The effect of universal toddlers-only hepatitis A virus vaccination program on seropositivity rate in unvaccinated toddlers: evidence for reduced virus circulation in the community. Pediatr Infect Dis J. 2009;28(5):391-3. <http://dx.doi.org/10.1097/INF.0b013e318190655c> PMID:19295466
 20. Ginsberg GM, Slater PE, Shouval D. Cost-benefit analysis of a nationwide infant immunization programme against hepatitis A in an area of intermediate endemicity. J Hepatol. 2001;34(1):92-9. [http://dx.doi.org/10.1016/S0168-8278\(00\)00007-6](http://dx.doi.org/10.1016/S0168-8278(00)00007-6) PMID:11211913

Spatial distribution and cluster analysis of a leishmaniasis outbreak in the south-western Madrid region, Spain, September 2009 to April 2013

D Gomez-Barroso^{1,2,3}, Z Herrador (zherrador@isciii.es)^{3,4,5}, J V San Martín^{5,6}, A Gherasim⁴, M Aguado⁶, A Romero-Maté⁶, L Molina⁶, P Aparicio^{4,5}, A Benito^{4,5}

1. Network Biomedical Research Centre in Epidemiology and Public Health (CIBERESP in Spanish), Madrid, Spain

2. Health Institute Carlos III (ISCIII), Madrid, Spain

3. These authors contributed equally to this article

4. National Centre for Tropical Medicine, ISCIII, Madrid, Spain

5. Network Biomedical Research on Tropical Diseases (RICET in Spanish), Madrid, Spain

6. Fuenlabrada University Hospital, Madrid, Spain

Citation style for this article:

Gomez-Barroso D, Herrador Z, San Martín JV, Gherasim A, Aguado M, Romero-Maté A, Molina L, Aparicio P, Benito A. Spatial distribution and cluster analysis of a leishmaniasis outbreak in the south-western Madrid region, Spain, September 2009 to April 2013. *Euro Surveill.* 2015;20(7):pii=21037. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21037>

Article submitted on 05 May 2014 / published on 19 February 2015

Since July 2009, there has been a community outbreak of leishmaniasis in south-west Madrid, Spain. The present study used the spatial distribution of cases to investigate the connection between the outbreak and a recently built peri-urban park. We included 157 cases of cutaneous (CL) and 90 cases of visceral (VL) leishmaniasis diagnosed at Fuenlabrada University Hospital between July 2009 and April 2013. CL and VL cases were geo-referenced and incidence rates by census tract were calculated. To identify high-risk areas, the spatial autocorrelation between individual cases was estimated. In a next step, areas where risk of disease was significantly increased were identified by cluster analysis. Higher incidence rates and the areas with highest intensity of CL and VL were located in the north-western part of the municipality. The most likely cluster of CL comprised three census tracks with relative risk (RR)=11.5 (95% confidence interval (CI): 9.2–13.6). Two additional significant VL clusters were detected, the most likely one with RR=9.2 (95% CI: 7.3–11.1). In addition, we found one significant VL cluster in the immigrant population (RR=12.8; 95% CI: 9.3–16.1). The spatial pattern of leishmaniasis transmission revealed a relation between the outbreak and the suspected risk area.

Background

Leishmaniasis is a parasitic disease caused by more than 20 protozoan species of the genus *Leishmania*. It is transmitted through the bite of female sandflies of the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World). An ecological system in which a *Leishmania* species is maintained indefinitely is usually formed by one principal reservoir host, e.g. dogs for *Leishmania infantum* in both the Old and New Worlds [1]. Recent reports indicate that in Spain, other animals such as wild carnivores, rabbits and hares may play a role in

the maintenance of the system, occasionally bringing the parasite from its enzootic focus into contact with humans [2,3].

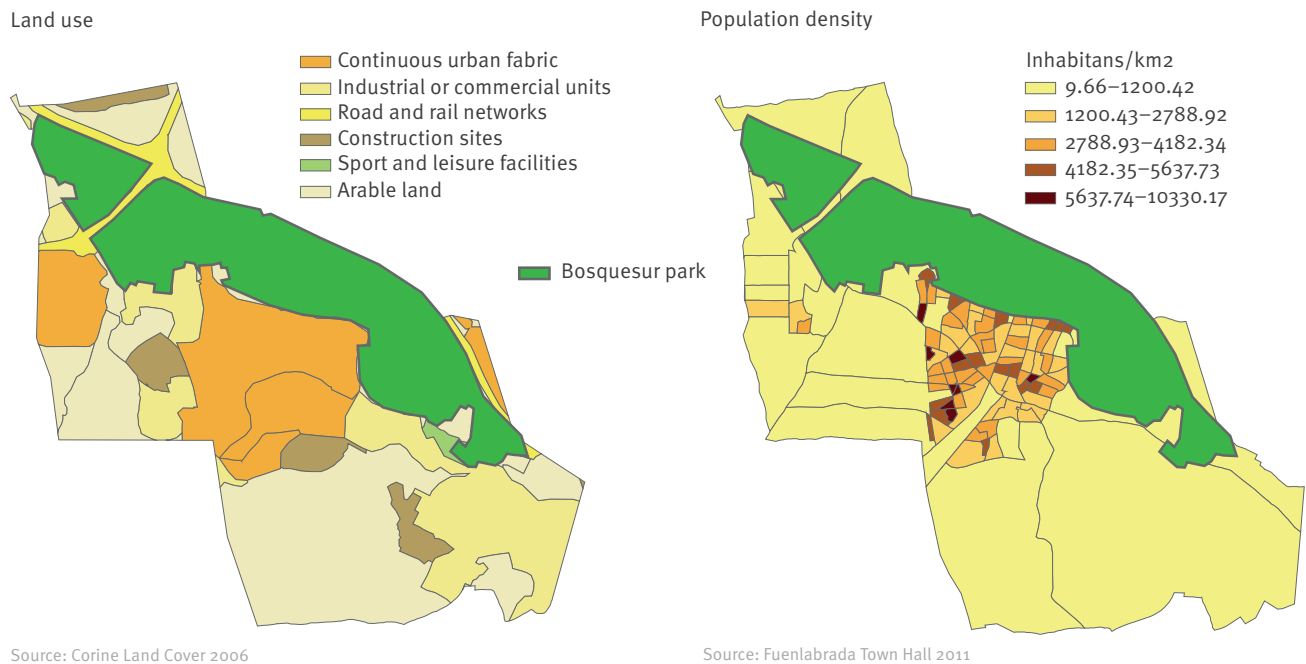
In Spain, leishmaniasis is endemic and both the visceral (VL) and the cutaneous (CL) forms are caused by *L. infantum*. Phlebotomine sandflies belonging to the subgenus *Larroussius* serve as vectors, while the dog is the main reservoir [4]. The real prevalence of leishmaniasis in Spain is unknown. Notification of leishmaniasis is not mandatory at national level (mandatory only in 12 of 17 regions) and there is no national leishmaniasis control programme [5]. Moreover, *Leishmanias* sp. cases are underascertained and underreported in Spain [6].

In the autonomous region of Madrid, notification of leishmaniasis has been mandatory since 1997 and is the most common zoonotic disease affecting infants and in patients with human immunodeficiency virus (HIV) infection [7,8]. Between 2000 and 2009, the annual incidence rate was around 0.5 per 100,000 population (between 12 and 25 leishmaniasis cases per year) [9]. During the last quarter of 2010, the case number increased fivefold. Subsequent research confirmed that an outbreak of leishmaniasis had been ongoing since July 2009 in the south-western area of the region of Madrid, with most cases occurring in the city of Fuenlabrada [5,10].

Most of the *L. infantum* isolates obtained from patients in the outbreak area presented an uncommon strain: the combined genotype L-920 [2]. The initial hypothesis to explain this outbreak postulated risk areas for bites by *Phlebotomus* sandflies infected with this new strain, creating a temporospatial clustering of infected individuals. Because leishmaniasis was found in only

FIGURE 1

Land use and population density, Fuenlabrada (Madrid), Spain, September 2009–April 2013



Maps were drafted using the free-licence source: Spanish National Institute of Geography. National Plan for Aerial Orthography (PNOA project). Available from: <http://www.ign.es/ign/layoutIn/actividadesFotoTelePNOA.do> [15].

7% of the dogs examined in the outbreak area, other reservoirs were suspected and investigated. Around 30% of the hares studied were positive for *Leishmania* parasites [3,5], suggestive of a sylvatic transmission cycle possibly linked to a recently built park called Bosquesur, adjacent to the urban area of Fuenlabrada [11].

Spatial pattern analysis has been found to be useful to better understand disease transmission of parasitic diseases when there is a strong correlation between the spatial distribution of the disease and its hosts [12,13]. We aimed to assess the spatial distribution of CL and VL cases and the cluster occurrence within the city of Fuenlabrada (Madrid) as well as the distribution of the cases in relation with the park Bosquesur.

Methods

Study area

Fuenlabrada is a city and municipality located in the Madrid metropolitan area in central Spain. It is the fourth biggest town in the community of Madrid and is located in the south-west, ca 22 km from the capital. The climate is Mediterranean, with an estimated average annual rainfall of 450–500 mm. The yearly mean maximum and minimum temperatures are 20.2°C and 7.6°C, respectively. The mean altitude of the area is 664 m above sea level and the municipality is divided into 108 census track units. Figure 1 shows the municipality's land cover (based on data from the European

Environment Agency [14]) and population density at census track level to describe the study area.

Cases

CL and VL data from September 2009 to April 2013 were supplied by the Internal Medicine and Dermatology Departments of Fuenlabrada University Hospital. Diagnosis was confirmed by direct observation of the parasite in a skin biopsy specimen or by a positive polymerase chain reaction (PCR) as well as by isolation in Novy-MacNeal-Nicolle (NNN) culture. Further details on laboratory methods are provided elsewhere [2]. Data contained information on a number of clinical, epidemiological and demographic variables; those considered in this study were sex, age, address of residence, migrant status and date of symptom onset. Frequencies and percentages were used to summarise data. The differences were assessed by Student's t-test and chi-square test for continuous and categorical variables, respectively.

Population

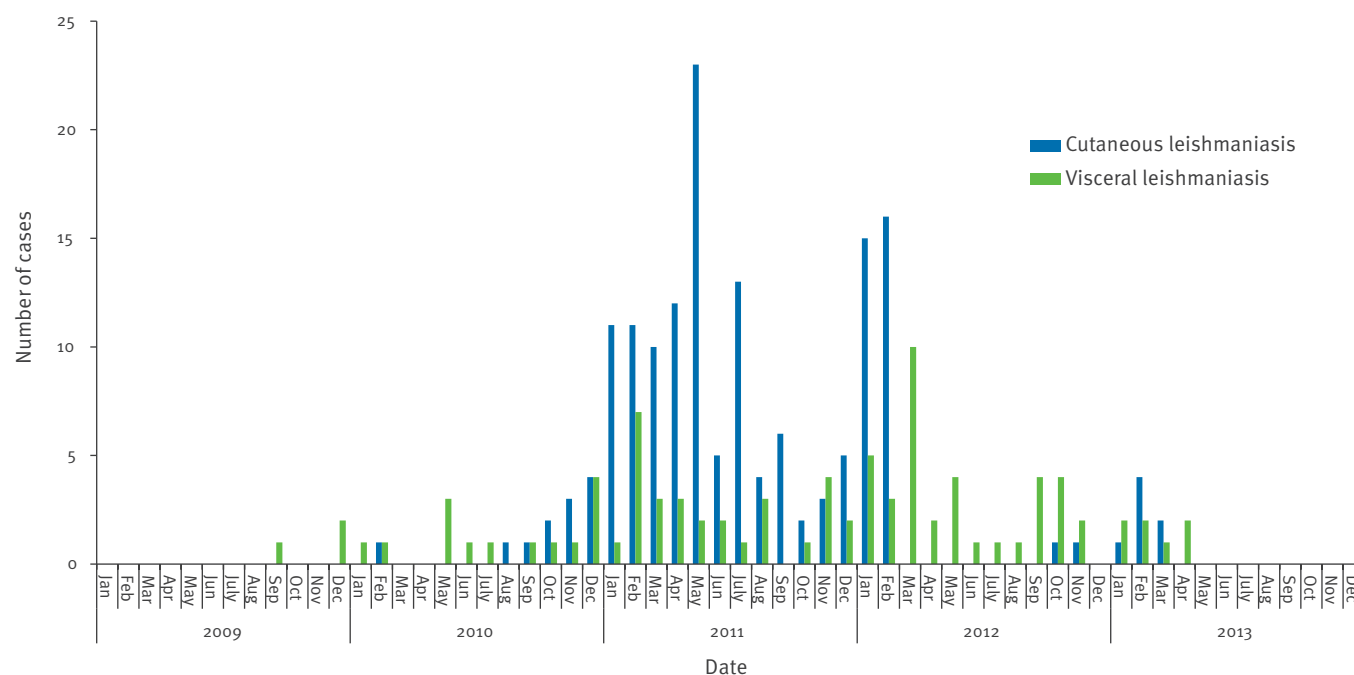
The population data was obtained from the 2011 annual statistic published by the City of Fuenlabrada, stratified by sex and age, at census track level [15]. Fuenlabrada had an area of 39.1 km² and an estimated population of 204,100 in 2011, of whom 75.3% were in the age group of 16–64 years and 15.4% were immigrants.

Spatial analysis

Geo-referencing of all VL and CL cases was carried out using Google Earth to obtain the geographical

FIGURE 2

Monthly distribution of cutaneous (n=157) and visceral (n=90) leishmaniasis cases in Fuenlabrada (Madrid), Spain, September 2009–April 2013



coordinates of patients' residences. In order to study the features of the territory, we obtained the map of Fuenlabrada by census track from the National Institute of Statistics (2011) [16] and downloaded images from the National Plan for Aerial Orthography (PNOA) project [17].

Spatial distribution

We calculated the incidence rates for CL and VL by census track adjusted by four age groups (0–14, 15–44, 45–64, ≥65 years) and by sex. We plotted the map with rates to understand the distribution of the disease at census track level. We calculated the local Moran's index in order to study the local indicator of spatial autocorrelation (LISA) between the rates. This index assesses local associations by comparing local averages to global average [18]. Its significance is estimated by generating a reference distribution using 999 random permutations. The LISA significance map includes the following categories: 'high–high' indicates clustering of high value rates (positive spatial autocorrelation), 'low–high' indicates that low value rates are adjacent to high value rates (negative spatial autocorrelation), 'low–low' indicates clustering of low value rates (positive spatial autocorrelation), 'high–low' indicates that high values are adjacent to low value rates (negative spatial autocorrelation), and 'not significant' indicates that there is no spatial autocorrelation.

In order to understand the risk distribution, we estimated the spatial smoothing distribution of CL and VL by means of the kernel density function. This function is based on the quadratic kernel function described

by Silverman [19]. This method is an interpolation and smoothing tool used to generalise the position of a point to an area. Kernel density estimation fits a curved surface over each case such that the surface is highest above the case and zero at a specified distance (the bandwidth) from the case.

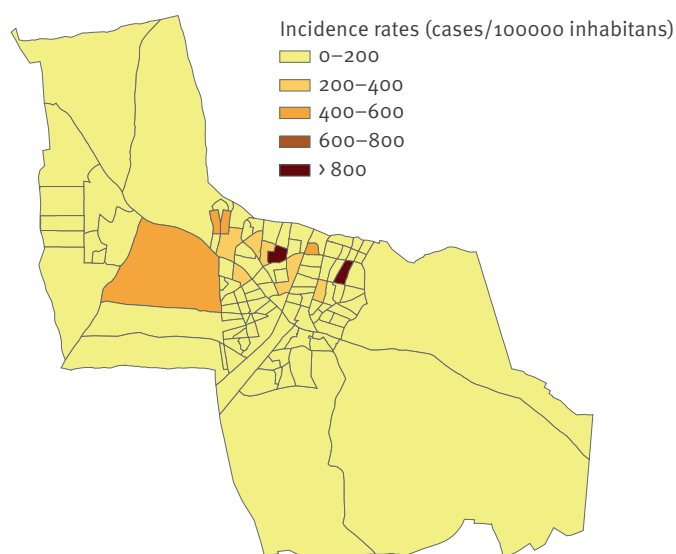
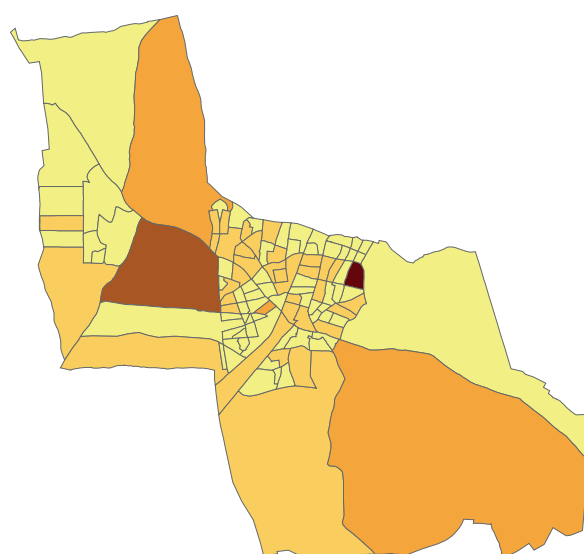
To estimate the distance to the park Bosquesur, which was the main suspected risk area according to previous investigations [3,11,20], we drew a polygon around this park. Distances to other landscape elements were also assessed. We created buffers with different distances: 500, 1,000 and 2,000 m around the park Bosquesur to measure the distances of the cases' addresses. Then, we calculated the average nearest neighbour index (ANNI) to calculate the distance between the location of each case and their nearest neighbour. This method was used as first approach to test whether or not the cases were clustering. If the average distance was below the average for a hypothetical random distribution, the distribution of the analysed characteristics was considered to be clustered. The index is expressed as the ratio of the observed distance divided by the expected distance. Thus, if the index is < 1, the pattern exhibits clustering, while index of > 1 indicates a trend towards dispersion. A significance level of 99% was chosen in the analysis.

Cluster analysis

Spatial clusters were analysed using the SaTScan spatial statistic estimator developed by Kulldorff [21]. To assess CL and VL spatial clusters in the entire population, and separately in the immigrant population, we

FIGURE 3

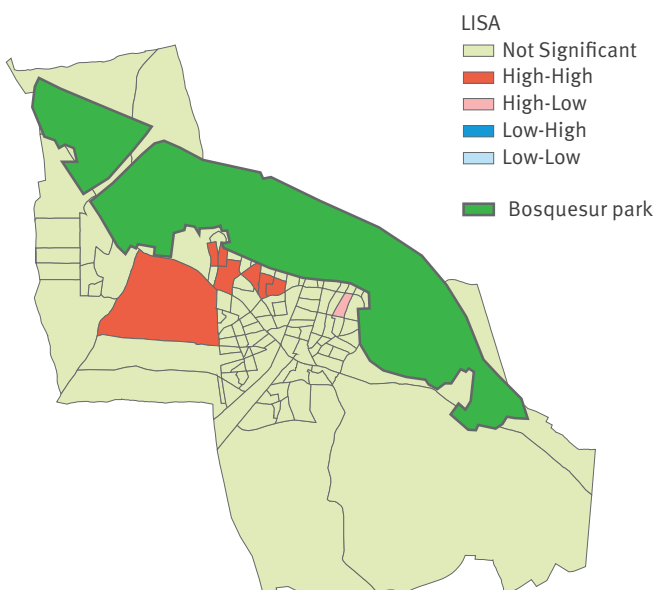
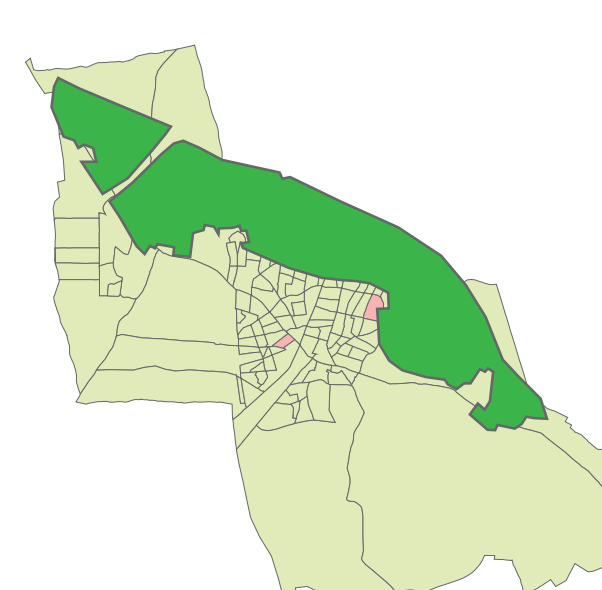
Incidence rates of cutaneous (n=157) and visceral (n=90) leishmaniasis, by census track, Fuenlabrada (Madrid), Spain, September 2009–April 2013

A. Cutaneous leishmaniasis**B. Visceral leishmaniasis**

Maps were drafted using the free-licence source: Spanish National Institute of Geography. National Plan for Aerial Orthography (PNOA project). Available from: <http://www.ign.es/ign/layoutIn/actividadesFotoTelePNOA.do> [15].

FIGURE 4

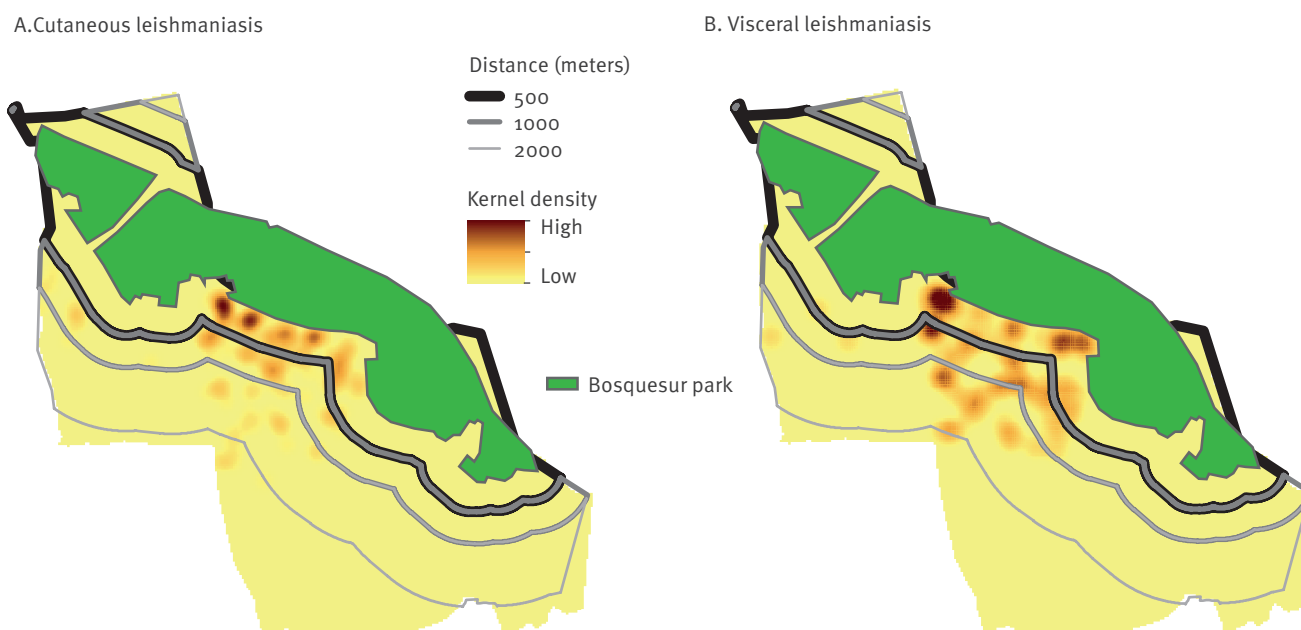
Local indicator of spatial autocorrelation map for cutaneous (n=157) and visceral (n=90) leishmaniasis rates in Fuenlabrada (Madrid), Spain, September 2009–April 2013

A. Cutaneous leishmaniasis**B. Visceral leishmaniasis**

Maps were drafted using the free-licence source: Spanish National Institute of Geography. National Plan for Aerial Orthography (PNOA project). Available from: <http://www.ign.es/ign/layoutIn/actividadesFotoTelePNOA.do> [15].

FIGURE 5

Kernel density for cutaneous (n=157) and visceral (n=90) leishmaniasis cases and buffer distances to the park Bosquesur, Fuenlabrada (Madrid), September 2009–April 2013



Maps were drafted using the free-licence source: Spanish National Institute of Geography. National Plan for Aerial Orthography (PNOA project). Available from: <http://www.ign.es/ign/layoutIn/actividadesFotoTelePNOA.do> [15].

used the scan statistic estimator to perform a purely spatial analysis, based on the assumption of a Poisson distribution. This method consists of creating a circular window which scans the entire study area. In our study we restricted the spatial window to a maximum radius of the average distance between cases (250 m). The radius of the centroid varies continuously in size from zero to the specified upper limit, in our case 250 m. The circle with maximum likelihood and containing more cases than expected is denominated the most likely cluster.

An increase in observed cases above the number expected was assessed using Monte Carlo test simulations (999 replications) with a 95% confidence interval. For the spatial analysis, we used Arcgis version 10.0, free software GeoDa and SaTScan. Data analysis was performed using SPSS version 18.0.

Results

Cases

From September 2009 to April 2013, a total of 157 CL and 90 VL cases were diagnosed at Fuenlabrada University Hospital. The distribution of CL cases over time (Figure 2) indicates that the first diagnosed CL cases appeared towards the end of 2010. CL cases climbed to a plateau during 2011, peaking twice, in May 2011 and January to February 2012. After the last

maximum, CL cases dropped to zero for the rest of 2012, and rose again at the beginning of 2013. The first VL cases were detected already in 2009. VL case numbers subsequently increased during 2011, reaching two peaks around the same time as the case numbers for CL. However, in contrast to the CL cases, the VL cases continued to be diagnosed throughout 2012.

52% CL and 72% VL cases were men ($p < 0.005$), with a median age of 48 and 46 years, respectively. The migration status was available for 246 of 247 cases. Eleven of 156 (7.6%) CL cases and 35 of 90 (39%) VL cases were immigrants ($p < 0.001$). Twenty-nine of the 46 immigrant cases were of Sub-Saharan origin (2/11 CL and 27/35 VL). No differences in sex and age distribution were found when comparing autochthonous and immigrant population.

Spatial analysis

Spatial distribution

The incidence rates of CL and VL in the different census tracks varied between zero and 1,003 and between zero and 613 cases per 100,000 population, respectively. Figure 3A shows the distribution of CL rates. Higher rates of CL (between 500 and 1,100 cases/100,000 population) were observed in three census tracks in the north of the municipality, while the highest rate for VL

TABLE 1

Distance to the park Bosquesur and distribution of cutaneous ($n = 157$) and visceral ($n = 90$) leishmaniasis cases, Fuenlabrada (Madrid), Spain, September 2009–April 2013

Distance	Cutaneous leishmaniasis		Visceral leishmaniasis	
	Cases	%	Cases	%
<500 m	82	52.2	37	41.1
500–1,000 m	40	25.5	28	31.1
1,000–2,000 m	20	12.7	21	23.3
>2,000 m	15	9.6	4	4.4
Total	157	100	90	100

was found in a different northern census track (Figure 3B).

The LISA for CL and VL rates are shown in Figure 4. Significant clusters of high values for CL and VL were detected: a CL hotspot ($p < 0.005$) in a census track located in the north-west of the municipality, and two significant VL hotspots ($p < 0.005$) in two census tracks with high values adjacent to census tracks with low value rates.

The average distance among the cases (bandwidth) was 250 m. Following spatial smoothing, areas with high intensity for CL were identified in the north of the city, close to the park Bosquesur (Figure 5A). The distribution of the VL cases is shown in Figure 5B; high intensity areas were located in the northern part of the municipality.

Table 1 summarises the cases distribution and distance to the park. We observed a decreasing trend in CL and VL occurrence in relation to the distance to the park. Up to 75% of CL cases and 70% of VL cases lived in places less than 1 km from this suspected risk area.

The ANNI was 0.505 ($p < 0.001$) for CL and 0.582 ($p < 0.001$) for VL throughout the study area. Both for CL and VL, the pattern exhibited clustering. The observed average distances were 98 and 270 m and the expected average distances were 193 and 463 m for CL and VL, respectively.

Cluster analysis

Four significant CL clusters were identified through spatial cluster analysis ($p < 0.001$, Figure 6A). The most likely cluster comprised three census tracks in which 24 cases were diagnosed during the study period, while the number of expected cases was 2.46 (relative risk (RR) = 11.50 and $p < 0.005$). Other secondary clusters were located in the north of the municipality.

The VL spatial cluster analysis detected two significant clusters ($p < 0.001$, Figure 6B; Table 2). The most likely cluster comprised one census track with eight cases and an expected number of cases of 0.95. The RR was 9.15 ($p < 0.005$). The other secondary cluster was located in the north-eastern part of the municipality.

When we analysed the data according to the migrant status, we found one significant cluster for VL in the immigrant population, with four observed cases, 0.35 expected cases and a RR of 12.75 ($p < 0.005$) (Figure 6C). This cluster included one census track located in the north of the municipality, different from the one detected for VL in overall population. No significant cluster of CL was identified through spatial cluster analysis in the immigrant population.

Discussion

Our study revealed the spatial characteristics of human CL and VL during an outbreak in Fuenlabrada (Madrid) using geographic information system (GIS) tools and spatial statistical analysis. Similar approaches have been used in Spain to investigate the spatial distribution of the sandfly vector and canine leishmaniasis [4,22]. However, to our knowledge this is the first attempt to implement spatial techniques to assess the distribution and cluster occurrence of human CL and VL cases in an outbreak in Europe.

We have described the evolution of the VL and CL cases in Fuenlabrada separately, as analysing them together may have resulted in misinterpretation. VL was more common in men than in women, while no sex differences were found for CL. This seems to be concordant with findings from previous epidemiological studies indicating that VL occurs more frequently among adult men [20,23]. Although it has been hypothesised that sex hormones play a role in the modulation of immunity against leishmaniasis [24], the explanation for this trend still remains uncertain.

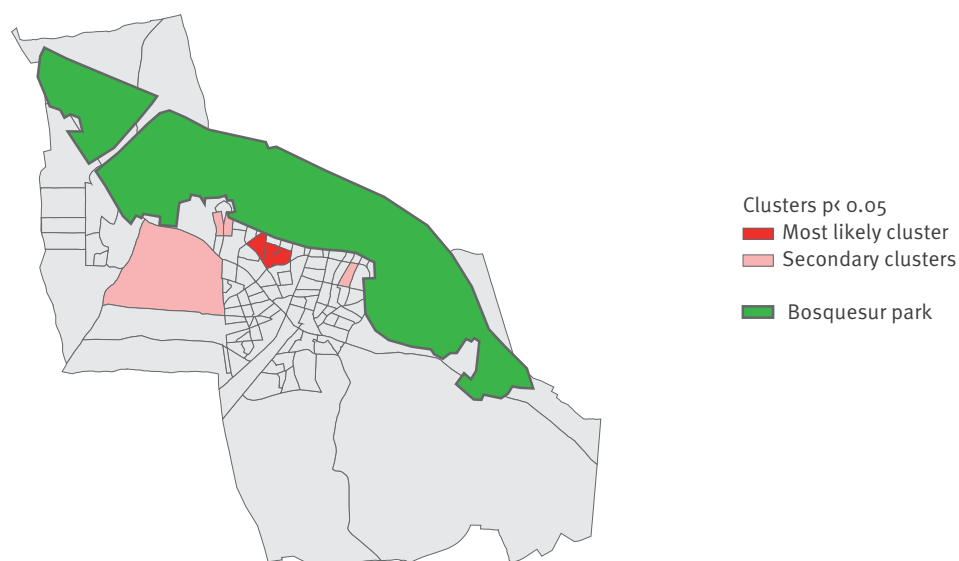
In our research, the census tracks with the highest incidence of CL were different from the ones with highest incidence of VL. It is difficult to determine whether the different distribution of CL and VL cases determined by disease onset, vector distribution, disease transmission pattern and/or presence of the host. Leishmaniasis is known to be a diverse and complex disease [1], therefore further studies may be needed to investigate this particular finding.

Although the spatial distribution of CL and VL leishmaniasis did not overlap perfectly in space, both were spatially clustered along the border of a park. Risk associated with other landscape elements within the municipality were analysed without significant results. Leishmaniasis is one of the main parasitic diseases of the world for which the transmission profile includes landscape elements and environment [25]. In our research, all spatial methods deployed showed that the northern peripheral census tracks were the most

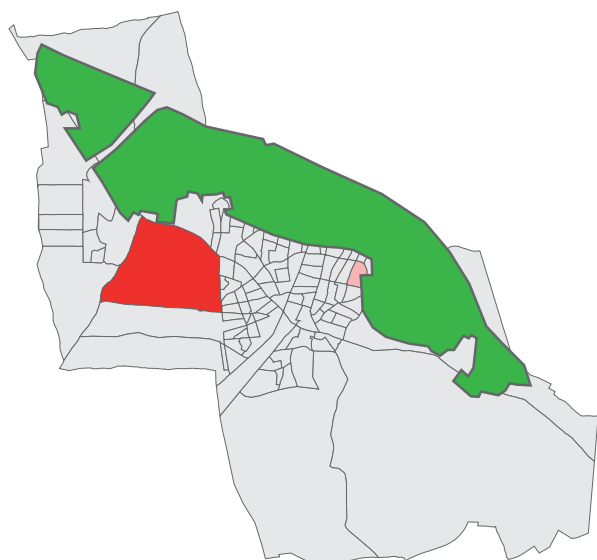
FIGURE 6

Significant clusters of cutaneous (n=61 cases) and visceral (n=13 cases) leishmaniasis in the overall and immigrant population, Fuenlabrada (Madrid), Spain, September 2009–April 2013

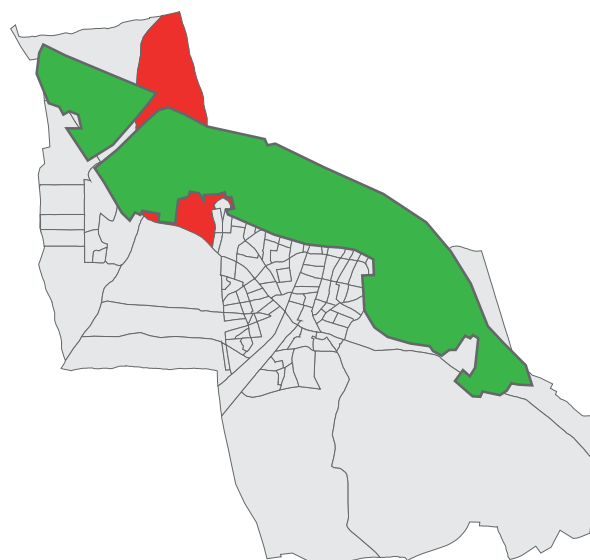
A. Cutaneous leishmaniasis



B. Visceral leishmaniasis



C. Visceral leishmaniasis immigrants



Maps were drafted using the free-licence source: Spanish National Institute of Geography. National Plan for Aerial Orthography (PNOA project). Available from: <http://www.ign.es/ign/layoutIn/actividadesFotoTelePNOA.do> [15].

TABLE 2

Significant clusters of cutaneous (n = 61 cases) and visceral (n = 13 cases) leishmaniasis, Fuenlabrada (Madrid), Spain, September 2009–April 2013

	Cluster	Number of census tracks	Observed	Expected	RR	LLR	p value
Cutaneous leishmaniasis	1	3	24	2.5	11.5	34.9	0.000
	2	1	11	1.0	11.9	16.8	0.000
	3	2	16	3.4	5.2	12.8	0.000
	4	1	10	1.5	7.09	10.6	0.000
Visceral leishmaniasis	1	1	8	1.0	9.2	10.3	0.000
	2	1	5	0.5	11.4	7.5	0.001
VL immigrants	1	1	4	0.4	12.8	6.3	0.002

LRR: log-likelihood ratio; RR: relative risk; VL: visceral leishmaniasis.

heavily affected. Moreover, CL and VL incidence rates and the distance to the park Bosquesur were spatially correlated. Finally, the cluster analysis also showed that the most likely CL and VL clusters were close to this park.

Our results were in accordance with previous research that emphasises that this disease can be associated with urbanisation close to vegetation areas [23,26,27]. Worldwide, leishmaniasis outbreaks have been related to human activities close to or within forested areas: road construction, building of dams, irrigation schemes and horticulture development, and establishment of new residential colonies lead to intrusion into the sylvatic cycle of the disease [27,28]. The park Bosquesur is a man-made natural area, located between the towns of Alcorcón, Leganés, Fuenlabrada, Getafe and Pinto. During the outbreak period, leishmaniasis cases were reported from these five towns [10]. Adjacent to this newly planted area, public construction work was carried out on one of the main roads (M-407) to Fuenlabrada in 2009–10. All these recent changes at peri-urban level may have altered the ecology of this area and the transmission dynamics of leishmaniasis.

The sum of observed cases in the four significant clusters of CL was 61 (39% of the total 157 CL cases included in the analysis), while the percentage of VL cases included in the significant clusters was even lower, 14% (n=13) of the 90 VL cases. This difference in case clustering can also be observed in the epidemiological curve (Figure 2), although we cannot draw conclusions in this regard as we did not carry out a temporospatial analysis. Other exposure pathways are also plausible. As shown by de Almeida et al., the pattern of leishmaniasis is not static and the disease may spread from one area of a municipality to another [29]. On the other hand, the use of a patient's home residence as a marker of the place of infection may not be accurate because contact between host and vector can have occurred outside the home.

The park has several recreational areas with footpaths and bicycle trails [30]. Social networks, recreational activities and other interactions between human settlements and the peri-urban natural environment may play a role in the distribution of the disease in this context [26,28]. Nevertheless, these establishments are attended mostly during daytime. First appearance of active *P. perniciosus* females occurs at sunset and the density peak is usually reached around 23:00–24:00 [5,11]. Therefore, the highest probability of transmission may be not associated with the presence of individuals inside the park, but rather in homes or outdoor places close to, but outside the park.

According to Arce et al., patients were asked during the outbreak investigation about recreational habits, but neither particular areas nor classic environmental risk factors were identified [10]. It should be noticed that only cases were assessed during the official investigation. We believe that the outbreak investigation would have benefited from a control group in order to evaluate exposures and risk factors.

A high percentage of VL cases (39%) were immigrants and we identified a cluster of VL in this particular group. This cluster did not overlap with the VL cluster detected in overall population. We also carried out the analysis in the non-immigrant population only (data not shown) and did not find any relevant difference compared to the analysis on entire population. According to official data from the local authorities, 15.4% of the Fuenlabrada population in 2011 were immigrants, of which 14.9% are from Sub-Saharan Africa [15]. The percentage of immigrants in the study area was similar to that of other urban areas in Spain [31]. Several factors could have been responsible for the high percentage of VL in immigrants: migration of non-immune people from areas where *L. infantum* is not endemic [1,32], differences in host immune responses [33], and differences in living habits and/or health-seeking behaviour. These factors should be explored in further investigations.

Our study has several limitations. One relates to the long incubation period of the disease, rendering determining and interpreting the information related to transmission site difficult. Control measures may have affected the spatial distribution of cases. We are aware of disinfection activities undertaken since 2011; although no information on the targeted sites is publicly available, we cannot discard their impact on the case distribution.

We chose Kulldorff's method for the spacial cluster analysis because it has several advantages [34]: it adjusts for population density and confounding variables (e.g. age and sex); there is no pre-selection bias since the clusters are selected without prior hypothesis on their location, size or time period; the statistical test takes into account multiple testing and delivers a single p value; if a cluster is detected, its location is specified.

In a molecular typing study, Chicharro et al. found that the outbreak was not caused by a single parasite strain, as four combined genotypes were found. At Fuenlabrada Hospital, the ITS-LOMBARDI type was isolated from all serotyped cases. This *L. infantum* ITS type has been present in this region since at least 1992 [2]. A high density of *P. perniciosus* has also been observed in the park [10]. In previous research carried out in central Spain, a correlation was found between vector density and cases living in areas between villages or at the limits of a village [22]. Future entomological and molecular typing studies could benefit from our results, as a combined methodology could allow more precise conclusions regarding the transmission patterns.

Conclusion

Although our study design did not allow establishing causal associations, the methodology can be considered useful in generating hypotheses during an outbreak investigation. Future work should examine the role of vector density, seroprevalence of *Leishmania* in canine and other possible reservoirs, climate variability, socio-economic conditions, land use and changes made by humans to the habitat over a longer time span in the study area. This will allow building accurate risk maps and targeting prevention and treatment interventions in these high-risk areas in a timely manner.

Acknowledgements

The authors gratefully acknowledge the financial support of the Health Institute Carlos III via the Tropical Diseases Research Network (RICET). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest

None declared.

Authors' contributions

Diana Gomez-Barroso - writing of the manuscript, epidemiological data analysis and interpretation of the study. Zaida Herrador - writing of the manuscript, epidemiological data analysis and interpretation of the study. Juan Victor San Martin - interpretation of the study and contributed to the revision of the draft manuscript. Alin Gherasim - epidemiological data analysis, interpretation of the study and contributed to the revision of the draft manuscript. Marta Aguado - contributed to the revision of the draft manuscript. Alberto Romero-Maté - contributed to the revision of the draft manuscript. Laura Molina - contributed to the revision of the draft manuscript - Pilar Aparicio - contributed to the revision of the draft manuscript; and Agustín Benito - interpretation of the study and contributed to the revision of the draft manuscript.

References

1. World Health Organization (WHO). Control of the leishmaniasis. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis, Geneva, 22-26 March 2010. WHO Tech Rep Ser, no. (2). Available from: http://whqlibdoc.who.int/trs/WHO_TRS_949_eng.pdf
2. Chicharro C, Llanes-Acevedo IP, García E, Nieto J, Moreno J, Cruz I. Molecular typing of *Leishmania infantum* isolates from a leishmaniasis outbreak in Madrid, Spain, 2009 to 2012. *Euro Surveill*. 2013;18(30):20545. <http://dx.doi.org/10.2807/1560-7917.ES2013.18.30.20545> PMID:23929179
3. Molina R, Jiménez MI, Cruz I, Iriso A, Martín-Martín I, Sevillano O, et al. The hare (*Lepus granatensis*) as potential sylvatic reservoir of *Leishmania infantum* in Spain. *Vet Parasitol*. 2012;190(1-2):268-71. <http://dx.doi.org/10.1016/j.vetpar.2012.05.006> PMID:22677135
4. Galvez R, Descalzo MA, Guerrero I, Miro G, Molina R. Mapping the current distribution and predicted spread of the leishmaniosis sand fly vector in the Madrid region (Spain) based on environmental variables and expected climate change. *Vector Borne Zoonotic Dis*. 2011;11(3):799-806. <http://dx.doi.org/10.1089/vbz.2010.0109> PMID:21417927
5. Centro de Coordinación de Alertas y Emergencias sanitarias (CCAES), Ministerio de Sanidad, Política Social e Igualdad (MSPSI). Evaluación del riesgo de transmisión de *Leishmania infantum* en España. [Risk assessment of *Leishmania infantum* transmission in Spain]. Madrid: MSPSI; 2012. Spanish. Available from: <http://www.msssi.gob.es/profesionales/saludPublica/ccayes/analisisituacion/doc/leishmania.pdf>
6. Garrote JI, Gutiérrez MP, Izquierdo RL, Dueñas MAI, Zarzosa P, Cañavate C, et al. Seroepidemiologic study of *Leishmania infantum* infection in Castilla-Leon, Spain. *Am J Trop Med Hyg*. 2004;71(2):403-6. PMID:15516634
7. Alvar J, Aparicio P, Aseffa A, Den Boer M, Cañavate C, Dedet J-P, et al. The relationship between leishmaniasis and AIDS: the second 10 years. *Clin Microbiol Rev*. 2008;21(4):334-59. <http://dx.doi.org/10.1128/CMR.00061-07> PMID:18400800
8. Manual de notificación del sistema de enfermedades de declaración obligatoria. Documentos técnicos de salud pública no. 69. [Manual of the notification system for notifiable diseases. Public health technical papers no. 69]. Madrid: Public Health Institute Community of Madrid; 2006. Spanish. Available from: http://www.madrid.org/cs/Satellite?c=CM_Publicaciones_FA&cid=1142284809709&idConsejeria=1109266187266&idListConsj=110926544710&idOrganismo=1142439320383&language=es&pagename=ComunidadMadrid%2FEstrutura&sm=1109266101003
9. Morbilidad por enfermedades de declaración obligatoria. Comunidad de Madrid. Año 2010. [Morbidity of compulsory notifiable diseases. Community of Madrid. Year 2010]. Boletín epidemiológico de la Comunidad de Madrid. 2011;17(11). Spanish. Available from: <http://www.madrid.org/cs/Satellite?blobcol=urldata&blobheader=application%2Fpdf&blobheadername1=Content-Disposition&blobheadervalue1=filename%3DNOviembre2011.pdf&blobkey=id&blobtable=MungoBlobs&blobwhere=1310910631705&ssbinary=true>
10. Arce A, Estirado A, Ordoñas M, Sevilla S, García N, Moratilla L, et al. Re-emergence of leishmaniasis in Spain: community outbreak in Madrid, Spain, 2009 to 2012. *Euro Surveill*. 2013;18(30):20546. <http://dx.doi.org/10.2807/1560-7917.ES2013.18.30.20546> PMID:23929177
11. Jiménez M, González E, Iriso A, Marco E, Alegret A, Fúster F, et al. Detection of *Leishmania infantum* and identification of blood meals in *Phlebotomus perniciosus* from a focus

- of human leishmaniasis in Madrid, Spain. *Parasitol Res.* 2013;112(3):2453-9. <http://dx.doi.org/10.1007/s00436-013-3406-3> PMID:23535889
12. Kulldorff M, Heffernan R, Hartman J, Assunção R, Mostashari F. A space-time permutation scan statistic for disease outbreak detection. *PLoS Med.* 2005;2(6):e59. <http://dx.doi.org/10.1371/journal.pmed.0020059> PMID:15719066
 13. Mott KE, Nuttall I, Desjeux P, Cattand P. New geographical approaches to control of some parasitic zoonoses. *Bull World Health Organ.* 1995;73(4):247-57. PMID:7743598
 14. European Environment Agency (EEA). Corine Land Cover 2006 raster data. Copenhagen: EEA. [Accessed: 28 Apr 2014]. Available from: <http://www.eea.europa.eu/data-and-maps/data/corine-land-cover-2006-raster>
 15. Población de Fuenlabrada, 2011. [Population of Fuenlabrada, 2011]. Fuenlabrada: City of Fuenlabrada. [Accessed 9 September 2013]. Spanish. Available from: <http://ayto-fuenlabrada.es/index.do?MP=3&MS=27&MN=2>
 16. National Statistics Institute. Population and Housing Census 2011. Madrid: Instituto Nacional de Estadística. [Accessed: 5 October 2013]. Available from: http://www.ine.es/en/censos2011_datos/cen11_datos_resultados_seccen_en.htm
 17. Spanish National Institute of Geography. Plan Nacional de Ortofotografía Aérea. [National Plan for Aerial Orthography]. Madrid: Instituto Geográfico Nacional. [Accessed: DATE]. Spanish. Available from: <http://www.ign.es/ign/layoutIn/actividadesFotoTelePNOA.do>
 18. Anselin L. Locals indicator of spatial association-LISA. *Geogr Anal.* 1995;27(4):93-115.
 19. Silverman BW. Density Estimation for Statistics and Data Analysis. London: Chapman and Hall/CRC; 1998.
 20. Suárez Rodríguez B, Isidoro Fernández B, Santos Sanz S, Sierra Moros MJ, Molina Moreno R, Astray Mochales J, et al. [Review of the current situation and the risk factors of Leishmania infantum in Spain]. *Rev Esp Salud Publica.* 2012;86(8):555-64. PMID:23325131
 21. Kulldorff M. A spatial scan statistic. *Commun Stat Theory Methods.* 1997;26(8):1481-96. <http://dx.doi.org/10.1080/03610929708831995>
 22. Gálvez R, Descalzo MA, Miró G, Jiménez MI, Martín O, Dos Santos-Brandao F, et al. Seasonal trends and spatial relations between environmental/meteorological factors and leishmaniosis sand fly vector abundances in Central Spain. *Acta Trop.* 2010;115(1-2):95-102. <http://dx.doi.org/10.1016/j.actatropica.2010.02.009> PMID:20171154
 23. Werneck GL, Costa CHN, Walker AM, David JR, Wand M, Maguire JH. The urban spread of visceral leishmaniasis: clues from spatial analysis. *Epidemiology.* 2002;13(6):364-7. <http://dx.doi.org/10.1097/00001648-200205000-00020> PMID:11964941
 24. Snider H, Lezama-Davila C, Alexander J, Satoskar AR. Sex hormones and modulation of immunity against leishmaniasis. *Neuroimmunomodulation.* 2009;16(4):106-13. <http://dx.doi.org/10.1159/000180265> PMID:19212130
 25. Valderrama-Ardila C, Alexander N, Ferro C, Cadena H, Marín D, Holford TR, et al. Environmental risk factors for the incidence of American cutaneous leishmaniasis in a sub-Andean zone of Colombia (Chaparral, Tolima). *Am J Trop Med Hyg.* 2010;82(4):243-50. <http://dx.doi.org/10.4269/ajtmh.2010.09-0218> PMID:20134000
 26. Gil JF, Nasser JR, Cajal SP, Juárez M, Acosta N, Cimino RO, et al. Urban transmission of American cutaneous leishmaniasis in Argentina: spatial analysis study. *Am J Trop Med Hyg.* 2010;82(6):433-40. <http://dx.doi.org/10.4269/ajtmh.2010.09-0113> PMID:20207869
 27. Ready PD. Leishmaniasis emergence and climate change. *Rev Sci Tech.* 2008 Aug;27(4):399-412. PMID:18819668
 28. Campbell-Lendrum D, Dujardin JP, Martinez E, Feliciangeli MD, Perez JE, Silans LN, et al. Domestic and peridomestic transmission of American cutaneous leishmaniasis: changing epidemiological patterns present new control opportunities. *Mem Inst Oswaldo Cruz.* 2001;96(4):159-62. <http://dx.doi.org/10.1590/S0074-02762001000200004> PMID:11285490
 29. de Almeida AS, Medronho R de A, Werneck GL. Identification of risk areas for visceral leishmaniasis in Teresina, Piauí State, Brazil. *Am J Trop Med Hyg.* 2011;84(9):681-7. <http://dx.doi.org/10.4269/ajtmh.2011.10-0325> PMID:21540375
 30. La Red de Centros de la Consejería de Medio Ambiente y Ordenación del Territorio. [Network of Centers from the Regional Ministry of Environment and Spatial Planning]. Madrid: Community of Madrid. [Accessed: 10 Jan 2014]. Spanish. Available from: http://www.madrid.org/cs/Satellite?c=CM_Actuaciones_FA&cid=1142314660051&idTema=1109265603340&language=es&pagename=ComunidadMadrid%2FEstructura&pv=1142343852186&segmento=1
 31. National Statistics Institute. Encuesta Nacional de Inmigrantes 2007. [National Immigrant Survey 2007]. Madrid: Instituto Nacional de Estadística. [Accessed: 10 Jan 2014]. Spanish. Available from: <http://www.ine.es/jaxi/menu.do?type=pcaxis&path=%2Ft20%2Fp319&file=inebase&L=0>
 32. Pavli A, Maltezos HC. Leishmaniasis, an emerging infection in travelers. *Int J Infect Dis.* 2010;14(10):e1032-9. <http://dx.doi.org/10.1016/j.ijid.2010.06.019> PMID:20952234
 33. Maache M, Azzouz S, Díaz de la Guardia R, Alvarez P, Gil R, de Pablos LM, et al. Host humoral immune response to Leishmania lipid-binding protein. *Parasite Immunol.* 2005;27(8):227-34. <http://dx.doi.org/10.1111/j.1365-3024.2005.00773.x> PMID:16048642
 34. Gomez-Barroso D, Varela C, Ramis R, Del Barrio JL, Simón F. Space-time pattern of hepatitis A in Spain, 1997-2007. *Epidemiol Infect.* 2012;140(6):407-16. <http://dx.doi.org/10.1017/S0950268811000811> PMID:21676358

Comparative safety evaluation of 7-valent and 13-valent pneumococcal vaccines in routine paediatric vaccinations in four Italian regions, 2009 to 2011

F Trotta (f.trotta@aifa.gov.it)^{1,2}, C Rizzo², C Santuccio¹, A Bella², the Pharmacovigilance Study Group on Pneumococcal Vaccination in Children³

1. Pharmacovigilance Unit, Italian Medicines Agency, Rome, Italy
2. National Centre of Epidemiology, National Institute of Health, Rome, Italy
3. The members of the group are listed at the end of the article

Citation style for this article:

Trotta F, Rizzo C, Santuccio C, Bella A, the Pharmacovigilance Study Group on Pneumococcal Vaccination in Children. Comparative safety evaluation of 7-valent and 13-valent pneumococcal vaccines in routine paediatric vaccinations in four Italian regions, 2009 to 2011. Euro Surveill. 2015;20(7):pii=21041. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21041>

Article submitted on 21 January 2014 / published on 19 February 2015

This study was aimed at estimating the risk of all types of adverse events following immunisation (AEFI), neurological events and convulsions following the co-administration of 13-valent pneumococcal polysaccharide conjugate vaccine (PCV13) with hexavalent vaccine. Paediatric spontaneous reports and exposure to vaccinations in four Italian regions were available. The estimated incidence rate ratio (IRR) for AEFI following co-administration of hexavalent vaccine with either PCV13 or PCV7 was 1.08 (95% confidence interval (CI): 0.91–1.28); the IRR for, respectively, neurological events and convulsion following co-administration of PCV13 with hexavalent vaccine were 1.27 (95% CI: 0.85–1.89) and 1.43 (95% CI: 0.70–2.91). Co-administration of PCV13 with hexavalent vaccine had a protective effect against AEFI (IRR=0.59; 95% CI: 0.49–0.72). This protective effect was not observed for neurological events or convulsions following co-administration of PCV13 with hexavalent vaccine compared with single administration (IRR=1.44; 95% CI: 0.77–2.67 and IRR=1.46; 95% CI: 0.50–4.25, respectively). We observed a trend of increased risk of neurological events or convulsions following PCV13 used in routine practice. Analysis of spontaneously reported data is a quick method to estimate associations between vaccines and less common adverse events. Given methodological limitations these findings cannot be conclusive and require further investigations.

Introduction

The 13-valent pneumococcal polysaccharide conjugate vaccine (PCV13) was introduced in Italy in mid-2010, fully replacing the use of 7-valent vaccine (PCV7) [1]. The PCV13 provides protection against an additional six serotypes (1, 5, 7F, 3, 6A, 19A) not included in PCV7.

Since 2005, pneumococcal vaccination has been recommended in the Italian national immunisation

programme (NIP), but only for risk groups (e.g. asplenia, immunocompromised subjects, patients with chronic diseases) [2,3]. However, it was in the following years increasingly offered also to other target groups, dependent on the vaccination policies in the individual regions [2]. Since 2008, pneumococcal vaccination has been included in the NIP free of charge for all newborns; it was administered as a three-dose schedule (during the first year of life) concomitantly with the hexavalent vaccine against diphtheria (D), tetanus (T), acellular pertussis (aP), *Haemophilus influenzae* type b (Hib), hepatitis B virus (HBV) and inactivated poliovirus (IPV) [4,5]. Thus, in routine practice, PCV and the hexavalent vaccine were usually administered concomitantly to children in a single vaccination session.

Clinical trials evaluating the effect of co-administration of PCV13 with the hexavalent vaccine showed a comparable safety and immunogenicity profile as those evaluating co-administration of PCV7 and the hexavalent vaccines when given in routine practice; among systemic adverse events, fever was more common in subjects vaccinated with PCV13 compared with PCV7 [6,7]. Like other vaccines, PCV can provoke fever, which could trigger a febrile seizure [7,9]. However, those clinical trials were not sized to detect less common or specific adverse events following immunisation (AEFI), and only involved a selected paediatric population (children with risk conditions were excluded). Therefore, post-marketing surveillance remains essential. The routine monitoring of spontaneous reports collected by the Italian Pharmacovigilance Network (IPN) in the first year after introduction of PCV13 in Italy showed a slightly higher frequency of serious AEFI with PCV13 than with PCV7; this trend was more evident for neurological events and when PCV13 was co-administered with the hexavalent vaccine [10]. This finding led us to

TABLE 1

Frequency of spontaneous reports of adverse events following vaccination, by seriousness and vaccine type administered alone or in co-administration, four regions in Italy, 2009 to 2011 (n=883)

	PCV7 alone	PCV13 alone	Hexavalent alone	PCV7 and hexavalent co-administered	PCV13 and hexavalent co-administered	Total
	2009 + Q1–Q2 2010	Q3–Q4 2010 + 2011	2009–2011	2009 + Q1–Q2 2010	Q3–Q4 2010 + 2011	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
All AEFI (n=883)						
Serious	4 (9)	6 (13)	14 (5)	27 (12)	56 (18)	107 (12)
Not serious	40 (91)	40 (87)	238 (93)	201 (86)	247 (81)	766 (87)
Undefined	0 (0)	0 (0)	4 (2)	4 (2)	2 (1)	10 (1)
Total	44 (100)	46 (100)	256 (100)	232 (100)	305 (100)	883 (100)
Neurological events (n=136)						
Serious	3 (50)	2 (67)	9 (36)	15 (38)	39 (63)	68 (50)
Not serious	3 (50)	1 (33)	15 (60)	23 (57)	23 (37)	65 (48)
Undefined	0 (0)	0 (0)	1 (4)	2 (5)	0 (0)	3 (2)
Total	6 (100)	3 (100)	25 (100)	40 (100)	62 (100)	136 (100)
Convulsions, febrile and afebrile (n=41)						
Serious	1 (100)	1 (100)	3 (50)	8 (67)	19 (90)	32 (78)
Not serious	0 (0)	0 (0)	3 (50)	4 (33)	2 (10)	9 (22)
Undefined	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	1 (100)	1 (100)	6 (100)	12 (100)	21 (100)	41 (100)
Administered doses (n)	209,098	169,069	243,802	802,126	979,446	Not applicable

AEFI: adverse events following immunisation; PCV: pneumococcal polysaccharide conjugate vaccine.

further investigate the safety of PCV13 in combination with the hexavalent vaccine.

The first objective of this study was to estimate the risk of all types of AEFI, neurological events, and convulsions following the co-administration of PCV13 with the hexavalent vaccine, using the co-administration of PCV7 with the hexavalent vaccine as a reference. The second objective was to compare the risk of all types of AEFI, neurological events, and convulsions following the co-administration of PCV13 with the hexavalent vaccine vs single administration of PCV13 and the hexavalent vaccine in different vaccine sessions.

Methods

AEFI reports and administered doses

We retrieved paediatric spontaneous reports to IPN after vaccination with PCV (7- and 13-valent) or the hexavalent vaccine occurring from 1 January 2009 and 31 December 2011. Data retrieval from IPN took place in mid-2012. No exclusion criteria for cases of AEFI were adopted. Details on the vaccinations received during the three-year period, such as the number of doses of PCV7, PCV13 and the hexavalent vaccine administered to the paediatric population (0–2 years-old) both as single or concomitant vaccination, were available from four Italian regions, Emilia-Romagna, Lombardy, Tuscany and Veneto, and pooled at regional

level. These regions are situated in the north and in the centre of Italy. All our analyses were limited to these regions, covering 22.6% of the resident Italian population and representing 77.8% of the spontaneous reports collected in the IPN during 2011 [11]. The corresponding paediatric population consisted of more than 217,000 children per year [12]. The birth cohort of children in 2012 had the following distribution by region: 41,397 in Emilia-Romagna, 96,602 in Lombardy, 32,473 in Tuscany, 46,588 in Veneto. The vaccine coverage (as completed vaccine course) during 2011 for DTaP, Hib, HBV, and IPV (included in the hexavalent vaccine) was at least 95% in all regions involved [13].

Each report was identified through a unique anonymised code and it was not possible to directly identify the person. We used information on the vaccinee (age, sex), the event(s) (type, date of onset, seriousness and outcome), vaccine(s) administered (type, trade name and date of administration), region and local healthcare facility indicated on the reporting form for the adverse drug reaction (ADR).

We considered two 18-month time periods to estimate the AEFI incidence rates (IR) of PCV7 and PCV13 (alone or co-administered with the hexavalent vaccine), from 1 January 2009 to 30 June 2010 for PCV7 and from 1 July 2010 to 31 December 2011 for PCV13. All participating regional pharmacovigilance centres were asked

to reconfirm the information reported for each case to exclude duplicates, increase data completeness and identify possible misclassification between PCV7 and PCV13. In Italy, during the period of interest, a single hexavalent vaccine was available.

Spontaneous ADR reports were grouped in three different categories, i.e. all the AEFI reported, neurological events, and convulsions. Neurological events were identified through the analysis of the reported AEFI and the related preferred terms coded according to the standardised medical terminology developed by the International Conference of Harmonization (MedDRA) [14]. Only cases with at least one preferred term leading to the primary MedDRA system organ classification (SOC) 'nervous system disorders' were considered as neurological events [15]. In addition, all serious cases were further evaluated (on the basis of the preferred terms, verbatim and other information included in the ADR reporting form) to confirm their inclusion/exclusion from the neurological events analysis.

All neurological reports were then evaluated to identify cases of convulsions (both febrile and afebrile) according to a pre-defined case definition. Cases reporting terms such as 'seizure' or 'convulsion' or 'convulsion and fever' were classified as convulsion/febrile convulsion by default. Cases with less specific terms (e.g. 'tonic-clonic movements', 'hypertonia', 'oculogyric crisis') were classified as convulsion only when two terms indicating loss of consciousness and generalised motor manifestation appeared together in the same report; this is in accordance with current case definition guidelines [16].

Statistical analysis

IR of AEFI were calculated by dividing the number of the AEFI reports by the number of administered vaccine doses (expressed per 100,000 doses). The administered doses in the group receiving single administrations of PCV13 and the hexavalent vaccine at different times were calculated as the sum of the administered doses of PCV13 alone and the hexavalent vaccine alone. Confidence intervals (95% CI) were calculated using the Poisson distribution. Incidence rate ratios (IRR) were estimated using univariate Poisson regression. IR and IRR were estimated for three AEFI groups: (i) all types of AEFI, (ii) neurological events, (iii) convulsions (febrile and afebrile). An attempt to stratify AEFI groups by seriousness of event was made. A standard pharmacovigilance definition for seriousness of cases was used:

"An adverse reaction corresponds to any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, is a congenital anomaly/birth defect [17]"

All events which did not meet the criteria for seriousness were thus considered as non-serious. Because seriousness was analysed as reported and was not modified during the analysis, we need to consider that misclassification may have occurred. This study was not intended to investigate risks by seriousness of events, and the stratified analyses should be viewed as a hypothesis generator only. Statistical analyses were carried out using STATA software version 11.2 (Stata Corporation, College Station, United States).

Results

ADR reports and administered doses

According to the NIP, administration of PCV (7- or 13-valent) together with the hexavalent vaccine was at least four times more frequent than single administration of PCV (7- or 13-valent) or the hexavalent vaccine (Table 1); the number of co-administered doses of PCV (7- or 13-valent) with the hexavalent vaccine was comparable.

Overall, 883 spontaneous reports of AEFI with PCV7, PCV13 or the hexavalent vaccine either as single or concomitant administration were retrieved in the IPN during the period 2009 to 2011 (Table 1); 107 reports were serious (12.1%), including two deaths of which one occurred two days after vaccination in a child suffering from perinatal hypoxic ischaemic encephalopathy and the other was sudden infant death syndrome. At least one neurological event was reported in 15.4% of the reports (136 of 883); 68 (50.0%) neurological events were serious. Of the 136 neurological events, 41 (30.1%) were cases of convulsions and 32 of the 41 were reported as serious. In particular, we found that of 41 cases of convulsion, 26 were reported as febrile convulsion, while the remaining 15 cases were afebrile convulsions; given the small sample we considered convulsion (both febrile and afebrile) as a single category in the analyses.

The majority of reports ($n=537$), 15.4% of which were serious ($n=83$), occurred after co-administration of PCV7 or PCV13 with the hexavalent vaccine ($n=232$ and $n=305$, respectively). The hexavalent vaccine alone was found to be administered in 256 AEFI reports, 5.5% of them serious ($n=14$), while we found only 90 reports following a single administration of PCV7 or PCV13, 10 of which were serious (four with PCV7 and six with PCV13).

Reports of any AEFI (serious and not serious) were equally frequent for any of the three vaccines, whether administered alone or concomitantly. In contrast, serious AEFI, neurological events and convulsions were observed more frequently when PCV13 and the hexavalent vaccine were administered together.

Overall, the demographic characteristics of children experiencing an AEFI were comparable across vaccine groups (Table 2). However, according to the vaccine

TABLE 2

Spontaneous reports of adverse events following vaccination, by sex, age group, and time to onset, four regions in Italy, 2009 to 2011 (n = 883)

	PCV7 alone	PCV13 alone	Hexavalent alone	PCV7 and hexavalent co-administered	PCV13 and hexavalent co-administered
	2009+ Q1–Q2 2010	Q3–Q4 2010 + 2011	2009–2011	2009+ Q1–Q2 2010	Q3–Q4 2010 + 2011
	n (%)	n (%)	n (%)	n (%)	n (%)
Sex^a					
Male	21 (48)	28 (61)	150 (59)	120 (52)	157 (51)
Female	23 (52)	18 (39)	106 (41)	111 (48)	148 (49)
Age group^a					
< 6 months	6 (14)	4 (9)	138 (54)	158 (68)	216 (71)
6–11 months	8 (19)	5 (11)	68 (27)	38 (16)	54 (18)
12–24 months	29 (67)	36 (80)	49 (19)	36 (16)	35 (11)
All AEFI					
Time to onset^b					
0 days	24 (56)	28 (62)	180 (70)	190 (82)	248 (82)
1 days	12 (28)	13 (29)	54 (21)	29 (13)	34 (11)
≥ 2 days	7 (16)	4 (9)	22 (9)	12 (5)	22 (7)
ADR outcome^b					
Resolved/improved	30 (68)	43 (94)	217 (85)	212 (91)	263 (69)
Unresolved ^c	0 (0)	1 (2)	1 (0)	5 (2)	9 (2)
Not available	14 (32)	2 (4)	38 (15)	15 (7)	109 (29)
Neurological events					
Time to onset^b					
0 days	5 (83)	3 (100)	21 (84)	30 (77)	49 (79)
1 days	0	0	1 (4)	7 (18)	10 (16)
≥ 2 days	1 (17)	0	3 (12)	2 (5)	3 (5)
ADR outcome^b					
Resolved/improved	4 (67)	3 (100)	22 (88)	38 (94)	52 (84)
Still not resolved	0	0	0	1 (3)	1 (2)
Not available	2 (33)	0	3 (12)	1 (3)	9 (14)
Convulsions (febrile and afebrile)					
Time to onset^b					
0 days	1 (100)	1 (100)	4 (66)	8 (66)	15 (71)
1 days	0	0	1 (17)	2 (17)	5 (24)
≥ 2 days	0	0	1 (17)	2 (17)	1 (5)
ADR outcome^b					
Resolved/improved	1 (100)	1 (100)	4 (67)	10 (84)	17 (81)
Still not resolved	0	0	0	1 (8)	0
Not available	0	0	2 (33)	1 (8)	4 (19)

ADR: adverse drug reaction; AEFI: adverse events following immunisation; ICSR: individual case safety report; PCV: pneumococcal polysaccharide conjugate vaccine.

^a The sum of the variables sex and age group differs from the overall population enrolled due to missing information reported on the ICSR.

^b The sum of the variables time to onset and ADR outcome differs from the overall population enrolled since such information is referred to the ADRs reported in each ICSR, and each ICSR may include more than one reaction.

^c At the time when the report was filed.

TABLE 3

Comparison of incidence ratios between PCV7 vs PCV13 given in co-administration with the hexavalent vaccine, four regions in Italy, 2009 to 2011 (n = 537)

	PCV7 and hexavalent Co-administered 2009 + Q1–Q2 2010		PCV13 and hexavalent co-administered Q3–Q4 2010 + 2011		PCV13 and hexavalent co-administered vs PCV7 and hexavalent co-administered	
Administered doses (n)		802,126		979,446		
	n	IR (95% CI)	n	IR (95% CI)	IRR	(95% CI)
All AEFI (n = 537)						
Serious	27	3.37 (2.22–4.90)	56	5.72 (4.32–7.42)	1.70	(1.07–2.69)
Not serious	201	25.06 (21.71–28.77)	247	25.22 (22.17–28.57)	1.01	(0.83–1.21)
Undefined	4	0.49 (0.13–1.28)	2	0.20 (0.02–0.74)	0.41	(0.07–2.23)
Total	232	28.92 (25.32–32.89)	305	31.14 (27.74–34.84)	1.08	(0.91–1.28)
Neurological events (n = 102)						
Serious	15	1.87 (1.05–3.08)	39	3.98 (2.83–5.44)	2.13	(1.17–3.86)
Not serious	23	2.87 (1.82–4.30)	23	2.35 (1.49–3.52)	0.82	(0.46–1.46)
Undefined	2	0.25 (0.03–0.90)	0		Not applicable	
Total	40	4.99 (3.56–6.79)	62	6.33 (4.85–8.11)	1.27	(0.85–1.89)
Convulsions, febrile and afebrile (n = 33)						
Serious	8	1.00 (0.43–1.96)	19	1.94 (1.17–3.03)	1.94	(0.85–4.44)
Not serious	4	0.49 (0.13–1.28)	2	0.20 (0.02–0.74)	0.41	(0.07–2.23)
Undefined	0	0	0	0	Not applicable	
Total	12	1.50 (0.77–2.61)	21	2.14 (1.33–3.28)	1.43	(0.70–2.91)

AEFI: adverse events following immunisation; CI: confidence interval; IR: incidence rate; PCV: pneumococcal polysaccharide conjugate vaccine.

strategy adopted in Italy, children up to five completed months of age accounted for ca 70% of reports following co-administration of PCV (7- or 13-valent) with the hexavalent vaccine, while the majority of reports following single administration of PCV7 (67%) or PCV13 (80%) occurred in children that were at least 12 months-old.

On average, onset of the AEFI was on the same day as vaccine administration in 75.4% of the reports, ranging from a minimum of 55.8% for single PCV7 to a maximum of 82.2% for the co-administration of PCV7 with the hexavalent vaccine. The majority of the AEFI reported (86.6%) resolved or improved. No difference in event onset or ADR outcome was found between the two types of co-administrations across different AEFI groups (Table 2).

PCV7 or PCV13 co-administered with the hexavalent vaccine: AEFI incidence rates and incidence rate ratios

Overall, no differences in IR (for all AEFI, neurological events or convulsions) were observed following PCV7 co-administration or PCV13 co-administration (Table 3). The IRR for all AEFI following the co-administration of PCV13 with the hexavalent vaccine compared with the co-administration of PCV7 with the hexavalent vaccine was 1.08 (95% CI: 0.91–1.28). Similarly, the IRR for neurological events or convulsion following co-administration of PCV13 with the hexavalent vaccine were

1.27 (95% CI: 0.85–1.89) and 1.43 (95% CI: 0.70–2.91), respectively. None of the three comparisons were statistically significant. When taking into account the seriousness of reactions, the IRR for all types of AEFI and for neurological events indicated an increased risk reaching statistical significance, and a similar risk trend was observed for serious convulsions (Table 3).

Co-administration of PCV13 with the hexavalent vaccine vs single administration of PCV13 and the hexavalent vaccine: AEFI incidence rates and incidence rate ratios

The AEFI IR for the co-administration of PCV13 with the hexavalent vaccine were compared with those observed when PCV 13 and the hexavalent vaccine were administered in different vaccination sessions. Overall, a lower significant IR for all AEFI was observed following the co-administration of PCV13 and the hexavalent vaccine (Table 4). In case of neurological events and convulsions the IR did not differ between the two groups. When considering the IRR, co-administration of PCV13 with the hexavalent vaccine was also associated with a smaller number of all types of AEFI compared with the single administration of PCV13 and the hexavalent vaccine at different times (IRR: 0.59, 95% CI: 0.49–0.72) (Table 4). This protective effect was not observed for neurological events or convulsions (IRR=1.44; 95% CI: 0.77–2.67 and IRR=1.46; 95% CI: 0.50–4.25, respectively). Taking into account the seriousness of events (serious AEFI overall, serious

TABLE 4

Comparison of incidence ratios of co-administration of PCV13 with the hexavalent vaccine vs separate administration in different vaccination sessions, four regions in Italy, 2009 to 2011 (n = 448)

	PCV13 and hexavalent administered in different vaccination sessions ^a Q3–Q4 2010 + 2011		PCV13 and hexavalent co-administered Q3–Q4 2010 + 2011		PCV13 and hexavalent co-administered in the same session vs single administration in different sessions	
Administered doses (n)	272,494		979,446		Not applicable	
	n	IR (95% CI)	n	IR (95% CI)	IRR	(95% CI)
All AEFI (n = 448)						
Serious	9	3.30 (1.51–6.27)	56	5.72 (4.32–7.42)	1.73	(0.86–3.50)
Not serious	133	48.81 (48.87–57.84)	247	25.22 (22.17–28.57)	0.52	(0.42–0.64)
Undefined	1	0.37 (0.005–2.04)	2	0.20 (0.02–0.74)	0.56	(0.05–6.14)
Total	143	52.48 (44.23–61.82)	305	31.14 (27.74–34.84)	0.59	(0.49–0.72)
Neurological events (n = 74)						
Serious	5	1.83 (0.59–4.28)	39	3.98 (2.83–5.44)	2.17	(0.85–5.50)
Not serious	7	2.57 (1.03–5.29)	23	2.35 (1.49–3.52)	0.91	(0.39–2.13)
Undefined	0	0	0	0	Not applicable	Not applicable
Total	12	4.40 (2.27–7.69)	62	6.33 (4.85–8.11)	1.44	(0.77–2.67)
Convulsions, febrile and afebrile (n = 25)						
Serious	2	0.73 (0.08–2.65)	19	1.94 (1.17–3.03)	2.64	(0.61–11.35)
Not serious	2	0.73 (0.08–2.65)	2	0.20 (0.02–0.74)	0.28	(0.04–1.97)
Undefined	0	0	0	0	Not applicable	Not applicable
Total	4	1.47 (0.39–3.76)	21	2.14 (1.33–3.28)	1.46	(0.50–4.25)

ADR: adverse drug reaction; AEFI: adverse events following immunisation; CI: confidence interval; IR: incidence rate; IRR: incidence rate ratio; PCV: pneumococcal polysaccharide conjugate vaccine.

^a The events following vaccine administration in different vaccination sessions were obtained as the sum of the number of ADR following single PCV13 (Q3–Q4 2010 and 2011) and the number of ADRs following a single hexavalent vaccine dose (Q3–Q4 2010 and 2011).

neurological events and serious convulsions), the IRR showed a trend of increased risk associated with co-administration, although statistical significance was not reached and CI were wide.

Discussion

In this study, we found a trend of a slightly increased risk for neurological events or convulsion after co-administration of PCV13 with the hexavalent vaccine when compared with the co-administration of PCV7 with the hexavalent vaccine. No increased risk emerged when the comparison concerns all types of AEFI. Two factors could be responsible for this observation. The first is the Weber effect, i.e. the increased attention paid by the healthcare professionals (HCPs) and the public to the launch of the new product (PCV13) in mid-2010 replacing the old product (PCV7) which may have led to an increased incidence of reported cases. The second factor is an overall increased vaccine reporting trend over the years in Italy, fuelled by the launch of several active pharmacovigilance projects [18].

The second relevant finding of this study regards the comparison between different vaccination strategies with PCV13 and the hexavalent vaccine (i.e. whether co-administered or not). Co-administration of these two vaccines showed a slightly increased risk trend for neurological events or convulsion. However, when

considering all AEFI, co-administration had a protective effect compared with single administration. Thus, the comparison between the two vaccination strategies seemed to favour co-administration, which is the current immunisation practice in Italy), over single administration. It should be pointed out that the protective effect of co-administration on all AEFI may have been influenced by the fact that the probability for an AEFI to be counted twice is higher when PCV13 and the hexavalent vaccine are given on different days: one AEFI may occur with PCV13 on one day and another AEFI may occur with the hexavalent vaccine on another day.

Only two studies have been published on the post-marketing surveillance of PCV, both based on data from the United States (US) and none aimed at evaluating PCV given in co-administration [19,20]. The first was a descriptive study by Wise et al., presenting ADR reports following PCV7 [19]; the second article by Tseng et al. was based on active surveillance data and investigated the association between pre-specified events (including febrile seizure) with PCV13 or PCV7; it did not find any signal of an increased risk for febrile convulsion [20]. However, this study did not report the CI for risk estimates of febrile seizure and did not consider the effect of co-administration of PCV with other vaccines or other vaccination strategies. Of note, Tseng et al. discussed an ancillary analysis in children receiving

PCV13 and influenza vaccine concomitantly; they found a risk trend for febrile seizure 1.35 times higher (95% CI: 0.93–2.00) than the risk after receiving PCV7 and influenza vaccine concomitantly [20].

A study based on active surveillance data was conducted in the US to investigate a signal of febrile seizures in young children after receipt of trivalent inactivated influenza vaccine (TIV) during the 2010/11 season. This study showed a risk of febrile seizure when TIV was co-administered with PCV13 that was higher than after single administration of TIV or PCV13 [21]. These findings were validated by an observational study conducted in the US which used prospective data collection and showed a higher risk of fever when TIV and PCV13 were co-administered than when either of these vaccines was administered without the other [22]. Increasing evidence suggests that co-administration and type of vaccine co-administered could play a role in the occurrence of an event such as convulsions following PCV. Overall, the results of our study are coherent with available literature data [19–22].

A strength of our study is that it used spontaneous reports from the general population exposed to vaccines without pre-defined exclusion/inclusion criteria. Moreover, our setting involved the whole paediatric population undergoing routine immunisation practice in the four regions included in our analysis. As these regions represent about a quarter of the country's total population, we consider our results to be representative for Italy as a whole.

The quality of spontaneous reporting data in Italy is high since an evaluation of each single case and validation of each report by a trained HCP is required [23]. Furthermore, all cases reporting neurological events were reviewed, and a predefined criterion to identify convulsions was applied.

The main limitation of this study resides in the nature of spontaneous reporting data. Although they allow the detection of less common events such as convulsions, they may contain partial information reported in a narrative way and collected heterogeneously. Moreover, the percentage of under-reporting could be significant, leading to a systematic under-estimation of cases; this would lead to decreased power of the study in detecting differences in the risks of AEFI, ultimately causing a lack of statistical significance and a wider CI. The higher risk estimates for serious cases can be expected to be affected by this methodological limitation. Seriousness has been analysed as indicated on the reports, not modified during the analysis, and could have introduced misclassification; the analyses by seriousness should thus be taken only as hypothesis-generating. Indeed, for a better interpretation of the study findings, it is advisable to refer to the overall risks within each ADR category. No risk adjustments for factors representing potential confounders (underlying diseases, age, sex) was feasible since this information

was not available at individual level for the subjects undergoing vaccination (only administered doses pooled at regional level were retrieved); however, age, sex, time of onset and ADR outcome can be considered to be balanced among the spontaneous reports in each vaccine group, and the influence of these covariates on the risk estimates could be residual. The lack of anamnesis for neurological conditions both in the AEFI reports and in each vaccinee's record is important missing information. Finally, we were not able to carry out any analyses by received dose since this information is not systematically included on the spontaneous report forms.

Even though safety surveillance based on spontaneously reported data is not intended to provide evidence on causality, it is a useful method to rapidly quantify associations between vaccines and less common adverse events. Moreover, preliminary findings based on surveillance data could help in designing further investigations to deliver more robust evidence.

Conclusion

Our analysis showed a trend of a slightly increased risk of neurological events or convulsions following vaccination with PCV13 compared with PCV7 when both were used in routine vaccination practice with the hexavalent vaccine. Similarly, we found an increased risk of neurological events or convulsions (although not reaching statistical significance) when PCV13 was co-administered with the hexavalent vaccine compared with single administration of both vaccines at different times. Given the limitations highlighted, our findings cannot be considered conclusive. Moreover, it should be underlined that such risks should be viewed in the context of the overall benefit of both vaccines.

While we continue monitoring reports of less common and potentially serious AEFI, further research should be conducted using different data sources that also account for dosing schedule, subjects' characteristics and co-morbidities. This study indicates that the evaluation of co-administration of PCV with other vaccines during a single session is a relevant issue for public health research. Our findings may also contribute to pooled estimates together with those of similar investigations.

Acknowledgements

Only public employees of the national or regional health authorities were involved in conceiving, planning, and conducting the study; no additional funding was received.

Disclaimer: The opinions expressed in this article do not necessarily reflect the view of the authors' institutions.

Conflict of interest

None declared.

Authors' contributions

All authors conceived the study; FT, CR, CS, AB designed the study and analysed the data; FT, CR, CS, AB wrote the manuscript. All authors contributed to the discussion and reviewed the manuscript. All authors saw, commented upon and approved the final version of the paper.

Members of the Pharmacovigilance Study Group on Pneumococcal Vaccination in Children

Francesco Trotta (Pharmacovigilance Unit, Italian Medicines Agency and National Centre of Epidemiology, National Institute of Health, Rome); Carmela Santuccio, Patrizia Felicetti (Pharmacovigilance Unit, Italian Medicines Agency, Rome); Antonino Bella, Caterina Rizzo (National Centre of Epidemiology, National Institute of Health, Rome); Valentino Conti, Giuseppe Monaco (The Lombardy Region); Francesca Russo, Giovanna Zanoni (The Veneto Region); Loredana Osbello, Maria Grazia Pascucci (The Emilia Romagna Region); Maria Parrilli, Marco Rossi (The Tuscany Region).

References

1. Indicazioni in merito alla somministrazione del vaccino antipneumococcico Prevenar 13 in età pediatrica. [Recommendations on the administration of PCV13 in the paediatric population]. Rome: Ministry of Health. 27 May 2010.
2. Piano nazionale vaccini 2005-2007. [National immunization schedules 2005-2007]. Rome: Ministry of Health. 31 Dec 2005. Italian. Available from: http://www.salute.gov.it/portale/documentazione/p6_2_2_1.jsp?lingua=italiano&id=543
3. Vaccinazione antipneumococcica in età pediatrica. [Antipneumococcal vaccination in paediatric age]. Rome: Ministry of Health. 19 Nov 2001. Italian. Available from: http://www.salute.gov.it/imgs/C_17_normativa_78_allegato.pdf
4. Alfonsi V, D'Ancona F, Giambi C, Nacca G, Rota MC, Regional Coordinators for Infectious Diseases and Vaccinations. Current immunization policies for pneumococcal, meningococcal C, varicella and rotavirus vaccinations in Italy. *Health Policy*. 2011;103(2-3):176-83. <http://dx.doi.org/10.1016/j.healthpol.2011.10.002> PMID:22030308
5. Piano nazionale prevenzione vaccinale 2012-2014. [National immunisation schedules 2012-2014]. Rome: Ministry of Health 5 April 2012 [Accessed: 14 Jan 2014]. Italian. Available from: http://www.salute.gov.it/imgs/C_17_pubblicazioni_1721_allegato.pdf
6. Esposito S, Tansey S, Thompson A, Razmpour A, Liang J, Jones TR, et al. Safety and immunogenicity of a 13-valent pneumococcal conjugate vaccine compared to those of a 7-valent pneumococcal conjugate vaccine given as a three-dose series with routine vaccines in healthy infants and toddlers. *Clin Vaccine Immunol*. 2010;17(6):1017-26. <http://dx.doi.org/10.1128/CVI.00062-10> PMID:20427630
7. Kieninger DM, Kueper K, Steul K, Juergens C, Ahlers N, Baker S, et al. Safety, tolerability, and immunologic noninferiority of a 13-valent pneumococcal conjugate vaccine compared to a 7-valent pneumococcal conjugate vaccine given with routine pediatric vaccinations in Germany. *Vaccine*. 2010;28(25):4192-203. <http://dx.doi.org/10.1016/j.vaccine.2010.04.008> PMID:20417262
8. Frequently Asked Questions about Febrile Seizures Following Childhood Vaccinations. Atlanta: Centers for Disease Control and Prevention. [Accessed: 14 Jan 2014]. Available from: <http://www.cdc.gov/vaccinesafety/Concerns/FebrileSeizures.html>
9. Cendes F, Sankar R. Vaccinations and febrile seizures. *Epilepsia*. 2011;52(Suppl 3):23-5. <http://dx.doi.org/10.1111/j.1528-1167.2011.03032.x> PMID:21542842
10. Santuccio C, Menniti-Ippolito F, Tartaglia L, Trotta F, Morlino D, Moretti U. Spontaneous Reporting of Adverse Reactions to Pneumococcal Vaccination in Children. *Drug Saf*. 2011;34(10):924-5.
11. Rapporto sulla sorveglianza postmarketing dei vaccini in Italia, 2011. [Report on the post-marketing surveillance of vaccines in Italy, 2011]. Rome: Agenzia Italiana del Farmaco (AIFA). [Accessed: 14 Jan 2014]. Italian. Available from: http://www.agenziafarmaco.gov.it/sites/default/files/Copertina%20+%20Rapporto%20vaccini%202011_o.pdf
12. Resident population in Italy. Rome: The National Institute for Statistics. [Accessed: 21 Sep 2014]. Italian. Available from: <http://demo.istat.it/index.html>
13. Coperture vaccinali. [Vaccine coverage]. Rome: Ministry of Health. 4 January 2008. [Accessed: 21 Sep 2014]. Italian. Available from: http://www.salute.gov.it/imgs/C_17_pagineAree_811_listaFile_itemName_15_file.pdf
14. Multidisciplinary Guidelines.M1 MedDRA Terminology. Geneva: The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). [Accessed: 14 Jan 2014]. Available from: <http://www.ich.org/products/guidelines/multidisciplinary/article/multidisciplinary-guidelines.html>
15. Introductory guide MedDRA Version 15.1. Geneva: The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). Sep2012. Available from: http://www.meddra.org/sites/default/files/guidance/file/intguide_15_1_English_o.pdf
16. Bonhoeffer J, Menkes J, Gold MS, de Souza-Brito G, Fisher MC, Halsey N, et al. Generalized convulsive seizure as an adverse event following immunization: case definition and guidelines for data collection, analysis, and presentation. *Vaccine*. 2004;22(5-6):557-62. <http://dx.doi.org/10.1016/j.vaccine.2003.09.008> PMID:14741144
17. European Directive. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. Official Journal of the European Union (OJ) L 311, 2001. Available from: http://www.echamp.eu/fileadmin/user_upload/Regulation/Directive_2001-82-EC_-_Consolidated_Version_.pdf
18. Trotta F, Alessandro A, Tartaglia L. Rapporto sul programma di farmacovigilanza attiva finanziato attraverso i fondi regionali disponibili per gli anni 2008 e 2009. [Report on the active pharmacovigilance programme funded with regional funds available during fiscal year 2008 and 2009]. Rome: Agenzia Italiana del Farmaco. 2013. [Accessed: 21 Nov 2013]. Italian. Available from: http://www.agenziafarmaco.gov.it/sites/default/files/AIFA_Rapporto%20Fondi_FV.pdf
19. Wise RP, Iskander J, Pratt RD, Campbell S, Ball R, Pless RP, et al. Post licensure safety surveillance for 7-valent pneumococcal conjugate vaccine. *JAMA*. 2004;292(14):1702-10. <http://dx.doi.org/10.1001/jama.292.14.1702> PMID:15479935
20. Tseng HF, Sy LS, Liul L, Qian L, Marcy SM, Weintraub E, et al. Post licensure surveillance for pre-specified adverse events following the 13-valent pneumococcal conjugate vaccine in children. *Vaccine*. 2013;31(22):2578-83. <http://dx.doi.org/10.1016/j.vaccine.2013.03.040> PMID:23579258
21. Tse A, Tseng HF, Greene SK, Vellozzi C, Lee GM, VSD Rapid Cycle Analysis Influenza Working Group. Signal identification and evaluation for risk of febrile seizures in children following trivalent inactivated influenza vaccine in the Vaccine Safety Datalink Project, 2010-2011. *Vaccine*. 2012;30(11):2024-31. <http://dx.doi.org/10.1016/j.vaccine.2012.01.027> PMID:22361304
22. Stockwell MS, Broder K, LaRussa P, Lewis P, Fernandez N, Sharma D, et al. Risk of fever after pediatric trivalent inactivated influenza vaccine and 13-valent pneumococcal conjugate vaccine. *JAMA Pediatr*. 2014;168(3):211-9. <http://dx.doi.org/10.1001/jamapediatrics.2013.4469> PMID:24395025
23. Bergvall T, Noren GN, Lindquist M. Great case reports, where do they come from? *Drug Saf*. 2013;36(9):858.

Assessment of human influenza pandemic scenarios in Europe

C Napoli¹, M Fabiani¹, C Rizzo (caterina.rizzo@iss.it)¹, M Barral², J Oxford³, J M Cohen⁴, L Niddam⁵, P Goryński⁶, A Pistol⁷, C Lionis⁸, S Briand⁹, A Nicoll¹⁰, P Penttinen¹⁰, C Gauci¹¹, A Bounekkar¹², S Bonnevey¹², A Beresniak¹³

1. Istituto Superiore di Sanità (ISS), Rome, Italy
2. Basque Institute for Agricultural Research and Development, Biscay, Spain
3. Retroscreen Virology, London, United Kingdom
4. Open Rome, Paris, France
5. Niddam European Community Lawyer, Budapest, Hungary
6. Polish National Institute of Public Health, Warsaw, Poland
7. Romanian National Institute of Public Health, Bucharest, Romania
8. University of Crete, Crete, Greece
9. World Health Organization (WHO), Geneva, Switzerland
10. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
11. Ministry of Health, Elderly and Community Care, Valletta, Malta
12. Université Claude Bernard Lyon 1, Lyon, France
13. Université Paris Descartes, Paris, France

Citation style for this article:

Napoli C, Fabiani M, Rizzo C, Barral M, Oxford J, Cohen JM, Niddam L, Goryński P, Pistol A, Lionis C, Briand S, Nicoll A, Penttinen P, Gauci C, Bounekkar A, Bonnevey S, Beresniak A. Assessment of human influenza pandemic scenarios in Europe. *Euro Surveill.* 2015;20(7):pii=21038. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21038>

Article submitted on 08 January 2014 / published on 19 February 2015

The response to the emergence of the 2009 influenza A(H1N1) pandemic was the result of a decade of pandemic planning, largely centred on the threat of an avian influenza A(H5N1) pandemic. Based on a literature review, this study aims to define a set of new pandemic scenarios that could be used in case of a future influenza pandemic. A total of 338 documents were identified using a searching strategy based on seven combinations of keywords. Eighty-three of these documents provided useful information on the 13 virus-related and health-system-related parameters initially considered for describing scenarios. Among these, four parameters were finally selected (clinical attack rate, case fatality rate, hospital admission rate, and intensive care admission rate) and four different levels of severity for each of them were set. The definition of six most likely scenarios results from the combination of four different levels of severity of the four final parameters (256 possible scenarios). Although it has some limitations, this approach allows for more flexible scenarios and hence it is far from the classic scenarios structure used for pandemic plans until 2009.

Introduction

Before the 2009 influenza A(H1N1) pandemic, most European Union (EU) Member States had developed preparedness plans in order to timely respond to an eventual pandemic. Many of these plans involve explicit or implicit planning assumptions on what can be expected during a pandemic and on how a pandemic virus might behave [1].

The response to the emergence of the 2009 influenza A(H1N1) pandemic was the result of a decade of

pandemic planning, largely centred on the threat of an avian influenza A(H5N1) pandemic. However, the influenza A(H5N1) and the 2009 pandemic influenza A(H1N1) viruses have markedly different characteristics in terms of mortality among confirmed cases and human-to-human transmission [2,3]. Moreover, the 2009 pandemic influenza A(H1N1) virus caused illness that did not require hospitalisation in the vast majority of cases, and was a highly transmissible virus among humans spreading to several countries within days [3,4].

In this situation, the severity assessment applied during the 2009 influenza A(H1N1) pandemic using a variety of indicators leading to a qualitative assessment in three levels (i.e. mild, moderate and severe) was not specific enough to guide interventions [5,6].

After the pandemic, the International Health Regulations (IHR) Pandemic Review Committee encouraged the World Health Organization (WHO) to develop and utilise measures to assess the severity of every influenza epidemic by applying, evaluating and refining tools to measure severity every year [7]. WHO has recently developed a new document for Pandemic Influenza Risk Management [8].

The 2009 influenza pandemic highlighted the importance of quantitatively defining different scenarios; severity should be assessed as early as possible during a pandemic and continually re-assessed as the pandemic evolves and new information becomes available.

This work has been conducted in the frame of the European Commission project FLURESP (Cost-effectiveness assessment of European influenza human pandemic alert and response strategies) with the aim to define a set of scenarios to be used for a future pandemic planning (www.fluresp.eu).

Methods

Literature review and selection of parameters

The literature search was conducted by consulting Medline, restricting it to articles published until December 2011. Seven different sets of keywords were considered (Table 1).

A systematic selection procedure was conducted in two steps by two researchers independently. In the first step, the major topics of the articles were assessed by title and abstract. In this phase of the selection procedure, all articles reporting epidemiological data on influenza pandemics were included. In case of doubt on the article's relevant information, the article was included in the second selection step.

In the second step, the full text articles, previously selected, were assessed. These articles were included in the review if they reported at least one of the following 13 parameters: basic reproductive number (R_0); clinical attack rate (CAR); age-specific CAR; case fatality rate (CFR); communicability/generation interval; modes of transmission; incubation period; timing and duration of pandemic; clinical consultation rate (CCR); hospital admission rate (HAR); intensive care admission rate (ICAR); work absenteeism; bed occupancy rate (BOR). If the articles included did not contain

information on at least one of the parameters listed above or if the study design was of low quality (e.g. small sample size, unclear definition of outcomes), they were excluded. Moreover, pertinent related citations were considered.

Of each article included in the review, the following data were recorded: year of the study, year of pandemic referring to, country, and described parameters.

International technical reports were obtained by consulting the websites of the European Centre for Disease Prevention and Control (ECDC) and WHO. Influenza pandemic preparedness plans for the European Union/European Economic Area (EU/EEA) countries were obtained from the ECDC website [9]. We also considered relevant studies based on mathematical modelling published in the literature but not retrieved through the search strategy.

Parameters collected through the literature review were subsequently discussed within the FLURESP Project by a panel of experts composed of collaborators from international (WHO and ECDC) and national public health organisations (from France, Italy, Spain and the United Kingdom) who selected the parameters to be used for defining scenarios.

Definition of severity profiles and scenarios

For each of the selected parameters, four severity profiles were defined. In order to set the profiles, ranges of variability for each of the parameters were categorised into a four-group scale, according to a quartile distribution. We then adjusted the ranges for each of the four groups, according to the suggestions made by the panel of experts. Based on the possible combination of the four severity profiles of each of the four parameters, a set of scenarios were defined.

Results

Parameters selected

From the literature review we collected information on 13 parameters as potential candidates for defining the pandemic scenario. These parameters were divided into eight *virus-related* (R_0 ; CAR; age-specific CAR; CFR; communicability/generation interval; modes of transmission; incubation period; timing and duration of pandemic) and five *health-system-related* (CCR; HAR; ICAR; work absenteeism; BOR).

The panel of experts was of the opinion that some of the parameters collected through the literature review were more relevant for mathematical modelling than for public health purposes, and others were considered less relevant for defining scenarios; consequently, all these were excluded: R_0 ; age-specific CAR; communicability/generation interval; modes of transmission; incubation period; timing and duration of pandemic. For example, R_0 (the average number of secondary infections produced by a single infected individual while

TABLE 1

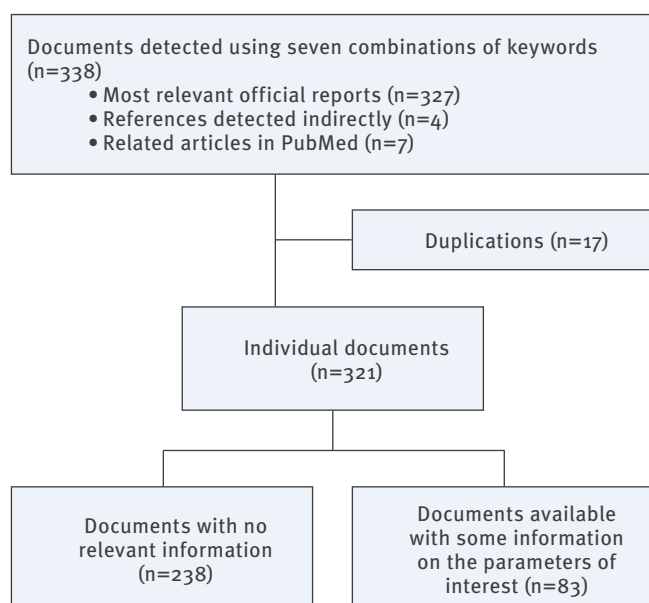
Sets of keywords used in searching scientific articles and number of articles retrieved for the study on pandemic influenza scenarios in Europe

Sets of keywords	Original articles	Reviews	Total
'Human influenza pandemic description'	20	6	26
'Influenza outbreak parameters estimation'	7	0	7
'Influenza scenario description'	2	0	2
'Influenza pandemic scenario'	88	15	103
'Influenza pandemic scenario description'	2	0	2
'Influenza pandemic parameter estimation'	3	0	3
'Influenza pandemic investigation'	148	22	170
Total	270	43	313

The search was restricted to articles published until December 2011.

FIGURE 1

Flowchart outlining the selection of documents for the study on pandemic influenza scenarios in Europe



The search was restricted to articles published until December 2011.

they are infectious, in an entirely susceptible population), incubation period, and the generation interval (defined as the mean duration between time of infection of a secondary infected individual and the time of infection of their primary infector), are measures of the degree of transmissibility of an infection and in combination might affect CAR. Age-specific CAR in most of the considered influenza pandemics were derived from studies conducted in small and selected communities not representative of the entire population, while the timing and duration of pandemic is expected to be from several weeks to a few months but will likely vary from country to country or within a single country. Therefore, these parameters were not considered in this study. Additionally, the contribution and clinical importance of potentially different modes of transmission of influenza are unknown and therefore were considered not relevant.

Thus, according to the opinion of the panel of experts, four parameters were selected to be used for defining scenarios for pandemic planning. The two *virus-related* parameters are listed below with their limitations:

- CAR, the proportion of the population with clinical symptoms over a specified period of time. Some individuals may not develop symptoms severe enough to be readily identified as acute respiratory infection (ARI) or influenza-like-illness (ILI). The measured CAR is thus not always the number of individuals who actually develop symptoms, and

may also include the number of individuals seeking healthcare.

- CFR, represented by the proportion of individuals who develop influenza symptoms, and die because of complications. The measured CFR could be affected by the laboratory confirmation that may be unavailable to validate the total number of cases. Moreover, the confirmation is likely biased to more severe cases. This results in an overestimation of the clinical severity of the disease, especially in case of people with underlying conditions that are at higher risk of death.

The *health-system-related* parameters deal with virulence (i.e. the ability of the virus to invade the tissues of the host and produce pathologic effects and complications) and impact (i.e. the effect on the health-care sector) of the virus on the population. The most relevant *health-system-resource* utilisation parameters used to define pandemic scenarios are listed below with their limitations:

- HAR, represented by the proportion of population hospitalised for confirmed influenza independently from the presence of complications. This measure is strongly affected by how the healthcare systems in different countries are structured.
- ICAR, the proportion of hospitalisations for confirmed influenza that are treated in an intensive care unit (ICU) for influenza complications.

The ICAR could also be related to the level of virulence of the virus, since it is a proxy for the level of severity.

Literature review

A total of 338 documents (including technical reports and scientific articles and reviews) were identified using our search strategy with the seven sets of keywords. Of these, 17 were duplicated articles and 238 showed no relevant information on the selected parameters (Figure 1). In conclusion, 83 articles and documents reporting information on the parameters listed above were considered for this study.

The year of publication of these documents ranges from 2003 to 2011 with more than half of the documents published between 2009 and 2010. When evaluating the performance of keywords' combinations selected, 26% (83/321) of the detected documents provided useful information on the parameters for defining scenarios. The largest number of documents was detected using three sets of keywords (293/321, 91%) (Table 1).

The keywords combination 'human influenza pandemic description' provided the highest proportion of useful documents (10/28, 36%).

The range estimates for the parameters derived from the 83 selected documents and their specific references are listed in Table 2.

TABLE 2

Range of values and references for the main parameters selected for the study on pandemic influenza scenarios in Europe

Parameters	Minimum	Maximum	References
Reproductive number (R_0)	0.99	3.75	[14, 34, 47–67]
Clinical attack rate (%)	0	50	[9,14,20,21–28,54,55,57,61,63,68,69,70–75,77–87]
Case fatality rate (%)	0	25	[9,14,20,27,31,42,54,57,59–62,66,70,71,73,75,82,83,88–98]
Generation interval (days)	1.6	4.1	[58,65,66]
Duration of infection (days)	1	21	[14,54,55,61,63]
Mode of transmission	NA	NA	[54,55,61,63,96,99,100]
Incubation period (days)	0.5	7	[14,20,26,33,48,54,63,66,69,76,99–102]
Pandemic duration (days)	0	180	[54,63,69,91,94,95,103]
Clinical consultation rate (%)	14	73	[24,52,54,55,61,76,104]
Hospital admission rate (%)	0	27.5	[24,31,32,42,52–55,61,63,65,69,70,71,75,77,82,88,90,91,94–96,105–107]
Intensive care admission rate (%)	0	34	[9,31,32,42,43,55,71,82,90,91,94–97]
Absenteeism (%)	0	40	[24,54,64,71,76,97,108]
Bed occupancy rate (%)	0	37	[109]

NA: not applicable.

Of the 83 relevant articles, 23 articles reported information on R_0 , with values ranging from 0.99 to 3.75. Thirty-four reported data on CAR, whose values ranged widely between 0 and 50%, while eight documents provided some information on age-specific CAR. For the CFR, 30 articles showed a range between 0 and 25%. Only three articles dealt with the generation interval, whose range was 1.6–4.1 days. The duration of infectiousness, reported in five articles, ranged between one and 21 days. Only seven articles provided generic descriptions of possible modes of transmission: all of them reported the respiratory route by droplets of infected secretions and/or hand-face contact after touching a contaminated person or surface. The incubation period, described in 12 articles, ranged from 0.5 to seven days, while the pandemic duration varied from 0 to 180 days according to seven articles. Moving to health system resource utilisation parameters, CCR ranged from 14% to 73% (seven articles); HAR ranged from 0% to 27.5% (26 articles); ICAR ranged from 0% to 34% (13 articles); work absenteeism ranged from 0 to 40% (seven articles); and BOR was between 0% and 37% of total critical care bed capacity according to one article.

Parameters collected from historical influenza pandemics

We also investigated parameters collected during the three significant influenza pandemics that occurred in the 20th century: 1918/19, 1957/58, and 1968/69 to 1969/70 (two waves) [5,10,11] (Table 3).

In some European countries (UK in particular) there were three waves associated with the 1918/19 pandemic [12]. In the UK, the wave structure of this pandemic is not well understood; the final 1919 wave may have been a separate pandemic of a different virus to the 1918 waves. The smallest of the waves was in July–August 1918, the largest second wave was from October 1918 to January 1919, and the third wave was from February to April 1919 [13]. Estimates of the national CAR vary in the UK, but suggest that nationally it was around 25% of the population (totalled over all waves). The highest CAR were observed in the young population. Estimates of the CFR are around 2%, relatively evenly spread across the population, though with an excess in young adults [12]. The 1957/58 pandemic had one wave. Estimates of the CAR vary, but suggest that nationally it was around 30% of the population.

TABLE 3

Relevant parameters collected in the four past influenza pandemics for the study on pandemic influenza scenarios in Europe

Season	Clinical attack rate (%)	Complication rate (%)	Hospital admission rate (%)	Case fatality rate (%)
1918/19	25	20	4	2–3
1957/58	30	2.7	<0.6	<0.2
1968/69–1969/70	35	2.7	<0.6	<0.2
2009/10	5	5–16 in at-risk groups <0.02–1 in healthy individuals	<0.02–1	<0.048 (influenza-like illness rate)

TABLE 4

Severity profile for the selected parameters, study on pandemic influenza scenarios in Europe

Parameters	Type of parameters	Severity profile (%)			
Clinical attack rate	Transmission	0–5	5–10	10–25	25–35
Case fatality rate	Virulence	0–0.01	0.01–0.05	0.05–0.8	0.8–2.5
Hospital admission rate	Use of medical resources	0–0.02	0.02–0.2	0.2–2	2–4
Intensive care admission rate		0–0.01	0.01–2.5	2.5–5	5–35

These severity profiles do not take into account any mitigation or control measures.

Estimates of the CFR are around 0.1–0.2%. These average figures mask the considerable variation by age, most deaths being in the older adult population. However, the highest number of cases was registered in the young individuals [14]. The 1968/69 pandemic came in two waves in Europe [15]. Estimates of the national CAR vary, but based on comparisons with the epidemic in the United States (US), it may have been around 35% of the population [12]. Estimates of the CFR are less than 0.2%. These average figures for mortality mask the considerable variation by age, again, with most deaths recorded in the older adult population.

Parameters collected from the 2009 influenza pandemic The recent influenza A(H1N1) pandemic in 2009/10 produced no major signal of excess deaths in the overall population [16], and most of the EU countries have reported data on those that have died from confirmed 2009 pandemic influenza A(H1N1) as a result of influenza A(H1N1) virus infection, but case ascertainment is unlikely to have been complete, and the true number is almost certainly higher [17–19].

Rates from the ILI surveillance systems across Europe showed that consultations were highest in the young. There was only one wave, except for the UK where two ‘waves’, one immediately following the other, were observed. There were high levels of background immunity among elderly.

In general, estimates of the CAR for the 2009 pandemic influenza A(H1N1) vary among countries: 0.01% in the central region of Portugal [20], 0.072% in Mexico [21], 18.3% in New Zealand [22], 30% in the Netherlands [23]. Across Europe the estimated CAR was 30% [24]. This variation reflects the different methods used to get the data: e.g. seroprevalence studies, epidemiological studies in different populations, mathematical models, etc. Other experiences in smaller groups of population provide additional results: 3.15% in a train in China [25], 4% in a primary school in China [26], 22% on a Peruvian Navy ship [27], 28.5% during an outbreak investigation in Nepal [28].

In the UK, figures used to track the epidemic suggest a CAR of 1–2% and modelling studies suggest that these estimates reflect only around 10% of those infected [29], which is consistent with the results of the

serological analysis of the first wave [30]. If only half of those infected were symptomatic, although possibly with very mild symptoms (as this is typical for influenza), the CAR would be around 5–10%. If so, estimates of the CFR are around 0.01% [31,32], but higher levels have been reported in the literature: up to 0.05% in the US [31], 0.1% in Spain [33], and 0.35% in Europe [24], 0.6% in Mexico [34]. In terms of age groups, mortality was spread evenly across the age groups although most cases were reported in the younger age groups.

Definition of severity profiles and scenarios

Table 4, shows the severity profile for each of the four selected parameters derived from the literature review and selected by the suggestions of the panel of experts.

With regard to CAR, the literature review reported data ranging from 0 to 50%. Nevertheless, since the maximum value of 50% refers to the extreme value reported during the 1889 ‘Russian’ pandemic [13,35–39], this value was excluded and, therefore, CAR maximum value was set at 35%.

Also for CFR, from the literature review the observed values ranged from 0% to 25% (Table 2). However, some of the data collected from the literature review were estimates of CFR derived from different populations (often representing high-risk groups) and source of information (mortality associated with the 2009 pandemic influenza A(H1N1) was estimated 15 times higher than reported laboratory-confirmed deaths) [40]. Moreover, when considering the influenza A(H5N1) avian influenza virus: CFR estimates reported by WHO for the ongoing outbreak is around 60% [41], even if, findings from a study based on surveillance and seroprevalence data published in 2008, reports estimates ranging from 14 to 33% [42]. For this reason we set the maximum level of the CFR at 2.5%, as most of values from recent pandemics ranged from 0.01 to 2.5% [13,14,32] (Table 3).

HAR depends on the level of virulence of the pandemic virus. However, its estimation may be affected by access to healthcare, proportion of chronic medical conditions in the population, pregnancy, and the virus characteristics (e.g. the level of pre-existing immunity, and pathogenicity of the virus itself). In our literature

FIGURE 2

Six possible scenarios identified by a combination of the selected parameters, study on pandemic influenza scenarios in Europe

A

Type of parameters	Parameters	Scenario 'seasonal-like'			
Transmission	CAR (%)	0–5	5–10	10–25	25–35
Virulence	CFR (%)	0–0.01	0.01–0.05	0.05–0.8	0.8–2.5
Use of medical resources	HAR (%)	0–0.02	0.02–0.2	0.2–2	2–4
	ICAR (%)	0–0.01	0.01–2.5	2.5–5	5–35

B

Type of parameters	Parameters	Scenario '2009 pandemic-like'			
Transmission	CAR (%)	0–5	5–10	10–25	25–35
Virulence	CFR (%)	0–0.01	0.01–0.05	0.05–0.8	0.8–2.5
Use of medical resources	HAR (%)	0–0.02	0.02–0.2	0.2–2	2–4
	ICAR (%)	0–0.01	0.01–2.5	2.5–5	5–35

C

Type of parameters	Parameters	Scenario 'community risk, low virulence'			
Transmission	CAR (%)	0–5	5–10	10–25	25–35
Virulence	CFR (%)	0–0.01	0.01–0.05	0.05–0.8	0.8–2.5
Use of medical resources	HAR (%)	0–0.02	0.02–0.2	0.2–2	2–4
	ICAR (%)	0–0.01	0.01–2.5	2.5–5	5–35

D

Type of parameters	Parameters	Scenario 'community risk, high virulence'			
Transmission	CAR (%)	0–5	5–10	10–25	25–35
Virulence	CFR (%)	0–0.01	0.01–0.05	0.05–0.8	0.8–2.5
Use of medical resources	HAR (%)	0–0.02	0.02–0.2	0.2–2	2–4
	ICAR (%)	0–0.01	0.01–2.5	2.5–5	5–35

E

Type of parameters	Parameters	Scenario 'high-risk groups/age groups'			
Transmission	CAR (%)	0–5	5–10	10–25	25–35
Virulence	CFR (%)	0–0.01	0.01–0.05	0.05–0.8	0.8–2.5
Use of medical resources	HAR (%)	0–0.02	0.02–0.2	0.2–2	2–4
	ICAR (%)	0–0.01	0.01–2.5	2.5–5	5–35

F

Type of parameters	Parameters	Scenario 'major event'			
Transmission	CAR (%)	0–5	5–10	10–25	25–35
Virulence	CFR (%)	0–0.01	0.01–0.05	0.05–0.8	0.8–2.5
Use of medical resources	HAR (%)	0–0.02	0.02–0.2	0.2–2	2–4
	ICAR (%)	0–0.01	0.01–2.5	2.5–5	5–35

CAR: clinical attack rate; CFR: case fatality rate; HAR: hospital admission rate; ICAR: intensive care admission rate.

The values of the parameters defining scenarios are highlighted in grey.

review we obtained values ranging from 0 to 27.5%. As the extreme value refer to pandemic 1918/19 in the US Army Camps, according to the opinions of the panel of experts set the maximum value to 4% as this value was collected in the general population [5].

For ICAR, it ranged from 0 to 35% in the literature review, and we considered the maximum of 35% as 'reasonable worst case' given that it was derived from an accurate evaluation of laboratory-confirmed influenza hospitalised cases conducted in the US [43].

Based on the above described ranges of values, it is possible to define 256 different scenarios. Among these, we selected six reasonable scenarios, according to the opinion of the panel of experts (Figure 2).

In detail, scenario A represents a 'seasonal-like' influenza outbreak; scenario B describes a situation in which the virus is quite diffusive, with an important HAR and a low virulence and ICAR, similar to the 2009 pandemic. The high HAR in scenario B could also represent high-risk groups (e.g. elderly, individuals with underlying conditions). Scenario C and D represent a situation in which the CAR is high with a low and a high virulence, respectively. Moreover, since the scenarios described above do not take into account the age profile of the population and the proportion of individuals

with chronic conditions, we consider a reasonable solution to retain scenario E and F that represent the worst case scenarios for at-risk groups.

Discussion

In our study we defined a set of scenarios that may be useful for pandemic planning. We used the combination of four severity profiles of four epidemiological parameters to identify 256 possible scenarios that can be adapted over time and are far from the classic scenarios structure used for pandemic plans up to the 2009 influenza pandemic [1]. Among the scenarios identified, on the basis of a literature review and of the opinion of the panel of experts, we selected the six most likely scenarios that synthesise the possible effect of an influenza outbreak with different characteristics (from a seasonal-like to a major event).

Historically, influenza pandemic planning has been based on an assessment of the 'reasonable worst case', derived from previous influenza seasons and pandemics in the 20th century, and thus has shown not to be appropriate during a moderate event, such as the 2009 pandemic [44]. Other experiences reported a modelling approach using a combination of indicators leading to a qualitative assessment in three levels (i.e. mild, moderate and severe) [5]; that approach was considered not to be specific enough to guide

interventions [6]. Moreover, mathematical modelling based on preliminary epidemiological data is useful in defining the impact and the mitigation measures to be implemented during a pandemic. However, these models are strongly affected by the epidemiological parameters used and, even if they are able to explore a wide range of values, they need a specific set of scenarios to produce reliable results. For this reason, the use of scenarios in pandemic planning is crucial.

In our literature review, most of the selected articles and documents were observational studies, mathematical simulations, or reviews. Information referring to different world regions, different population subgroups and different influenza pandemics (mostly the 2009 influenza pandemic) over 100 years-period made comparison of results difficult. In fact, our results showed that most of the parameters values vary a lot between different countries and in different pandemics. For example, mortality rates often vary by age: age-specific mortality rates for 1957/58 and 1968/69 show a U-shaped pattern with a slightly increased CFR in the very young and an increasing one with older age [45]. On the other hand, during the 1918 pandemic, a higher mortality rate was observed in young adults followed by lower rates in other age groups [35]. Moreover, variation in epidemiological parameters could also reflect differences in the surveillance systems (e.g. in case of different case definitions, time lag between influenza confirmation and death, etc.) and diagnostic methods. This heterogeneous information presented in the documents did not allow us to use parts of the data (e.g. the absolute number of deaths was neglected where the corresponding denominator to calculate CFR was missing). It should also be noted that we did not consider age groups and the proportion of people with other underlying conditions that are strictly related to the vulnerability of the population to a pandemic virus [6]. Finally, estimates for the 2009/10 pandemic are likely to change as further data and studies become available after the literature review was conducted (December 2011).

The experience of the 2009 influenza pandemic showed that the EU countries had prepared for a pandemic of high severity but appeared unable to adapt their national and subnational responses adequately to a more moderate event. Knowledge of past pandemics is of substantial help when planning for a future one [46] and indeed the epidemiological aspects of the three 20th century influenza pandemics (1918/20, 1957/58, 1968/69) are of outstanding importance. However, modelling studies based on epidemiological parameters collected during the 20th century pandemics overestimated the impact of the 2009/10 pandemic [8]. Furthermore, society has undergone major changes since 1918 (the scenario on which most pandemic plans and models before and during the 2009 pandemic have been based) and even since 1968, with an increased availability of ICUs and clinical countermeasures (such as vaccines, antivirals, etc.).

Thus, in June 2013, the WHO published the 'Pandemic Influenza Risk Management' [8]. The approach taken in this document introduces a risk-based approach to pandemic influenza risk management and encourages countries to develop flexible plans, and to conduct risk assessments in order to prioritise the development of risk management programmes tailored to the hazards present. Our results are in line with the 'WHO Pandemic Influenza Risk Management' [8] and provide a description of possible scenarios of pandemic influenza considering key epidemiological parameters. The described scenarios allow severity assessments and provide the basis for developing flexible risk management plans over the course of a pandemic.

In the context of the FLURESP Project, the proposed scenarios have been used to select potential response strategies (clustered and ranked according to performance and efficiency using a multi-criteria analysis) in order to conduct cost-effectiveness evaluations to compare cost and performance of response strategies for each proposed scenario.

Our study, although not based on a standardised procedure, is supported by an extensive literature review and suggestions derived from a panel of experts.

In conclusion, our study provides an original template to categorise human influenza pandemic scenarios, useful for pandemic planning. Before using its outcomes, limitations should be taken into account by public health authorities dealing with pandemic planning. This study is the first step of the FLURESP project, whose objective is to define adequate public health responses and measures according to each scenario presented in this paper and to compare performance and cost-effectiveness of such measures.

Acknowledgements

This work was possible thanks to research funding provided by the EU DG-Sanco for the FLURESP Project (Grant agreement no. 2010-11-01). We thank Dr Peter G Grove and Dr Guy Walker for their useful insights.

Conflicts of interest

None declared.

Authors' contributions

The work presented here was carried out in collaboration between all authors. C.N. M.F. and C.R. contributed to the data collection, performed the analysis and drafted the manuscript. C.N., M.F., C.R., A.Bo., S.Bo., M.B., J.O., J.M.C., L.N., P.G., A.P., C.L. and C.G. interpreted the results. S.Br., A.N. and P.P. contributed to the methodological approach. A.Be. conceived the overall study aims and design. All authors have contributed to, seen and approved the manuscript.

References

1. World Health Organization (WHO). WHO global influenza preparedness plan. Geneva: WHO. 2005. [Accessed April 2013]. Available from: http://www.who.int/csr/resources/publications/influenza/WHO_CDS_CSR_GIP_2005_5.pdf
2. World Health Organization. Epidemiology of WHO-confirmed human cases of avian influenza A(H5N1) infection. *Wkly Epidemiol Rec.* 2006;81(26):249-57. PMID:16812929
3. World Health Organization (WHO). Pandemic Influenza A (H1N1). Geneva: WHO. 2011. [Accessed April 2013]. Available from: http://www.who.int/csr/resources/publications/swineflu/h1n1_donor_032011.pdf
4. United Kingdom Department of Health. UK Influenza Pandemic Preparedness Strategy 2011. London: Department of Health. 10 Nov 2011. [Accessed April 2013]. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/213717/dh_131040.pdf
5. Ajelli M, Gonçalves B, Balcan D, Colizza V, Hu H, Ramasco JJ, et al. Comparing large-scale computational approaches to epidemic modeling: agent-based versus structured metapopulation models. *BMC Infect Dis.* 2010;10(1):190. <http://dx.doi.org/10.1186/1471-2334-10-190> PMID:20587041
6. World Health Organization. Considerations for assessing the severity of an influenza pandemic. *Wkly Epidemiol Rec.* 2009;84(22):197-202. Last accessed April 2013. Available from: <http://www.who.int/wer/2009/wer8422/en/index.html> PMID:19480086
7. World Health Organization (WHO). Strengthening response to pandemics and other public-health emergencies. Geneva: WHO. 2011. [Accessed April 2013]. Available from: http://www.who.int/ihr/publications/RC_report/en/index.html
8. World Health Organization (WHO). Pandemic Influenza Risk Management. WHO Interim Guidance. Geneva: WHO. 2013. [Accessed July 2014]. Available from: http://www.who.int/influenza/preparedness/pandemic/GIP_PandemicInfluenzaRiskManagementInterimGuidance_Jun2013.pdf?ua=1
9. European Centre for Disease Control and Prevention (ECDC). ECDC health information. Pandemic (H1N1) 2009 planning assumptions to end of May 2010 for EU/EEA countries. Stockholm: ECDC. 11 Nov 2009. [Accessed April 2013]. Available from: [http://www.ecdc.europa.eu/en/healthtopics/Documents/091111_Pandemic_\(H1N1\)_2009_Planning_Assumptions_for_EU_EEA_countries.pdf](http://www.ecdc.europa.eu/en/healthtopics/Documents/091111_Pandemic_(H1N1)_2009_Planning_Assumptions_for_EU_EEA_countries.pdf)
10. Yu H, Feng L, Peng Z, Feng Z, Shay DK, Yang W. Estimates of the impact of a future influenza pandemic in China. *Influenza Other Respi Viruses.* 2009;3(5):223-31. <http://dx.doi.org/10.1111/j.1750-2659.2009.00093.x> PMID:21462394
11. Miller MA, Viboud C, Olson DR, Grais RF, Rabaa MA, Simonsen L. Prioritization of influenza pandemic vaccination to minimize years of life lost. *J Infect Dis.* 2008;198(3):305-11. <http://dx.doi.org/10.1086/589716> PMID:18558871
12. Department of Health. Scientific Pandemic Influenza Advisory Committee (SPI): Subgroup on Modelling. Modelling Summary. 13 Jun 2013. London: Department of Health. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/208264/SPI-M_Modelling_Summary_13_06_13.pdf
13. Nguyen-Van-Tam JS, Hampson AW. The epidemiology and clinical impact of pandemic influenza. *Vaccine.* 2003;21(16):1762-8. [http://dx.doi.org/10.1016/S0264-410X\(03\)00069-0](http://dx.doi.org/10.1016/S0264-410X(03)00069-0) PMID:12686091
14. World Health Organization (WHO). Pandemic influenza preparedness and response. Geneva: WHO. 2009. [Accessed April 2013]. Available from: http://whqlibdoc.who.int/publications/2009/9789241547680_eng.pdf
15. Rizzo C, Bella A, Viboud C, Simonsen L, Miller MA, Rota MC, et al. Trends for influenza-related deaths during pandemic and epidemic seasons, Italy, 1969-2001. *Emerg Infect Dis.* 2007;13(5):694-9. <http://dx.doi.org/10.3201/eid1305.061309> PMID:17553246
16. Yang L, Chan KP, Cowling BJ, Chiu SS, Chan KH, Peiris JS, et al. Excess mortality associated with the 2009 pandemic of influenza A(H1N1) in Hong Kong. *Epidemiol Infect.* 2012;140(09):1542-50. <http://dx.doi.org/10.1017/S0950268811002238> PMID:22074735
17. O'Dowd A. Swine flu claims first European death, as cases worldwide rise to 36,000. *BMJ.* 2009;338(jun16 3):b2470. <http://dx.doi.org/10.1136/bmj.b2470> PMID:19531555
18. Nishiura H. Case fatality ratio of pandemic influenza. *Lancet Infect Dis.* 2010;10(7):443-4. [http://dx.doi.org/10.1016/S1473-3099\(10\)70120-1](http://dx.doi.org/10.1016/S1473-3099(10)70120-1) PMID:20610325
19. Nishiura H. The virulence of pandemic influenza A (H1N1) 2009: an epidemiological perspective on the case-fatality ratio. *Expert Rev Respir Med.* 2010;4(3):329-38. <http://dx.doi.org/10.1586/ers.10.24> PMID:20524916
20. Duque V, Cordeiro E, Mota V, Vaz J, Morais C, Rodrigues F, et al. The early days of pandemic (H1N1) 2009 virus infection in the central region of Portugal. *Rev Port Pneumol.* 2010;16(6):870-9. PMID:21067692
21. Chowell G, Echevarría-Zuno S, Viboud C, Simonsen L, Tamerius J, Miller MA, et al. Characterizing the epidemiology of the 2009 influenza A/H1N1 pandemic in Mexico. *PLoS Med.* 2011;8(5):e1000436. <http://dx.doi.org/10.1371/journal.pmed.1000436> PMID:21629683
22. Bandaranayake D, Huang QS, Bissielo A, Wood T, Mackereth G, Baker MG, et al. Risk factors and immunity in a nationally representative population following the 2009 influenza A(H1N1) pandemic. *PLoS ONE.* 2010;5(10):e13211. <http://dx.doi.org/10.1371/journal.pone.0013211> PMID:20976224
23. Hak E, Meijboom MJ, Buskens E. Modelling the health-economic impact of the next influenza pandemic in The Netherlands. *Vaccine.* 2006;24(44-46):6756-60. <http://dx.doi.org/10.1016/j.vaccine.2006.05.065> PMID:16797797
24. European Centre for Disease Control and Prevention (ECDC). Planning assumption for first wave of pandemic H1N1 2009 in Europe. Stockholm: ECDC. 29 Jul 2009. [Accessed April 2013]. Available from: http://www.ecdc.europa.eu/en/activities/sciadvic/_layouts/forms/Review_DisForm.aspx?ID=88&List=a3216f4c-f040-4f51-9f77-a96046dbfd72
25. Cui F, Luo H, Zhou L, Yin D, Zheng C, Wang D, et al. Transmission of pandemic influenza A (H1N1) virus in a train in China. *J Epidemiol.* 2011;21(4):271-7. <http://dx.doi.org/10.2188/jea.JE20100119> PMID:21646746
26. Huai Y, Lin J, Varma JK, Peng Z, He J, Cheng C, et al. A primary school outbreak of pandemic 2009 influenza A (H1N1) in China. *Influenza Other Respi Viruses.* 2010;4(5):259-66. <http://dx.doi.org/10.1111/j.1750-2659.2010.00150.x> PMID:20795308
27. Centers for Disease Control and Prevention (CDC). Outbreak of 2009 pandemic influenza A (H1N1) on a Peruvian Navy ship - June-July 2009. *MMWR Morb Mortal Wkly Rep.* 2010;59(6):162-5. PMID:20168295
28. Mahato RK, Bhandari GP, Shrestha JM, Basnet P. Pandemic influenza A (H1N1) 2009 outbreak investigation in Nepal. *J Nepal Health Res Counc.* 2010;8(2):75-7. PMID:21876566
29. Baguelin M, Hoschler K, Stanford E, Waight P, Hardelid P, Andrews N, et al. Age-specific incidence of A/H1N1 2009 influenza infection in England from sequential antibody prevalence data using likelihood-based estimation. *PLoS ONE.* 2011;6(2):e17074. <http://dx.doi.org/10.1371/journal.pone.0017074> PMID:21373639
30. 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.* 2010;375(9720):1100-8. [http://dx.doi.org/10.1016/S0140-6736\(09\)62126-7](http://dx.doi.org/10.1016/S0140-6736(09)62126-7) PMID:20096450
31. Presanis AM, De Angelis D, Hagy A, Reed C, Riley S, Cooper BS, et al. The severity of pandemic H1N1 influenza in the United States, from April to July 2009: a Bayesian analysis. *PLoS Med.* 2009;6(12):e1000207. <http://dx.doi.org/10.1371/journal.pmed.1000207> PMID:19997612
32. Presanis AM, Lipsitch M, De Angelis, Swine Flu Investigation Team, New York City Department of Health and Mental Hygiene, Hagy A, et al. The severity of pandemic H1N1 influenza in the United States, April - July 2009. *PLoS Curr.* 2009;1:RRN1042. PMID:20029614
33. Santa-Olalla Peralta P, Cortes García M, Martínez Sánchez EV, Nogareda Moreno F, Limia Sánchez A, Pachón Del Amo I, et al. [Enhanced surveillance of initial cases of pandemic influenza (H1N1) 2009 infection in Spain, April-June 2009]. *Rev Esp Salud Publica.* 2010;84(5):529-46. [Spanish]. <http://dx.doi.org/10.1590/S1135-57272010000500007> PMID:21203718
34. World Health Organization (WHO). Public health measures during the influenza A(H1N1) 2009 pandemic 2011. Geneva: WHO. 2011. [Accessed April 2013]. Available from: http://whqlibdoc.who.int/hq/2011/WHO_HSE_GIP_ITP_2011.3_eng.pdf
35. Monto AS. Influenza: quantifying morbidity and mortality. *Am J Med.* 1987;82(6):6A;20-5. [http://dx.doi.org/10.1016/0002-9343\(87\)90556-0](http://dx.doi.org/10.1016/0002-9343(87)90556-0) PMID:3591814
36. Mann PG, Pereira MS, Smith JW, Hart RJ, Williams WO, Dunhill B. A five-year study of influenza in families. Joint Public Health Laboratory Service/Royal College of General Practitioners working group. *J Hyg (Lond).* 1981;87(02):191-200. <http://dx.doi.org/10.1017/S0022172400069400> PMID:7288174
37. Longini IM Jr, Halloran ME, Nizam A, Yang Y. Containing pandemic influenza with antiviral agents. *Am J Epidemiol.* 2004;159(7):623-33. <http://dx.doi.org/10.1093/aje/kwh092> PMID:15033640

38. Fleming DM. The contribution of influenza to combined acute respiratory infections, hospital admissions, and deaths in winter. *Commun Dis Public Health*. 2000;3(1):32-8. PMID:10743316
39. Carrat F, Vergu E, Ferguson NM, Lemaître M, Cauchemez S, Leach S, et al. Time lines of infection and disease in human influenza: a review of volunteer challenge studies. *Am J Epidemiol*. 2008;167(7):775-85. <http://dx.doi.org/10.1093/aje/kwm375> PMID:18230677
40. Dawood FS, Iuliano AD, Reed C, Meltzer MI, Shay DK, Cheng PY, et al. Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *Lancet Infect Dis*. 2012;12(9):687-95. [http://dx.doi.org/10.1016/S1473-3099\(12\)70121-4](http://dx.doi.org/10.1016/S1473-3099(12)70121-4) PMID:22738893
41. World Health Organization (WHO). Influenza at the Human-Animal Interface. Geneva: WHO. [Accessed April 2013]. Available from: www.who.int/csr/disease/avian_influenza/
42. Li FC, Choi BC, Sly T, Pak AW. Finding the real case-fatality rate of H5N1 avian influenza. *J Epidemiol Community Health*. 2008;62(6):555-9. <http://dx.doi.org/10.1136/jech.2007.064030> PMID:18477756
43. Skarbinski J, Jain S, Bramley A, Lee EJ, Huang J, Kirschke D, et al. Hospitalized patients with 2009 pandemic influenza A (H1N1) virus infection in the United States--September-October 2009. *Clin Infect Dis*. 2011;52(1) Suppl 1:S50-9. <http://dx.doi.org/10.1093/cid/ciq021> PMID:21342900
44. Reed C, Biggerstaff M, Finelli L, Koonin LM, Beauvais D, Uzunian A, et al. Novel framework for assessing epidemiologic effects of influenza epidemics and pandemics. *Emerg Infect Dis*. 2013;19(1):85-91. <http://dx.doi.org/10.3201/eid1901.120124> PMID:23260039
45. Simonsen L, Clarke MJ, Schonberger LB, Arden NH, Cox NJ, Fukuda K. Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. *J Infect Dis*. 1998;178(1):53-60. <http://dx.doi.org/10.1086/515616> PMID:9652423
46. Miller MA, Viboud C, Balinska M, Simonsen L. The signature features of influenza pandemics--implications for policy. *N Engl J Med*. 2009;360(25):2595-8. <http://dx.doi.org/10.1056/NEJMp0903906> PMID:19423872
47. Balcan D, Hu H, Gonçalves B, Bajardi P, Poletto C, Ramasco JJ, et al. Seasonal transmission potential and activity peaks of the new influenza A(H1N1). A Monte Carlo likelihood analysis based on human mobility. *BMC Med*. 2009;10:745. doi: 10.1186/1741-7015-7-45.
48. Chowell G, Ammon CE, Hengartner NW, Hyman JM. Estimation of the reproductive number of the Spanish flu epidemic in Geneva, Switzerland. *Vaccine*. 2006;24(44-46):6747-50. <http://dx.doi.org/10.1016/j.vaccine.2006.05.055> PMID:16782243
49. Chowell G, Nishiura H, Bettencourt LM. Comparative estimation of the reproduction number for pandemic influenza from daily case notification data. *J R Soc Interface*. 2007;4(12):155-66. <http://dx.doi.org/10.1098/rsif.2006.0161> PMID:17254982
50. Chowell G, Viboud C, Munayco CV, Gómez J, Simonsen L, Miller MA, et al. Spatial and temporal characteristics of the 2009 A/H1N1 influenza pandemic in Peru. *PLoS ONE*. 2011;6(6):e21287. <http://dx.doi.org/10.1371/journal.pone.0021287> PMID:21712984
51. Colizza V, Barrat A, Barthelemy M, Valleron AJ, Vespignani A. Modeling the worldwide spread of pandemic influenza: baseline case and containment interventions. *PLoS Med*. 2007;4(1):e13. <http://dx.doi.org/10.1371/journal.pmed.0040013> PMID:17253899
52. Marchbanks TL, Bhattarai A, Fagan RP, Ostroff S, Sodha SV, Moll ME, et al.; Pennsylvania Working Group. An outbreak of 2009 pandemic influenza A (H1N1) virus infection in an elementary school in Pennsylvania. *Clin Infect Dis*. 2011;52(1) Suppl 1:S154-60. <http://dx.doi.org/10.1093/cid/ciq058> PMID:21342888
53. Novel influenza A(H1N1) investigation team. Description of the early stage of pandemic (H1N1) 2009 in Germany, 27 April-16 June 2009. *Euro Surveill*. 2009;14(31):19295. PMID:19660249
54. Pandemic Flu Response Review Team. Cabinet Office (UK). The 2009 Influenza Pandemic: an independent official review of the UK response to the 2009 influenza pandemic. London: Cabinet Office. July 2010. [Accessed April 2013]. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/61252/the2009influenzapandemic-review.pdf
55. Polish Ministry of Health. Krajowy plan działań dla polski na wypadek wystąpienia kolejnej pandemii grypy [Polish National Action Plan in the event of the next influenza pandemic]. 10 Aug 2005. [Accessed April 2013]. Available from: http://www2.mz.gov.pl/wwwfiles/ma_struktura/docs/zalacznik_r_26.pdf
56. Simón Méndez L, de Mateo Ontañón S, Larrauri Cámara A, Jiménez-Jorge S, Vaqué Rafart J, Pérez Hoyos S; en representación del Sistema de Vigilancia de Gripe en España (SVGE). [Transmissibility and severity of the pandemic influenza A (H1N1) 2009 virus in Spain]. *Gac Sanit*. 2011;25(4):296-302. [Spanish] PMID:21543138
57. Valleron AJ, Cori A, Valtat S, Meurisse S, Carrat F, Boëlle PY. Transmissibility and geographic spread of the 1889 influenza pandemic. *Proc Natl Acad Sci USA*. 2010;107(19):8778-81. <http://dx.doi.org/10.1073/pnas.1000886107> PMID:20421481
58. Viboud C, Tam T, Fleming D, Handel A, Miller MA, Simonsen L. Transmissibility and mortality impact of epidemic and pandemic influenza, with emphasis on the unusually deadly 1951 epidemic. *Vaccine*. 2006;24(44-46):6701-7. <http://dx.doi.org/10.1016/j.vaccine.2006.05.067> PMID:16806596
59. World Health Organization (WHO). WHO checklist for influenza pandemic preparedness planning. Geneva: WHO. 2005. [Accessed April 2013]. Available from: http://whqlibdoc.who.int/hq/2005/WHO_CDS_CSR_GIP_2005.4.pdf
60. World Health Organization (WHO). Pandemic influenza preparedness Framework 2011. Geneva: WHO. 2011. [Accessed April 2013]. Available from: http://whqlibdoc.who.int/publications/2011/9789241503082_eng.pdf
61. Turkish Ministry of Health. Directorate General of Primary Health Care. Pandemic Influenza National Action Plan. Ankara: Ministry of Health. 2005.
62. Franco-Paredes C, Hernandez-Ramos I, Del Rio C, Alexander KT, Tapia-Conyer R, Santos-Preciado JJ. H1N1 influenza pandemics: comparing the events of 2009 in Mexico with those of 1976 and 1918-1919. *Arch Med Res*. 2009;40(8):669-72. <http://dx.doi.org/10.1016/j.arcmed.2009.10.004> PMID:20304254
63. Malta Health Division. Influenza Pandemic Contingency Plan for the Maltese Islands. Malta. 2007.
64. Lee VJ, Chen MI. Effectiveness of neuraminidase inhibitors for preventing staff absenteeism during pandemic influenza. *Emerg Infect Dis*. 2007;13(3):449-57. <http://dx.doi.org/10.3201/eid1303.060309> PMID:17552099
65. Lessler J, Cummings DA, Fishman S, Vora A, Burke DS. Transmissibility of swine flu at Fort Dix, 1976. *J R Soc Interface*. 2007;4(15):755-62. <http://dx.doi.org/10.1098/rsif.2007.0228> PMID:17412677
66. Lessler J, Reich NG, Cummings DA, New York City Department of Health and Mental Hygiene Swine Influenza Investigation Team. Outbreak of 2009 pandemic influenza A (H1N1) at a New York City school. *N Engl J Med*. 2009;361(27):2628-36. <http://dx.doi.org/10.1056/NEJMo0906089> PMID:20042754
67. Mills CE, Robins JM, Lipsitch M. Transmissibility of 1918 pandemic influenza. *Nature*. 2004;432(7019):904-6. <http://dx.doi.org/10.1038/nature03063> PMID:15602562
68. Jackson C, Vynnycky E, Mangtani P. Estimates of the transmissibility of the 1968 (Hong Kong) influenza pandemic: evidence of increased transmissibility between successive waves. *Am J Epidemiol*. 2010;171(4):465-78. <http://dx.doi.org/10.1093/aje/kwp394> PMID:20007674
69. Carrat F, Luong J, Lao H, Sallé AV, Lajaunie C, Wackernagel H. A 'small-world-like' model for comparing interventions aimed at preventing and controlling influenza pandemics. *BMC Med*. 2006;23:4:26.
70. Correia AM, Queirós L, Dias J. Pandemic influenza A (H1N1) in the North of Portugal: how did the autumn-winter wave behave? *Rev Port Pneumol*. 2010;16(6):880-6. PMID:21067693
71. United Kingdom Department of Health. Cabinet Office. Swine Flu. UK Planning Assumptions. London: Department of Health. 3 Sep 2009. [Accessed April 2013]. Available from: http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/documents/digitalasset/dh_104843.pdf
72. Doyle TJ, Hopkins RS; Transmission Investigation Team. Low secondary transmission of 2009 pandemic influenza A (H1N1) in households following an outbreak at a summer camp: relationship to timing of exposure. *Epidemiol Infect*. 2011;139(01):45-51. <http://dx.doi.org/10.1017/S095026881000141X> PMID:20561391
73. Gomez J, Munayco C, Arrasco J, Suarez L, Laguna-Torres V, Aguilar P, et al. Pandemic influenza in a southern hemisphere setting: the experience in Peru from May to September, 2009. *Euro Surveill*. 2009;14(42):19371. PMID:19883547
74. Gordon A, Saborío S, Videá E, López R, Kuan G, Balmaseda A, et al. Clinical attack rate and presentation of pandemic H1N1 influenza versus seasonal influenza A and B in a pediatric cohort in Nicaragua. *Clin Infect Dis*. 2010;50(11):1462-7. <http://dx.doi.org/10.1086/652647> PMID:20420502
75. Iuliano AD, Reed C, Guh A, Desai M, Dee DL, Kutty P, et al. Notes from the field: outbreak of 2009 pandemic influenza A (H1N1) virus at a large public university in Delaware, April-May 2009. *Clin Infect Dis*. 2009;49(12):1811-20. <http://dx.doi.org/10.1086/649555> PMID:19911964

76. Jhung MA, Swerdlow D, Olsen SJ, Jernigan D, Biggerstaff M, Kamimoto L, et al. Epidemiology of 2009 pandemic influenza A (H1N1) in the United States. *Clin Infect Dis*. 2011;52(1) Suppl 1:S13-26. <http://dx.doi.org/10.1093/cid/ciq008> PMID:21342884
77. Lee BY, Bailey RR, Wiringa AE, Assi TM, Beigi RH. Antiviral medications for pregnant women for pandemic and seasonal influenza: an economic computer model. *Obstet Gynecol*. 2009;114(5):971-80. <http://dx.doi.org/10.1097/AOG.0b013e3181bdfbfe> PMID:20168096
78. Leung YH, Li MP, Chuang SK. A school outbreak of pandemic (H1N1) 2009 infection: assessment of secondary household transmission and the protective role of oseltamivir. *Epidemiol Infect*. 2011;139(01):41-4. <http://dx.doi.org/10.1017/S0950268810001445> PMID:20561390
79. Li T, Liu Y, Di B, Wang M, Shen J, Zhang Y, et al. Epidemiological investigation of an outbreak of pandemic influenza A (H1N1) 2009 in a boarding school: serological analysis of 1570 cases. *J Clin Virol*. 2011;50(3):235-9. <http://dx.doi.org/10.1016/j.jcv.2010.11.012> PMID:21195022
80. Mayoral Cortés JM, Ruiz Fernández J, Pachón Díaz J, Navarro Marí JM, Puell Gómez L, Pérez Morilla E, et al. [Infection by the pandemic virus (H1N1) 2009 in Andalusia]. *Rev Esp Salud Publica*. 2010;84(5):517-28. <http://dx.doi.org/10.1590/S1135-57272010000500006> PMID:21203717
81. Nougairède A, Ninove L, Zandotti C, Salez N, Mantey K, Resseguier N, et al. Novel virus influenza A (H1N1sw) in South-Eastern France, April-August 2009. *PLoS ONE*. 2010;5(2):e9214. <http://dx.doi.org/10.1371/journal.pone.0009214> PMID:20174643
82. Office General delà santé Publique, Confédération Suisse: Plan suisse de pandémie Influenza. available at: <http://www.bag.admin.ch/influenza/01120/01134/03058/index.html?lang=fr>, Last accessed April 2013.
83. Smith RD, Keogh-Brown MR, Barnett T. Estimating the economic impact of pandemic influenza: An application of the computable general equilibrium model to the U.K. *Soc Sci Med*. 2011;73(2):235-44. <http://dx.doi.org/10.1016/j.socscimed.2011.05.025> PMID:21708419
84. van Genugten ML, Heijnen ML, Jager JC. Pandemic influenza and healthcare demand in the Netherlands: scenario analysis. *Emerg Infect Dis*. 2003;9(5):531-8. <http://dx.doi.org/10.3201/eid0905.020321> PMID:12737735
85. Wielders CC, van Lier EA, van 't Klooster TM, van Gageldonk-Lafeber AB, van den Wijngaard CC, Haagsma JA, et al. The burden of 2009 pandemic influenza A(H1N1) in the Netherlands. *Eur J Public Health*. 2012;22(1):150-7. <http://dx.doi.org/10.1093/eurpub/ckq187> PMID:21183472
86. Wilson N, Mansoor O, Baker M. Estimating the impact of the next influenza pandemic on population health and health sector capacity in New Zealand. *N Z Med J*. 2004;118(1211):U1346.
87. Yates L, Pierce M, Stephens S, Mill AC, Spark P, Kurinczuk JJ, et al. Influenza A/H1N1v in pregnancy: an investigation of the characteristics and management of affected women and the relationship to pregnancy outcomes for mother and infant. *Health Technol Assess*. 2010;14(34):109-82. <http://dx.doi.org/10.3310/hta14340-02> PMID:20630123
88. Barry JM, Viboud C, Simonsen L. Cross-protection between successive waves of the 1918-1919 influenza pandemic: epidemiological evidence from US Army camps and from Britain. *J Infect Dis*. 2008;198(10):1427-34. <http://dx.doi.org/10.1086/592454> PMID:18808337
89. Centers for Disease Control and Prevention (CDC). Outbreak of 2009 pandemic influenza A (H1N1) at a school - Hawaii, May 2009. *MMWR Morb Mortal Wkly Rep*. 2010;58(51):1440-4. PMID:20057351
90. Cherifi S, Reynders M, Theunissen C. Hospital preparedness and clinical description of the 2009 influenza A (H1N1) pandemic in a Belgian tertiary hospital. *J Hosp Infect*. 2011;77(2):118-22. <http://dx.doi.org/10.1016/j.jhin.2010.09.032> PMID:21216036
91. Di Giambenedetto S, Zileri Dal Verme L, Sali M, Farina S, Di Cristo V, Manzara S, et al. Clinical presentation, microbiological features and correlates of disease severity of 2009 pandemic influenza A (H1N1) infection. *Eur J Clin Microbiol Infect Dis*. 2011;30(4):541-9. <http://dx.doi.org/10.1007/s10096-010-1116-7> PMID:21104285
92. Donaldson LJ, Rutter PD, Ellis BM, Greaves FE, Mytton OT, Pebody RG, et al. Mortality from pandemic A/H1N1 2009 influenza in England: public health surveillance study. *BMJ*. 2009;339(dec10 1):b5213. <http://dx.doi.org/10.1136/bmj.b5213> PMID:20007665
93. Fujikura Y, Kawana A, Kato Y, Mizuno Y, Kudo K. [Clinical features in fatal Spanish influenza: Japanese Army Hospital medical records investigation]. *Kansenshogaku Zasshi*. 2010;84(2):165-70. [Japanese]. PMID:20420160
94. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, et al. Hospitalized Patients with 2009 H1N1 Influenza in the United States, April-June 2009. *N Engl J Med*. 2009;361(20):1935-44. doi: 10.1056/NEJMoa0906695.
95. Louie JK, Acosta M, Winter K, Jean C, Gavali S, Schechter R, et al. California Pandemic (H1N1) Working Group. Factors associated with death or hospitalization due to pandemic 2009 influenza A(H1N1) infection in California. *JAMA*. 2009;302(17):1896-902. <http://dx.doi.org/10.1001/jama.2009.1583> PMID:19887665
96. Al Hajjar S, McIntosh K, Sami Al Hajjara and Kenneth McIntoshb. The first influenza pandemic of the 21st century. *Ann Saudi Med*. 2010;30(1):1-10. PMID:20103951
97. Santa-Olalla Peralta P, Cortes-García M, Vicente-Herrero M, Castrillo-Villamandos C, Arias-Bohigas P, Pachon-del Amo I, et al. Risk factors for disease severity among hospitalised patients with 2009 pandemic influenza A (H1N1) in Spain, April - December 2009. *Euro Surveill*. 2010;15(38):19667. PMID:20929651
98. Shieh WJ, Blau DM, Denison AM, Deleon-Carnes M, Adem P, Bhatnagar J, et al. 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. *Am J Pathol*. 2010;177(1):166-75. <http://dx.doi.org/10.2353/ajpath.2010.100115> PMID:20508031
99. Han K, Zhu XP, He F, Liu LG, Zhang LJ, Ma HL, et al. [Non-airborne transmission during outbreak of pandemic (H1N1) 2009 among tour group members, China, June 2009]. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2010;31(9):1046-9. [Chinese]. PMID:21162874
100. Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet*. 2004;363(9409):587-93. [http://dx.doi.org/10.1016/S0140-6736\(04\)15589-X](http://dx.doi.org/10.1016/S0140-6736(04)15589-X) PMID:14987882
101. Cao B, Li XW, Mao Y, Wang J, Lu HZ, Chen YS, et al. Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in China. *N Engl J Med*. 2009;361(26):2507-17. <http://dx.doi.org/10.1056/NEJMoa0906612> PMID:20007555
102. Lessler J, Reich NG, Brookmeyer R, Perl TM, Nelson KE, Cummings DA. Incubation periods of acute respiratory viral infections: a systematic review. *Lancet Infect Dis*. 2009;9(5):291-300. [http://dx.doi.org/10.1016/S1473-3099\(09\)70069-6](http://dx.doi.org/10.1016/S1473-3099(09)70069-6) PMID:19393959
103. Yang TH, Chu D, Hu BS, Hung YT, Chou P. Early experience of the pandemic influenza H1N1 2009 epidemic in Taiwan. *J Chin Med Assoc*. 2011;74(7):298-304. <http://dx.doi.org/10.1016/j.jcma.2011.05.005> PMID:21783094
104. Yuan J, Zhang L, Xu W, Shen J, Zhang P, Ma H. Reported changes in health-related behaviours in Chinese urban residents in response to an influenza pandemic. *Epidemiol Infect*. 2009;137(07):988-93. <http://dx.doi.org/10.1017/S0950268809002726> PMID:19426571
105. AlMazroa MA, Memish ZA, AlWadey AM. Pandemic influenza A (H1N1) in Saudi Arabia: description of the first one hundred cases. *Ann Saudi Med*. 2010;30(1):11-4. PMID:20103952
106. Balter S, Gupta LS, Lim S, Fu J, Perlman SE. Pandemic (H1N1) 2009 surveillance for severe illness and response, New York, USA, April-July 2009. *Emerg Infect Dis*. 2010;16(8):1259-64. <http://dx.doi.org/10.3201/eid1608.091847> PMID:20678320
107. van Genugten ML, Heijnen ML. The expected number of hospitalisations and beds needed due to pandemic influenza on a regional level in the Netherlands. *Virus Res*. 2004;103(1-2):17-23. <http://dx.doi.org/10.1016/j.virusres.2004.02.007> PMID:15163483
108. Martinese F, Keijzers G, Grant S, Lind J. How would Australian hospital staff react to an avian influenza admission, or an influenza pandemic? *Emerg Med Australas*. 2009;21(1):12-24. <http://dx.doi.org/10.1111/j.1742-6723.2008.01143.x> PMID:19254308
109. Ercole A, Taylor BL, Rhodes A, Menon DK. Modelling the impact of an influenza A/H1N1 pandemic on critical care demand from early pathogenicity data: the case for sentinel reporting. *Anaesthesia*. 2009;64(9):937-41. <http://dx.doi.org/10.1111/j.1365-2044.2009.06070.x> PMID:19645759