Impact factor 4.659

Eurosurveillance

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

Vol. 19 | Weekly issue 39 | 2 October 2014

RAPID COMMUNICATIONS

SURVEILLANCE AND OUTBREAK REPORTS

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Influenza season in Réunion dominated by influenza B virus circulation associated with numerous cases of severe disease, France, 2014

E Brottet (elise.brottet@ars.sante.fr)[1], D Vandroux[2], B A Gauzere[2], E Antok[3], M C Jaffar-Bandjee[4], A Michault[5], L Filleul[1]
1. Cire océan Indien (Cire OI), Institut de Veille Sanitaire (InVS), Saint Denis, Réunion, France
2. Intensive Care Unit, Centre Hospitalier Universitaire, Saint-Denis, Réunion, France
3. Intensive Care Unit, Centre Hospitalier Universitaire, Saint-Pierre, Réunion, France
4. Laboratory of virology, Centre Hospitalier Universitaire, Saint-Denis, Réunion, France
5. Laboratory of Biology, Centre Hospitalier Universitaire, Saint-Pierre, Réunion, France

Citation style for this article:
Brottet E, Vandroux D, Gauzere BA, Antok E, Jaffar-Bandjee MC, Michault A, Filleul L. Influenza season in Réunion dominated by influenza B virus circulation associated with numerous cases of severe disease, France, 2014. Euro Surveill. 2014;19(39):pii=20916. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20916

Article submitted on 15 September 2014 / published on 2 October 2014

The 2014 seasonal influenza in Réunion, a French overseas territory in the southern hemisphere, was dominated by influenza B. Resulting morbidity impacted public health. Relative to the total number of all-cause consultations over the whole season, the rate of acute respiratory infection (ARI) consultations was 6.5%. Severe disease occurred in 32 laboratory-confirmed influenza cases (31.7 per 100,000 ARI consultations), 16 with influenza B. The observed disease dynamics could present a potential scenario for the next European influenza season.

Réunion is a French overseas territory located in the southern hemisphere between Madagascar and Mauritius in the Indian Ocean. On this island, influenza is monitored through a sentinel practitioner network [1]. Influenza activity generally increases during the austral winter, corresponding to summer in Europe. At the end of May 2014 the proportion of general practitioners' (GP) consultations for acute respiratory infections (ARI) relative to all-cause consultations increased, signalling the beginning of the influenza season. We describe the characteristics of the epidemic.

Surveillance of influenza in Réunion

Influenza surveillance sentinel network

In Réunion, 56 general practitioners (GPs) and two paediatricians (comprising all together 7.2% of the total GPs on the island) participate in the influenza surveillance sentinel network. Activities of the sentinel GPs represent 4.9% of total activities of all GPs on the island. They report on a weekly basis the total number of all-cause and acute respiratory infections (ARI) consultations, with ARI defined as a sudden onset of fever (≥38°C) and cough, which are associated or not with other symptoms, such as for example breathing difficulty or headache [2].

In addition to the weekly proportion of ARI among sentinel consultations, a weekly estimated number of ARI consultations is extrapolated from the total number of consultations in Réunion, which is itself derived from health insurance data [3].

Furthermore, every physician of the sentinel network collects a nasal swab from the first two patients of the week who present with ARI symptoms since less than three days. All swabs are analysed by the hospital laboratory by reverse transcription-polymerase chain reaction (RT-PCR) for influenza A, influenza A(H1N1) pdm09 and influenza B viruses. In a second step, a subset of the isolates that are positive for influenza A, but not H1N1 are tested for H3N2 by the national influenza centre.

Reporting of cases with severe influenza

In addition to the primary healthcare surveillance, all severe influenza cases defined as patients with a laboratory-confirmed influenza infection (positive RT-PCR for influenza virus) admitted for more than 24 hours to an intensive care unit (ICU) are reported by clinicians through a standardised form. Demographic and clinical data are collected as well as risk factors/comorbidities and vaccination status. All the ICUs (adults and paediatrics) present on the island (n=4), which cover the total population, participate in this surveillance and swab all patients with severe ARI.

Confirmation of laboratory findings and genetic and antigenic characterisation

Every year, a randomly-selected sample is sent to the national influenza centre in mainland France for confirmation and genetic and antigenic characterisation. In 2014, this sample consisted of 11 swabs from sentinel surveillance (2%) and 15 swabs (47%) from intensive care unit (ICU).

Analysis of epidemics relative to prior influenza seasons

Epidemic periods are defined as an increase of the number of ARI consultations above the mean of such consultations during the same periods in the previous years (the 2010–2013 mean was taken for comparison to 2014) and an increase of the positive rate for influenza viruses (proportion of persons testing positive for influenza virus among all persons tested for influenza) superior to 50%.

Historical data from 2010 to 2014, which had been collected by the influenza surveillance system annually, including during epidemic periods, were compared. For each year, including epidemic periods, the number of cases with severe influenza relative to the numbers of ARI consultations was calculated both for the year in question and for the year's respective epidemic periods. The influenza surveillance system remained the same during the five years studied in terms of population covered and sampling protocol.

Influenza epidemic in 2014 in Réunion

Description of the epidemic

The proportion of ARI among the all-cause GP consultations increased at the end of May 2014 (week 22) and reached 9.3% in the first week of July (week 27). For this week, the estimated number of consultations due to ARI was 11,800 (Figure). Between the beginning of the epidemic on 26 May 2014 (week 22) and the end on 27 July 2014 (week 30), the number of patients with ARI who consulted a physician was estimated at 69,500, which represents a cumulative rate of 6.5% (69,500/1,070,000) of total consultations at GPs.

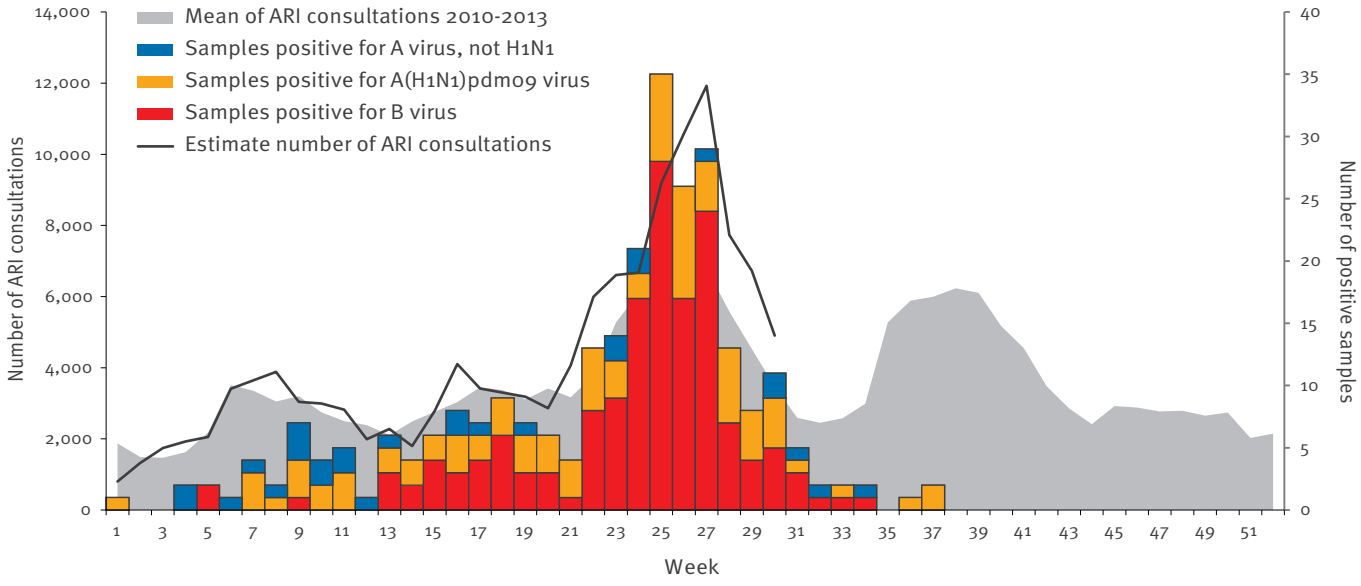
During the epidemic, 62% (169/273) of samples were positive for influenza. Among the 169 identified viruses, 118 (70%) were influenza B, 44 (26%) influenza A(H1N1)pdm09 and seven (4%) influenza A but not H1N1. In a second step, a subset of influenza A not H1N1 isolates were all confirmed as influenza A(H3N2) viruses. From the sample of genetically and antigenically characterised viruses (n=26), 13 influenza A viruses were A(H1N1)pdm09 and 10 influenza B viruses belonged to the Yamagata lineage. These viruses were of the same strains than those targeted by the 2014 seasonal vaccine for the southern hemisphere (B/Massachusetts/2/2012 for the influenza B viruses and A/California/7/2009 for influenza A).

Characteristics of laboratory-confirmed influenza cases with severe disease

Among the 32 cases identified with severe disease in 2014 (Table 1), 16 were infected with influenza B virus, 13 with A(H1N1)pdm09 virus and three with A(H3N2) virus. Patients with influenza B were older than those affected by A(H1N1)pdm09 virus (mean of 58 years vs 42 years respectively, p=0.03). Five of 13 influenza A(H1N1)pdm09 cases had no comorbidities compared to one of 16 influenza B cases. Most (n=24) of the cases with severe disease who presented at least one risk factor/comorbidity were not vaccinated, although the information was not available for four of 32 total patients. Among the 32 cases with severe influenza, nine deaths occurred including four infected with influenza B virus and five with influenza A(H1N1)pdm09 virus (including two patients with no risk factors).

Figure

Weekly estimation of acute respiratory infections consultations and number of samples positive for influenza viruses, Réunion, France, 2014



ARI: acute respiratory infection.

Table 1

Characteristics of cases with severe influenza, Réunion, France, 2014 (n=32)

Characteristic	Influenza virus type			Total (n = 32)
	B (n=16)	A(H1N1)pdm09 (n=13)	A non-H1N1/A(H3N2)[a] (n = 3)	
Sex				
Male/female	12/4	6/7	3/0	21/11
Age				
Mean age in years (range)	58 (5–82)	42 (1–71)	53 (19–76)	51 (1–82)
Age groups in years				
0–4	0	1	0	1
5–14	1	1	0	2
15–64	10	8	2	20
≥65	5	3	1	9
Risk factors/comorbidities				
Respiratory disease	7	3	0	10
Diabetes	2	4	2	8
Cardiac disease	5	4	2	11
Pregnancy	0	2	0	2
Hepatic disease	2	0	0	2
None	1	5	0	6
More than one risk factor/comorbidities	8	4	2	14
Indicators or signs of severity				
Respiratory assistance	13	8	2	23
With acute respiratory distress syndrome	10	7	1	18
With extracorporeal membrane oxygenation	0	5	0	5
Death	4	5	0	9
Influenza vaccination				
Vaccinated	0	0	0	0
Unvaccinated	15	11	2	28
Not specified	1	2	1	4

[a] The influenza A subtype is not H1N1 and likely to be A(H3N2).

Table 2

Indicators of influenza surveillance, Réunion, France, 2010–2014

Year		2010	2011	2012	2013	2014
Epidemic period (week numbers)		35–45	22–29	23–30 and 35–39	23–30	22–30
Predominant influenza viruses		A(H1N1)pdm09	A(H3N2)	A(H3N2) + B	A(H1N1)pdm09	B
Number of laboratory-confirmed influenza cases with severe disease	Annual	14	8	9	18	32
	During epidemic period	12	5	4	15	22
Number of deaths in ICU	Annual	7	1	4	7	9
	During epidemic period	7	0	1	7	7
Estimated number of ARI consultations during epidemic period		74,000	50,300	97,000	53,400	69,500
Incidence rate of cases with severe influenza for 100,000 ARI consultations	Annual[a]	18.9	15.9	9.3	33.7	46.0
	During epidemic period[b]	16.2	9.9	4.1	28.1	31.7

ARI: acute respiratory infection; ICU: intensive care unit.

[a] The annual rate = annual number of cases with severe disease/estimated number of ARI consultations during the epidemic period that year.

[b] The rate during the epidemic = number of cases with severe disease during the epidemic period/estimated number of ARI consultations during the epidemic period.

Comparison of the 2014 epidemic to previous influenza seasons

The estimated annual incidence of cases with severe influenza observed in Réunion in 2014 is the highest since 2010, with 46 cases per 100,000 consultations for ARI (Table 2).

Discussion

It is the first time since 2009 (date of the set-up of virological surveillance) that an influenza epidemic is mainly due to influenza B virus in Réunion.

Compared to the four past years, the 2014 seasonal influenza outbreak on the island has had a higher impact on public health in terms of related morbidity and the incidence of cases in ICU presenting with severe disease [4], which is the highest since 2010. This result cannot be linked to a surveillance bias as we have had the same complete monitoring records since 2010 with the participation of all ICUs in Réunion. Furthermore, during the last five influenza seasons, we contacted ICU doctors weekly to obtain information about cases with severe influenza.

The characterisation of viruses circulating this season showed that influenza B and influenza A(H1N1)pdm09 viruses were of the strains covered by the seasonal influenza vaccine for the southern hemisphere in 2014 [5]. Furthermore, most patients in ICU with risk factors were not vaccinated. The last estimation of the immunisation coverage for influenza among vulnerable people targeted by vaccination in 2013 was 39.7% in Réunion vs 50.1% in mainland France [6].

The influenza pattern and the types of viruses observed in Réunion are similar to those of other countries in the Indian Ocean this year, specifically Madagascar where influenza B virus Yamagata lineage was identified in June and July. In contrast, in the Pacific area (Australia, New Zealand) and South America, influenza A viruses predominated [7].

To our knowledge, few reports have described cases of severe disease due to influenza B virus [8]. Our results, presenting the individual characteristics of cases of severe disease, in clinical and virological terms, contribute to an enhanced knowledge of the burden of influenza B. The cases with most severe influenza in ICU infected by influenza B virus were not vaccinated, and had at least one risk factor/comorbidity, particularly respiratory disease. Moreover, these patients were older than patients infected with A(H1N1)pdm09 virus.

Despite the fact that Réunion represents a small area in the southern hemisphere, the typical pattern of influenza outbreaks has already been shown to be of interest for Europe [2]. The epidemic features observed in Réunion during the influenza season 2014 could be similar in the upcoming 2014/15 season in Europe [9,10]. An increase of influenza immunisation coverage among targeted groups could prevent a number of cases of severe illness. Promoting specific awareness and information on vaccination in the population for which influenza vaccination is recommended could avoid severe cases in ICU in mainland France or Europe for the 2014/15 influenza season.

Acknowledgements

We are thankful to all the sentinel network practitioners, the practitioners of the intensive care unit and biological laboratories. We want also to thank Emmanuel Belchior and Isabelle Bonmarin for French influenza expertise and reviewing of this publication.

Conflict of interest

None declared.

Authors' contributions

All authors contributed to the interpretation of the results, the revision of the draft manuscript and approved the final version. EB conducted the data analysis and wrote the manuscript; DV, BAG and EA was involved in the data collection in ICU; MCJB was responsible for the viral laboratory analyses; AM was involved in the laboratory analysis; LF was involved in the design of the influenza surveillance system and participated in the writing of the manuscript.

References

1. D'Ortenzio E, Do C, Renault P, Weber F, Filleul L. Enhanced influenza surveillance on Réunion Island (southern hemisphere) in the context of the emergence of influenza A(H1N1)v. Euro Surveill. 2009;14(23):pii=19239.
2. Filleul L, Brottet E, Gauzere BA, Winer A, Vandroux D, Michault A, et al. Réunion, a sentinel territory for influenza surveillance in Europe. Euro Surveill. 2012;17(27):pii= 20212.
3. Renault P, D'Ortenzio E, Kermarec F, Filleul L. Pandemic influenza 2009 on Réunion Island: A mild wave linked to a low reproduction number. Version 2. PLoS Curr. 2010 Jan 19 [revised 2010 Jan 19];2:RRN1145.
4. Vandroux D, Brottet E, Ursulet L, Angue M, Jabot J, Filleul L, et al. New patterns of A(H1N1)pdm09 influenza in the Southern Hemisphere. Intensive Care Med. 2014;40(1):131-2. http://dx.doi.org/10.1007/s00134-013-3143-1
5. World Health Organization (WHO). Recommended composition of influenza virus vaccines for use in the 2014 southern hemisphere influenza season. Geneva:WHO;26 Sep 2013 (revised 11 October 2013).
6. Brottet E, Ristor B, Simoes J, Polycarpe D, Filleul L. Couverture vaccinale antigrippale à la Réunion de 2009 à 2013. BVS océan Indien. 2014;22:11-12. French. Available from: http://www.invs.sante.fr/fr/Publications-et-outils/Bulletin-de-veille-sanitaire/Tous-les-numeros/Ocean-indien-Reunion-Mayotte/Bulletin-de-veille-sanitaire-ocean-Indien.-N-22-Juin-2014
7. World Health Organization (WHO). FluNet. Influenza Laboratory Surveillance Information by the Global Influenza Surveillance and Response System (GISRS). Geneva: WHO. Available from: http://gamapserver.who.int/gareports/Default.aspx?ReportNo=1
8. 8. Paul Glezen W, Schmier JK, Kuehn CM, Ryan KJ, Oxford J. The burden of influenza B: a structured literature review. Am J Public Health. 2013;103(3):e43-51. http://dx.doi.org/10.2105/AJPH.2012.301137
9. Cottrell SL, Moore C, Dexter L, Thomas DR, Salmon RL. Unusually high impact of influenza B during the early 2012-2013 influenza season in Wales--epidemiology and clinical analysis of the first 100 cases. Influenza Other Respir Viruses. 2013;7(6):1013-6. http://dx.doi.org/10.1111/irv.12151
10. Mook P, Ellis J, Watson JM, Thompson C, Zambon M, McMenamin J, et al. Public health implications of influenza B outbreaks in closed settings in the United Kingdom in the 2007/08 influenza season. Euro Surveill. 2008;13(38):pii=18986.

Outbreak of adenovirus type 1 severe pneumonia in a French intensive care unit, September–October 2012

N Cassir (cassirmadim@gmail.com)[1,2,3], S Hraiech[1,3,4], A Nougairede[5,6], C Zandotti[6], P E Fournier[1,2], L Papazian[1,4]

1. Unité de Recherche sur les Maladies Infectieuses Tropicales et Emergentes, UM63 CNRS 7278, IRD 189, Inserm U1095, IHU Méditerranée-Infection, Aix-Marseille Université, Marseille, France
2. Comité de Lutte contre les Infections Nosocomiales, Hôpital Nord, Assistance Publique-Hôpitaux de Marseille, Marseille, France
3. These authors contributed equally to the work
4. Service de Réanimation des Détresses Respiratoires et Infections Sévères, Hôpital Nord, Assistance Publique-Hôpitaux de Marseille, Marseille, France
5. Aix Marseille Université, IRD French Institute of Research for Development, EHESP French School of Public Health, EPV UMR_D 190 'Emergence des Pathologies Virales', 13385, Marseille, France
6. Laboratoire de Microbiologie Clinique, Hôpital de la Timone, Assistance Publique-Hôpitaux de Marseille, Marseille, France

Citation style for this article:
Cassir N, Hraiech S, Nougairede A, Zandotti C, Fournier PE, Papazian L. Outbreak of adenovirus type 1 severe pneumonia in a French intensive care unit, September–October 2012. Euro Surveill. 2014;19(39):pii=20914. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20914

Article submitted on 20 August 2013 / published on 2 October 2014

We herein describe and analyse the first outbreak of severe pneumonia caused by human adenovirus type 1 (HAdV C type 1), which included immunocompetent patients in an intensive care unit (ICU) of Marseille, France, and occurred between September and October 2012. Seven successive patients were diagnosed by HAdV specific real-time polymerase chain reaction with a positive bronchoalveolar lavage. After the collection of nasopharyngeal swabs from healthcare workers, three nurses working night shifts tested positive for HAdV C including one that had exhibited respiratory signs while working one week before the outbreak. She was the most likely source of the outbreak. Our findings suggest that HAdV-1 could be considered as a possible cause of severe pneumonia even in immunocompetent patients with a potential to cause outbreaks in ICUs. HAdV rapid identification and typing is needed to curtail the spread of this pathogen. Reinforcing hand hygiene with antiseptics with demonstrated activity against non-enveloped viruses and ensuring that HCWs with febrile respiratory symptoms avoid direct patient contact are critical measures to prevent transmission of HAdV in healthcare settings.

Introduction

Human adenoviruses (HAdVs) cause a broad spectrum of clinical presentations that range from asymptomatic to mild, severe and life-threatening infections. To date, more than 50 different types [1] of HAdVs have been identified, and they are grouped in seven different species (A–G) on the basis of biochemical and molecular characteristics. Specific types have been linked to distinct clinical syndromes; HAdV-3, and HAdV-7 are common causes of severe pneumonia in neonates and children under five years of age [2]. HAdV-4 is among the most important causes of acute respiratory distress syndrome (ARDS) in new military recruits [3]. HAdV-11- and HAdV-14-associated febrile respiratory disease outbreaks have been reported in both children and adults [4,5]. Recently, HAdV-55 has been described as an emerging cause of community-acquired pneumonia in China [6]. Extra-pulmonary presentations have also been described and associated with specific types (i.e., infantile gastroenteritis: HAdV-40 and HAdV-41; epidemic keratoconjunctivitis: HAdV-8, HAdV-37 and HAdV-54; haemorrhagic cystitis: HAdV-11, HAdV-34 and HAdV-35) [7–9].

Transmission of HAdV may occur via aerosol droplets, by the faecal-oral route and by contact with contaminated fomites. In particular, adenoviruses may persist on human skin for many hours and survive for long periods on environmental surfaces [10]. One of their characteristics is their resistance to lipid disinfectants because they are non-enveloped. Thus, they are inactivated by high concentrations of alcohols (ethanol, isopropanol, *n*-propanol) or chlorine-based products. The virucidal activity of chlorhexidine is known to be low against adenoviruses [11]. Moreover, washing hands with soap and water has been described to be ineffective in eliminating adenovirus from the culture-positive hands of physicians and patients, indicating that mechanical removal was incomplete [12].

Outbreaks of HAdV pneumonia have been described in military and long-term care settings [13,14]. In a recent review about viral outbreaks in neonatal intensive care units (ICUs), HAdV outbreaks corresponded to 9.4% of all viral outbreaks and had the highest mortality rate, 35.4% [15].

We herein report an outbreak of HAdV C type 1 respiratory infections involving healthcare workers (HCWs) in a medical intensive care unit and describe the measures

undertaken to control the outbreak and prevent new cases from occurring.

Methods

Setting

In September 2012, a HAdV-positive bronchoalveolar lavage (BAL) was identified from a patient hospitalised in the medical ICU in our university hospital. In the following outbreak, we defined our case-patients as patients hospitalised at the ICU with a HAdV-positive BAL associated with a clinical worsening of respiratory status or deterioration of their chest X-rays. The outbreak duration was defined as the time between the first and last case identification.

The 14-bed ICU serves critically ill adults with a particular recruitment of lung-transplanted patients, and patients with severe respiratory failure and infections. BALs from patients hospitalised in the ICU are routinely tested with a respiratory virus panel including influenza A and B viruses, human respiratory syncytial viruses A and B, human rhinoviruses, human metapneumoviruses, human adenoviruses, human parainfluenzaviruses (1, 2, 3 and 4), and human coronaviruses (229^E, OC43 and HKU1). All rooms are individually high-efficiency particulate air (HEPA)-filtered.

Laboratory Investigations

Samples (BAL, nasopharyngeal swabs) for molecular detection of HAdV were analysed at the Virology Laboratory of the public hospitals of Marseille. Total nucleic acids extraction was performed using the EZ1-XL Biorobot with the Virus Mini Kit 2.0 (Qiagen, Hilden, Germany) from 200µl of BAL. Quantitative molecular detection of HAdV was performed using the commercial Adenovirus R-gene kit (Argene, BioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions.

The HAdV species detected (A, B, C, D, E, F or G) was determined using six real-time polymerase chain reaction (PCR) assays as previously described [16]: briefly, 10µl of total nucleic acids extract was used to perform a real-time PCR using a C1000Touch/CFX96 thermocycler (Bio-Rad, Marnes-la-Coquette, France), the Fast qPCRMastermix – No ROX kit (Eurogentec, Angers, France) and a standard cycling protocol recommended by the manufacturer.

The type of the HAdV C was determined by sequencing the hexon hypervariable region as described previously [17]: in short, 5µl of total nucleic acids extract was used to perform a PCR using the Platinum PCR SuperMix High Fidelity kit (Life Technologies, Saint-Aubin, France), the primers AdV_HVR_PCR_C_F1 (5'-ATGATGCCGCAGTGGTCTTAC-3'), and AdV_HVR_PCR_C_R1 (5'-ATTAAAGGACTGGTCGTTGGTGTC-3') and a standard cycling protocol (annealing temperature: 58°C). Amplicons were sequenced using the following previously described primers (AdV_HVR_PCR_C_F2: 5'-ACG ACG TRA CCA CAG ACC G-3'; AdV_HVR_PCR_C_R2: 5'-GCC ACC ACT CGC TTG TTC AT-3'; AdV_HVR_Seq_CF652: 5'-GGM GAA TCT CAG TGG WAY GAA-3'; AdV_HVR_Seq_F1183: 5'-TAY TTT TCY ATG TGG AAK CAG GC-3'; AdV_HVR_Seq_R1148: 5'-TGR TAK GAM AGC TCT GTG TTT CTG-3'; AdV_HVR_Seq_R744: 5'-ATA NGA WCC RTA RCA TGG TTT CAT-3'). Data from sequencing reactions were combined for analysis and edited using the Sequencer 5.1 software (Gene Codes Corporation, Ann Arbor, USA). All types found using this method were HAdV type 1 (results were subjected to BLAST analysis [18]. We used Clustal X [19] to align these sequences with other homologous sequences of HAdV C (HAdV-1 : AC_000017, AF534906, FJ943633, FJ943635, FJ943621, and DQ336392; HAdV-2 : AC_000007 and J01917; HAdV-5: AC_000008 and AY601635; HAdV-6: HQ413315). Based on this alignment of the hexon hypervariable region, two primers (forward: 5'-ATGCTCAGGCTCCTTTGGCAGG-3'; reverse: 5'-TCAGCTTCATTCCACTGAGATTCT CC-3'; PCR-product length: 148 bp) were designed for a region with high inter- type variability to specifically detect the HAdV-1, and a SYBR Green real-time PCR assay was performed with 10µl of total nucleic acids extract, the Quantitec SYBR Green PCR kit (Qiagen), a C1000Touch/CFX96 thermocycler (Bio-Rad) and a standard protocol (annealing temperature: 60°C) with a melting-curve analysis. The specificity of the assay was assessed with two samples positive for HAdV-2 and three samples positive for HAdV-B: all were negative (data not shown).

Infection Control Measures

The infection control team was alerted after confirmation of the first case-patient. The initial intervention in the ICU consisted of placing the patients who tested positive for HAdV under contact and droplet precautions as recommended by the French Society of Hospital Hygiene [20], and limiting visits from their relatives. Other infection control measures that were implemented included the recommended use of alcohol-based hand rub with a product (Hanlabs bloc'K, Christeyns, Vertou, France) evaluated for its effectiveness against non-enveloped viruses according to the European Norm (EN) 14476. Reinforcement of hand hygiene consisted in daily information and surveillance of hand hygiene compliance. Daily environmental surfaces disinfection with a prepared sodium hypochlorite aqueous solution was implemented. Qualitative assessment of the compliance with these measures was carried out by the presence of an infection control team member supervising practices once daily until the end of the outbreak. All HCWs were reminded that if they had respiratory signs or a fever that they should avoid coming to work or when working wear a surgical mask at all times until being asymptomatic.

In addition, nasopharyngeal swabs were collected from HCWs working at the ICU. Among all 101 HCWs (13 physicians and 88 nurses), 43% (13 physicians and 30 nurses) voluntarily accepted to be sampled for HAdV testing. An information sheet about HAdV transmission

that included measures to prevent secondary cases in household contacts was given to all HCWs and to the relatives of the patients.

Results

Outbreak description

The first case-patient of the HAdV outbreak was identified on 15 September and the last one 19 days later, on 4 October 2012 (Figure). During the outbreak period, seven (5 men, 2 women) of 30 patients admitted to the ICU had a positive BAL for HAdV. We assumed that six of the seven infections were nosocomially-acquired because the time from hospital admission to an HAdV-positive sample was greater than the incubation period for HAdV (range 2-14 days) or prior BAL samples had been negative [21].

Of the seven patients, three had recently received lung transplants; the remaining four had been admitted to the ICU for acute respiratory failure. One patient suffered from lung cancer. The average age was 48.5 years old (range 31– 67). The time from admission to the ICU to positive BAL ranged from one to 51 days. All positive patients were mechanically ventilated at the time of the sampling. Lung-transplanted patients were treated with methylprednisolone and tacrolimus (Table 1). Clinical worsening of respiratory status at the time of the generation of the positive sample was observed in six of the patients, whereas one patient was asymptomatic and HAdV was diagnosed in a routine control BAL. However, for this patient we observed a deterioration of their chest radiography with the increase in interstitial diffuse opacities. Of the six patients who had a clinical degradation, four had a BAL also positive for bacteria, suggesting a co-infection. For further two patients, HAdV was the only pathogen identified in their BAL. Three patients died at the ICU. Death occurred six days after the positive sample for one patient and 20 and 23 days after the symptom onset for the other two respectively.

The HAdV species was determined for six of seven patients, and the result was C for all of them. We identified HAdV-1 in the BAL of five patients by real-time PCR (Table 2). Furthermore, all sequences of the hexon hypervariable region were 100% identical (GenBank accession number: KM610306), suggesting a common source of infection. The HAdV type was not identified for two patients because of a very low quantity of HAdV DNA detected using our quantitative assay (data not shown). No other patients positive for HAdV C were identified during the outbreak in the hospital. Case-patients were distributed all-over the ICU. It was confirmed that the three positive nurses took care of all those patients. Only two bronchoscopes were used during the outbreak period, and those who performed the procedure were identified for each BAL performed. There was neither bronchoscope- nor user-associated HAdV PCR positivity (data not shown).

Figure

Timeline of diagnosis and outcomes for patients with human adenovirus type 1 severe pneumonia during their stay in the intensive care unit, human adenovirus type 1 outbreak, Marseille, France, September–October 2012 (n=7)

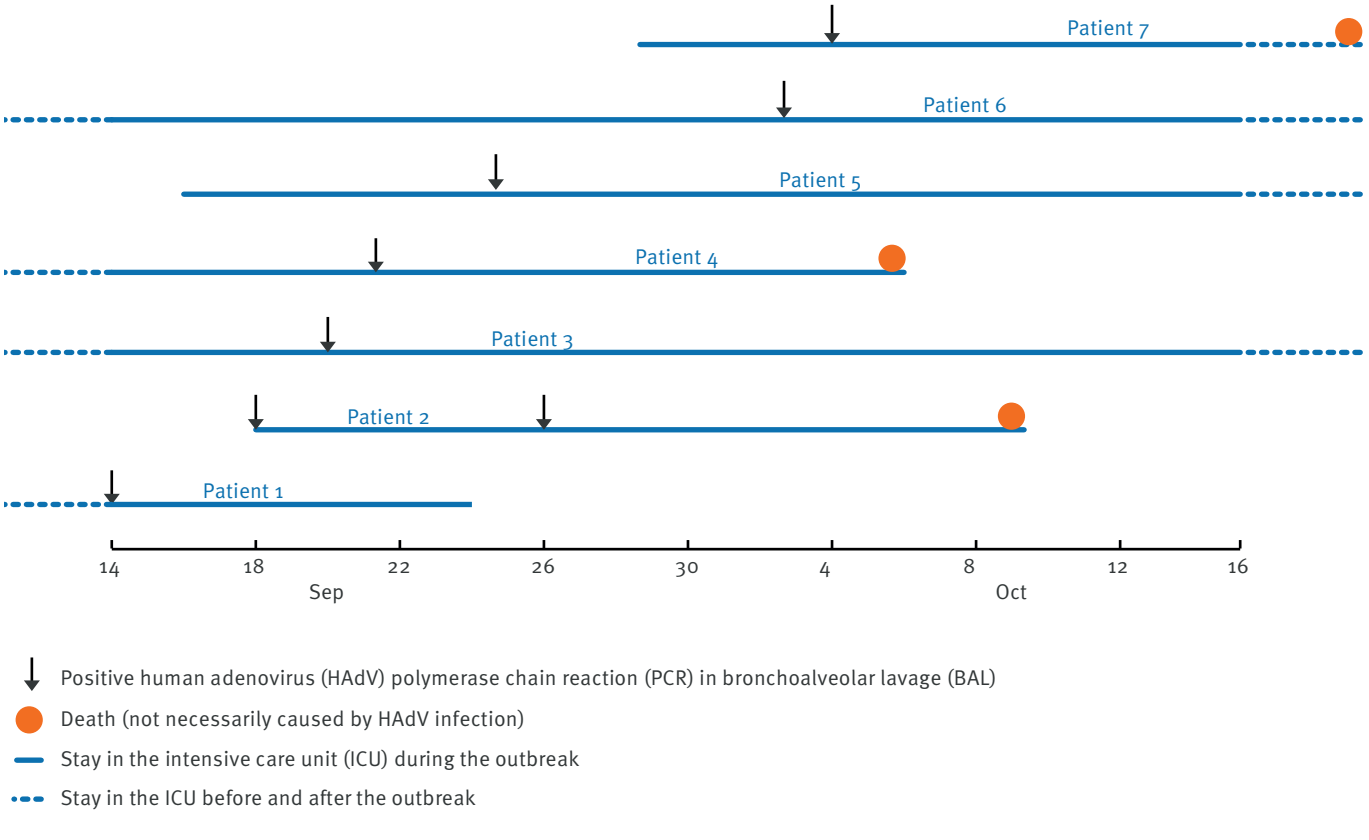


Table 1

Characteristics and clinical outcomes of patients with human adenovirus type 1 severe pneumonia, human adenovirus type 1 outbreak, Marseille, France, September–October 2012 (n=7)

Chronological order of positive BAL	Age (years)	Sex	Time from admission to BAL positivity (days)	Diagnosis at admission	Diagnosis at the time of BAL	Associated bacteriological infection documented in BAL	Chest X-ray or computed tomography	Outcome at discharge
1	53	M	26	Lung transplantation	Severe pneumonia	None	Increase of bilateral alveolar opacities	Alive
2	49	F	1	Aspiration pneumonia/ARDS	Severe ARDS and septic shock	None	Bilateral diffused opacities	Death
3	52	M	51	Right lobectomy for lung abscess	Severe pneumonia	*Pseudomonas aeruginosa*	Right alveolar opacities	Alive
4	67	M	49	Nosocomial pneumonia	Ventilator weaning failure	MRSA	Increase of basal left alveolar opacities	Death
5	45	F	10	Lung transplantation	ARDS	MRSA	Bilateral diffused opacities	Alive
6	31	M	26	Septic shock mediastinitis	Severe pneumonia	*Enterobacter cloacae*	Left lung alveolar opacities	Alive
7	43	M	3	Lung transplantation	Asymptomatic, routine BAL	None	Increase of right lung alveolar opacities	Death

ARDS: acute respiratory distress syndrome; BAL: bronchoalveolar lavage; F: females; M:males; MRSA: meticillin-resistant *Staphylococcus aureus*.

Table 2

Laboratory results for infected patients (n=7) and healthcare workers (n=3) in the intensive care unit, human adenovirus type 1 outbreak, Marseille, France, September–October 2012

		Sample	Specific HAdV q-real-time PCR	Species	HAdV type
Patients	1	BAL	Positive	C	1
	2	BAL	Positive	C	1
	3	BAL	Positive	C	n.a.
	4	BAL	Positive	C	1
	5	BAL	Positive	C	1
	6	BAL	Positive	C	1
	7	BAL	Positive	n.a.	n.a.
HCWs	1	Nasopharyngeal swab	Positive	C	n.a.
	2	Nasopharyngeal swab	Positive	n.a.	n.a.
	3	Nasopharyngeal swab	Positive	n.a.	n.a.

HCWs: healthcare workers; BAL: bronchoalveolar lavage; HAdV: human adenovirus ; PCR: polymerase chain reaction; n.a.: not available.

Testing of healthcare workers

Nasopharyngeal swabs were collected from 43 HCWs (all asymptomatic when sampled) and three tested positive for HAdV. They were all nurses working night shifts at the time of the outbreak. Interestingly, for one of them (nurse 1 in Table 2), identification to the species level was possible, and a C species was identified. Moreover, after systematic interviews with all HCWs who had had contact with the first identified case-patient within two weeks before the outbreak started, it appeared that nurse 1 was the only one who reported having fever with mild respiratory symptoms one week before. Upper or lower respiratory tract infection could not be determined in the absence of chest X-ray. She came to work without wearing a surgical mask.

None of the HCWs reported having relatives with fever or respiratory signs during the outbreak period.

Effectiveness of Infection Control Measures

Infection control measures were initiated on the day after the first acute patient had had onset of respiratory failure, when the virological results were received. Of the six case-patients who emerged after implementing control measures, five had either been subsequently infected or already been in incubation. We could not include patient two as a case-contact in this outbreak because she was positive for HAdV already at admission, suggesting an acquisition outside our hospital. Neither HCWs nor relatives developed symptoms after the first onset in patients. Surveillance showed better hand hygiene compliance over time.

No other HAdV-positive patient was diagnosed at the ICU during the 12 months of follow-up.

Discussion

We here described an outbreak of severe pneumonia caused by HAdV in a French ICU.

Recent studies have raised concerns about viral infections as a common cause of community-acquired and nosocomial pneumonia [22,23]. Availability of efficient molecular diagnostic tests and increased immunosuppressed population may have contributed to this trend. In a recent South African study using a multiplex real-time PCR for the identification of common viruses in patients hospitalised with severe acute respiratory illness, 13% were positive for HAdV [24]. In another study, the prevalence of HAdV was 3.8% among Sub-Saharan African children with severe pneumonia [25].

Our second patient was diagnosed one day after admission. She had been transferred from a peripheral hospital in the northwest of Marseille, suggesting a regional circulation of the virus. At the time of the outbreak, no other cases of HAdVs were reported in Marseille public hospitals (data not shown). Nevertheless, HAdV typing is not commonly performed for respiratory samples and thus, community-wide outbreaks of adenovirus are not easily detected. Previous reports are limited to those occurring in hospital, school, or military settings [3,26,27]. Rapid HAdV identification and typing is needed to curtail the spread of this pathogen, as shown in a recent investigation of the transmission of HAdV-14 from infected hospitalised patients to HCWs [14].

Virus detection does not necessarily mean clinically manifest disease [28]. However, in a recent population-based study, it was shown that when a viral infection is present either alone or during the same hospitalisation together with a bacterial infection, it is associated with an increased risk of mortality, ARDS, multi-system organ failure and septic shock. Interestingly, co-infections showed the strongest association with each adverse outcome [29]. In our study, bacterial co-infections were found in four of the seven patients. Severe immunosuppression remains a leading risk factor for disseminated HAdV disease and is associated with high mortality [30]. In particular, HAdV-1, HAdV-2 and HAdV-5, belonging to species HAdV C, were described as being frequently associated with disseminated disease in highly immunosuppressed patients [30,31]. In our study, there were three lung-transplanted patients and three immunocompetent patients; three patients died (1 lung-transplanted, 2 immunocompetent), and three improved without specific treatment for HAdV or modification of their immunosuppressive therapy. The relatively small number of patients precludes any comparative study with controls. Nonetheless, the HAdV PCR positivity in the BAL suggests a replication in the lungs that favours the hypothesis of an active infection.

As part of the measures implemented to prevent onward transmission in our hospital, we recommended a reinforcement of hand hygiene. Particularly, the substitution of the previous alcohol-based hand rub with a product evaluated for its effectiveness against non-enveloped viruses seemed critical for the control of the outbreak. In fact, the virucidal activity of the previously used alcohol-based hand rub was not tested, and it is not mandatory for a product to be listed as effective for hygienic hand disinfection by the French Society for Hospital Hygiene [32]. However, as previously observed, replacement of hand and surface disinfectant with a product proven to be active against non-enveloped viruses is essential in order to interrupt the chain of transmission during adenoviral infection outbreaks [33]. The second critical measure implemented to control our outbreak was to remind all HCWs that if they had respiratory signs or a fever that they should avoid coming to work or when working wear a surgical mask at all times until free of signs and symptoms.

We reported here, to the best of our knowledge, the first HAdV-1 pneumonia outbreak in mechanical ventilated critically ill patients involving immunocompetent patients. In this particular population, cross-transmission can occur during aerosol-generating procedures (i.e., bronchoscopy, nasotracheal suctioning) or by endotracheal tube contamination from hands during medication. The nurse who worked while exhibiting

respiratory signs and symptoms without wearing a mask might have been the source of the outbreak herein reported, but the route of transmission and a causal link could not be determined. Moreover, the fact that less than half of HCWs were tested for HAdV most likely results in an underestimation of viral circulation among this population during the outbreak.

Conclusion

Our findings suggest that HAdV C type 1 could be considered as a possible cause of severe pneumonia even in immunocompetent patients with a potential to cause outbreaks in ICUs. HAdV rapid identification and typing is needed to curtail the spread of this pathogen. Reinforcing hand hygiene with antiseptics with demonstrated activity against non-enveloped viruses and ensuring that HCWs with febrile respiratory symptoms avoid direct patient contact are critical measures to prevent transmission of HAdV in healthcare settings.

Acknowledgements

We acknowledge Georgette Grech for her technical assistance.

Conflict of interest

None declared.

Authors' contributions

NC wrote the manuscript, performed the outbreak investigation, analysed and interpreted the patient data. SH was a major contributor in writing the manuscript and took care of the patients. AN wrote the manuscript and performed the microbiological investigations. CZ helped perform the microbiological investigation. PEF wrote the manuscript. LP took care of the patients and wrote the manuscript. All authors read and approved the final manuscript.

References

1. Walsh MP, Seto J, Jones MS, Chodosh J, Xu W, Seto D. Computational analysis identifies human adenovirus type 55 as a re-emergent acute respiratory disease pathogen. J Clin Microbiol. 2010;48(3):991-3. http://dx.doi.org/10.1128/JCM.01694-09
2. Carballal G, Videla C, Misirlian A, Requeijo PV, Aguilar M del C. Adenovirus type 7 associated with severe and fatal acute lower respiratory infections in Argentine children. BMC Pediatr. 2002;2:6. http://dx.doi.org/10.1186/1471-2431-2-6
3. Kolavic-Gray SA, Binn LN, Sanchez JL, Cersovsky SB, Polyak CS, Mitchell-Raymundo F, et al. Large epidemic of adenovirus type 4 infection among military trainees: epidemiological, clinical, and laboratory studies. Clin Infect Dis. 2002;35(7):808-18. http://dx.doi.org/10.1086/342573
4. Kajon AE, Dickson LM, Metzgar D, Houng H-S, Lee V, Tan B-H. Outbreak of febrile respiratory illness associated with adenovirus 11a infection in a Singapore military training cAMP. J Clin Microbiol. 2010;48(4):1438-41. http://dx.doi.org/10.1128/JCM.01928-09
5. Trei JS, Johns NM, Garner JL, Noel LB, Ortman BV, Ensz KL, et al. Spread of adenovirus to geographically dispersed military installations, May-October 2007. Emerg Infect Dis. 2010;16(5):769-75. http://dx.doi.org/10.3201/eid1605.091633
6. Cao B, Huang G-H, Pu Z-H, Qu J-X, Yu X-M, Zhu Z, et al. Emergence of community-acquired adenovirus type 55 as a cause of community-onset pneumonia. Chest. 2014;145(1):79-86. http://dx.doi.org/10.1378/chest.13-1186
7. Filho EP, da Costa Faria NR, Fialho AM, de Assis RS, Almeida MMS, Rocha M, et al. Adenoviruses associated with acute gastroenteritis in hospitalized and community children up to 5 years old in Rio de Janeiro and Salvador, Brazil. J Med Microbiol. 2007;56(Pt 3):313-9. http://dx.doi.org/10.1099/jmm.0.46685-0
8. Kaneko H, Suzutani T, Aoki K, Kitaichi N, Ishida S, Ishiko H, et al. Epidemiological and virological features of epidemic keratoconjunctivitis due to new human adenovirus type 54 in Japan. Br J Ophthalmol. 2011;95(1):32-6. http://dx.doi.org/10.1136/bjo.2009.178772
9. Hofland CA, Eron LJ, Washecka RM. Hemorrhagic adenovirus cystitis after renal transplantation. Transplant Proc. 2004;36(10):3025-7. http://dx.doi.org/10.1016/j.transproceed.2004.10.090
10. Sattar SA, Springthorpe VS, Tetro J, Vashon R, Keswick B. Hygienic hand antiseptics: should they not have activity and label claims against viruses? Am J Infect Control. 2002;30(6):355-72. http://dx.doi.org/10.1067/mic.2002.124532
11. Kampf G, Kramer A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. Clin Microbiol Rev. 2004;17(4):863-93. http://dx.doi.org/10.1128/CMR.17.4.863-893.2004
12. Jernigan JA, Lowry BS, Hayden FG, Kyger SA, Conway BP, Gröschel DH, et al. Adenovirus type 8 epidemic keratoconjunctivitis in an eye clinic: risk factors and control. J Infect Dis. 1993;167(6):1307-13. http://dx.doi.org/10.1093/infdis/167.6.1307
13. Klinger JR, Sanchez MP, Curtin LA, Durkin M, Matyas B. Multiple cases of life-threatening adenovirus pneumonia in a mental health care center. Am J Respir Crit Care Med. 1998;157(2):645-9. http://dx.doi.org/10.1164/ajrccm.157.2.9608057
14. Lessa FC, Gould PL, Pascoe N, Erdman DD, Lu X, Bunning ML, et al. Health care transmission of a newly emergent adenovirus serotype in health care personnel at a military hospital in Texas, 2007. J Infect Dis. 2009;200(11):1759-65. http://dx.doi.org/10.1086/647987
15. Civardi E, Tzialla C, Baldanti F, Strocchio L, Manzoni P, Stronati M. Viral outbreaks in neonatal intensive care units: What we do not know. Am J Infect Control. 2013;41(10):854-6. http://dx.doi.org/10.1016/j.ajic.2013.01.026
16. Watzinger F, Suda M, Preuner S, Baumgartinger R, Ebner K, Baskova L, et al. Real-time quantitative PCR assays for detection and monitoring of pathogenic human viruses in immunosuppressed pediatric patients. J Clin Microbiol. 2004;42(11):5189-98. http://dx.doi.org/10.1128/JCM.42.11.5189-5198.2004
17. Biere B, Schweiger B. Human adenoviruses in respiratory infections: sequencing of the hexon hypervariable region reveals high sequence variability. J Clin Virol. 2010;47(4):366-71. http://dx.doi.org/10.1016/j.jcv.2010.01.005
18. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403-10. http://dx.doi.org/10.1016/S0022-2836(05)80360-2
19. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23(21):2947-8. http://dx.doi.org/10.1093/bioinformatics/btm404
20. Société francaise d'hygiène hospitalière: prévention de la transmission croisée par voie respiratoire : air ou gouttelettes. Hygiènes. 2013;21(1):1-40.
21. Lynch JP, Fishbein M, Echavarria M. Adenovirus. Semin Respir Crit Care Med. 2011;32(4):494-511. http://dx.doi.org/10.1055/s-0031-1283287
22. Johnstone J, Majumdar SR, Fox JD, Marrie TJ. Viral infection in adults hospitalized with community-acquired pneumonia: prevalence, pathogens, and presentation. Chest. 2008;134(6):1141-8. http://dx.doi.org/10.1378/chest.08-0888
23. Chiche L, Forel J-M, Papazian L. The role of viruses in nosocomial pneumonia. Curr Opin Infect Dis. 2011;24(2):152-6. http://dx.doi.org/10.1097/QCO.0b013e328343b6e4
24. Pretorius MA, Madhi SA, Cohen C, Naidoo D, Groome M, Moyes J, et al. Respiratory viral coinfections identified by a 10-plex real-time reverse-transcription polymerase chain reaction assay in patients hospitalized with severe acute respiratory illness--South Africa, 2009-2010. J Infect Dis. 2012;206(Suppl 1):S159-65. http://dx.doi.org/10.1093/infdis/jis538
25. Berkley JA, Munywoki P, Ngama M, Kazungu S, Abwao J, Bett A, et al. Viral etiology of severe pneumonia among Kenyan infants and children. JAMA. 2010;303(20):2051-7. http://dx.doi.org/10.1001/jama.2010.675
26. Hong JY, Lee HJ, Piedra PA, Choi EH, Park KH, Koh YY, et al. Lower respiratory tract infections due to adenovirus in hospitalized Korean children: epidemiology, clinical features,

and prognosis. Clin Infect Dis. 2001;32(10):1423-9. http://dx.doi.org/10.1086/320146

27. Xie L, Yu X-F, Sun Z, Yang X-H, Huang R-J, Wang J, et al. Two adenovirus serotype 3 outbreaks associated with febrile respiratory disease and pharyngoconjunctival fever in children under 15 years of age in Hangzhou, China, during 2011. J Clin Microbiol. 2012;50(6):1879-88. http://dx.doi.org/10.1128/JCM.06523-11
28. Luyt C-E. Virus diseases in ICU patients: a long time underestimated; but be aware of overestimation. Intensive Care Med. 2006;32(7):968-70. http://dx.doi.org/10.1007/s00134-006-0203-9
29. Miggins M, Hasan A, Hohmann S, Southwick F, Casella G, Schain D, et al. The potential influence of common viral infections diagnosed during hospitalization among critically ill patients in the United States. PLoS ONE. 2011;6(4):e18890. http://dx.doi.org/10.1371/journal.pone.0018890
30. Echavarría M. Adenoviruses in immunocompromised hosts. Clin Microbiol Rev. 2008;21(4):704-15. http://dx.doi.org/10.1128/CMR.00052-07
31. Ganzenmueller T, Buchholz S, Harste G, Dammann E, Trenschel R, Heim A. High lethality of human adenovirus disease in adult allogeneic stem cell transplant recipients with high adenoviral blood load. J Clin Virol. 2011;52(1):55-9. http://dx.doi.org/10.1016/j.jcv.2011.06.005
32. Société francaise d'hygiène hospitalière: Liste positive désinfectants. Hygiènes. 2009;17(3):1-24.
33. Chaberny IE, Schnitzler P, Geiss HK, Wendt C. An outbreak of epidemic keratoconjunctivtis in a pediatric unit due to adenovirus type 8. Infect Control Hosp Epidemiol. 2003;24(7):514-9. http://dx.doi.org/10.1086/502247

Large outbreak of *Salmonella* Thompson related to smoked salmon in the Netherlands, August to December 2012

I Friesema (ingrid.friesema@rivm.nl)[1,2], A de Jong[2,3], A Hofhuis[1,2], M Heck[4], H van den Kerkhof[5], R de Jonge[6], D Hameryck[3], K Nagel[3], G van Vilsteren[3], P van Beek[5], D Notermans[4], W van Pelt[1]

1. Epidemiology and Surveillance Unit, Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
2. These authors contributed equally to this work
3. Dutch Food and Consumer Product Safety Authority (NVWA), Utrecht, the Netherlands
4. Laboratory for Infectious Diseases and Perinatal Screening, Centre for Infectious Disease Control, RIVM, Bilthoven, the Netherlands
5. Preparedness and Response Unit, Centre for Infectious Disease Control, RIVM, Bilthoven, the Netherlands
6. Centre for Zoonoses and Environmental Microbiology, Centre for Infectious Disease Control, RIVM, Bilthoven, the Netherlands

Citation style for this article:
Friesema I, de Jong A, Hofhuis A, Heck M, van den Kerkhof H, de Jonge R, Hameryck D, Nagel K, van Vilsteren G, van Beek P, Notermans D, van Pelt W. Large outbreak of Salmonella Thompson related to smoked salmon in the Netherlands, August to December 2012. Euro Surveill. 2014;19(39):pii=20918. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20918

Article submitted on 20 June 2013 / published on 2 October 2014

On 15 August 2012, an increase in the number of *Salmonella* Thompson cases was noticed by the *Salmonella* surveillance in the Netherlands. A case–control study was performed, followed by a food investigation. In total 1,149 cases were laboratory-confirmed between August and December 2012 of which four elderly (76–91 years) were reported to have died due to the infection. The cause of the outbreak was smoked salmon processed at a single site. The smoked salmon had been continuously contaminated in the processing lines through reusable dishes, which turned out to be porous and had become loaded with bacteria. This is the largest outbreak of salmonellosis ever recorded in the Netherlands. The temporary closure of the processing site and recall of the smoked salmon stopped the outbreak. An estimated four to six million Dutch residents were possibly exposed to the contaminated smoked salmon and an estimated 23,000 persons would have had acute gastroenteritis with *S.* Thompson during this outbreak. This outbreak showed that close collaboration between diagnostic laboratories, regional public health services, the national institute for public health and the food safety authorities is essential in outbreak investigations.

Introduction

In the Netherlands, an estimated 35,000 cases of salmonellosis occurred in 2009, which equals around 212 cases per 100,000 inhabitants [1]. Overall, based upon data from the Dutch laboratory surveillance network for gastroenteric pathogens, the incidence of salmonellosis in the Netherlands has decreased since 1997, with some peaks due to outbreaks [2]. Almost every year, 15 to 20 outbreaks of salmonellosis are detected at a regional or national level. The largest outbreaks recorded in the Netherlands up to the time of this report, were an excess of 540 confirmed cases of *Salmonella* Enteritidis in 2003 most likely caused by increased importation of contaminated eggs, during the avian influenza outbreak [3]; and an outbreak of *S.* Typhimurium phage type 561 with 224 laboratory-confirmed cases due to contaminated hard, raw milk cheese in 2006 [4]. Whereas *S.* Typhimurium together with *S.* Enteritidis are the most common serotypes responsible for salmonellosis in the Netherlands, infections with *S.* Thompson are rare with zero to seven cases per year laboratory-confirmed within the laboratory surveillance network in the past ten years [2]. In the literature, the number of reports on outbreaks due to *S.* Thompson are limited with distinct implicated sources as cilantro [5], rucola lettuce [6], bread contaminated by an ill food handler [7], roast beef [8], egg albumen [9], and cow's milk [9].

On 15 August 2012 (week 33), the National Institute for Public Health and the Environment (RIVM) noticed an increase in the number of *S.* Thompson cases in the Dutch laboratory surveillance network. That week, 11 cases and two weeks earlier four cases were detected at the RIVM, scattered over the country. This prompted an outbreak investigation to identify the source, in order to take subsequent actions to prevent further cases. In October 2012, as the outbreak was ongoing, a preliminary report was published [10]. In this final report, all available data were combined to describe the complete outbreak.

Methods

Laboratory surveillance network

The Dutch laboratory surveillance network, established in 1987, is based on 16 regional public health laboratories, which send *Salmonella* isolates from patients to the RIVM for further typing, covering ca 64% of the

Dutch population [11]. Each isolate is accompanied by a standardised completed request form with information about the submitting laboratory, basic demographics of the patient and the isolate. At the RIVM, the isolates are serotyped and the results and background information are filed in the laboratory registry system. Additionally, during this outbreak, Dutch diagnostic laboratories outside the surveillance network, were encouraged to submit *Salmonella* group C isolates, to which *S.* Thompson belongs, in order to get a more complete picture of the outbreak.

Epidemiological investigation

Cases were defined as residents of the Netherlands with an *S.* Thompson isolate cultured from any sample type, confirmed at the RIVM between August and December 2012. Between 16 August (week 33) and 28 September (week 39) when the source was identified, the regional public health services were requested once a week to contact the new cases of that week within their region after obtaining consent from the doctor in attendance, to administer an extensive questionnaire. This questionnaire covered consumption of different meats, fish, dairy products, vegetables and fruits, snacks, establishments where food was purchased, contact with a person with diarrhoea and contact with animals during the seven days before onset of illness. Furthermore, information about the symptoms, onset of illness and hospitalisation was asked. An adapted questionnaire was also sent to controls from the general population in the same period (week 33–39). From the database with a random sample of the Dutch population available at the RIVM, four controls from the same or neighbouring municipality with comparable year of birth, and sex were drawn. The control questionnaire was sent by mail. An envelope with a freepost number was included to return the questionnaire.

On 28 September (week 39) the source was found, namely smoked salmon, and a recall was started to remove the product from the market. As the number of submitted isolates kept rising after the recall, a supplemental study was performed between 19 October and 22 November (week 42–47), in order to monitor the course of the outbreak and to check whether smoked salmon was still the cause. Cases with a first date of illness after 5 October 2012 (more than one week after the start of the recall) or unknown onset of illness were contacted with a short questionnaire. The cases were asked when they fell ill, whether they had eaten fish or seafood and if so, where they had bought or eaten it, when, and what type of fish or seafood. Cases were also requested to indicate whether they had been in contact with another patient with similar symptoms in the week before falling ill.

Food and trace-back investigations

The Dutch Food and Consumer Product Safety Authority (NVWA), which is responsible for product tracing during foodborne outbreaks in the Netherlands, performed a trace-back investigation based on the results of the case–control study. Subsequently, samples were taken at the processing site of a Dutch producer of smoked salmon that emerged from this investigation as a likely source of the outbreak.

Microbiological investigation

Salmonella isolates submitted to the RIVM were serotyped based on O- and H-group antigens according to the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* [12]. A subset of isolates of *Salmonella enterica* subsp. *enterica* Thompson from patients and food samples were subjected to molecular typing by means of pulsed-field gel electrophoresis (PFGE) according to the PulsNet International protocol [13]. Restriction enzyme *XbaI* was used for digestion of DNA. The banding patterns, i.e. DNA fingerprints, were compared using BioNumerics 6.6 (Applied Maths, Sint-Martens-Laten, Belgium) with tolerance and optimisation both set at 1%. To confirm our PFGE analysis, strains were sent to Centers for Disease Control and Prevention (CDC) in Atlanta for DNA fingerprinting.

International inquiry

An urgent inquiry was sent out by the RIVM on 23 August 2012 to European Union (EU) Member States via the Epidemic Intelligence Information System (EPIS), managed by the European Centre for Disease Prevention and Control (ECDC). Member States were asked to report any increase in the number of cases of *S.* Thompson in their countries. On 1 October a notice was sent to all National Focal Points in Europe via the Early Warning and Response System (EWRS).

Statistical analyses

All statistical analyses were performed using SAS 9.2 (SAS institute Inc., USA). In the case–control analysis, variables with a p-value of less than 0.20 in the univariate logistic regression were entered into the multivariate analysis. A final model was determined by backward elimination of variables, until all variables in the model had reached significance ($p<0.05$), adjusted for age and sex, and the model was significant. Day of onset was mainly available for the cases completing the extensive questionnaire or the supplemental questionnaire, and only rarely for the other cases via the form accompanying the *Salmonella* isolate presented for serotyping. The epidemic curve of the frequencies of cases by dates of onset was biased, as the questionnaires were completed at the beginning of the outbreak (extensive questionnaire) or at the end of the outbreak (supplemental questionnaire). Therefore, for cases without known day of onset but with a date of sampling, an estimated day of onset was calculated based on the median number of days between date of onset and sampling for the cases in the same period (whereby the time of the outbreak was divided in three periods: beginning, middle and end). This time span between onset and sampling was estimated using data from cases with both dates available.

Results

Descriptive epidemiology

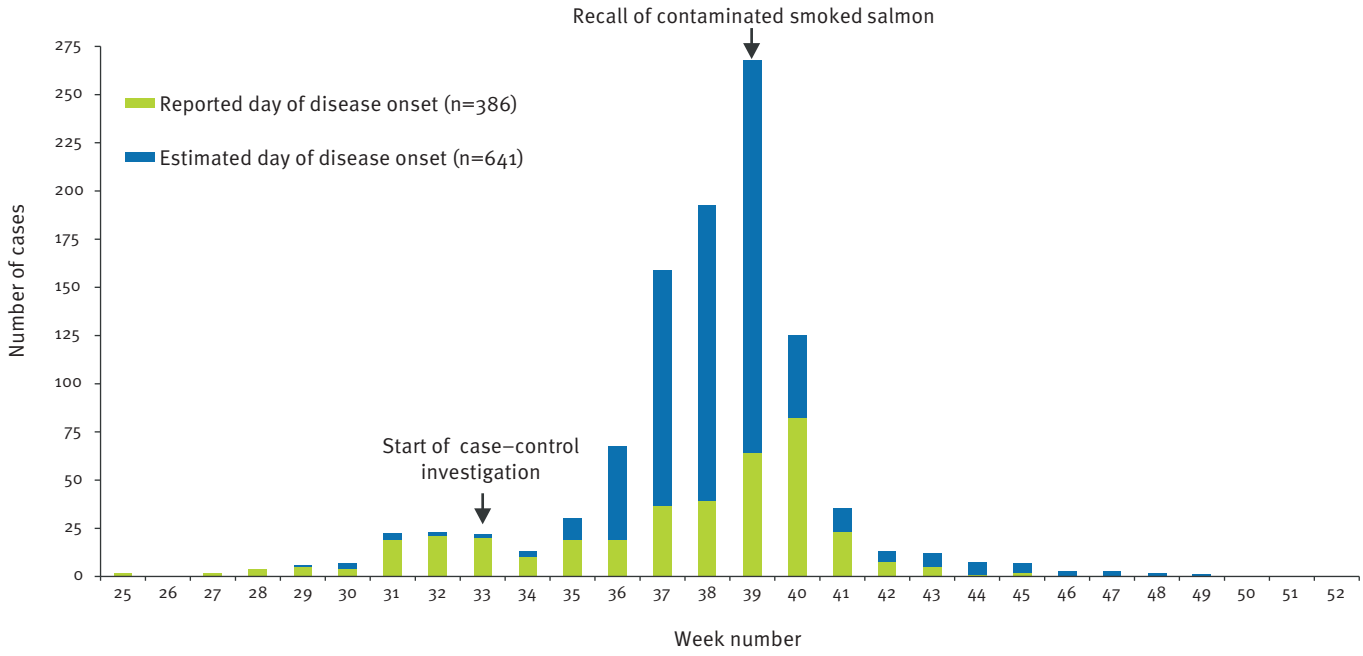
In total 1,149 cases were laboratory-confirmed at the RIVM between August and December 2012 of which 812 within the national surveillance network laboratories and 337 within Dutch diagnostics laboratories outside the surveillance network, which had been encouraged to submit *Salmonella* group C isolates. Two men and two women aged between 76 and 91 years were reported to have died due to the *S.* Thompson infection. The Figure shows the epidemic curve according to reported and estimated day of disease onset. The first isolates of the outbreak arrived at the RIVM on 27 July (week 30), and the last isolates arrived on 27 and 28 December (week 52). Serotyping at the RIVM lasted between two and 21 days (median seven days), although the preliminary results (before being checked and made available by RIVM) were frequently ready up to two days earlier than the median seven days. The peak of isolate entries was in week 41. Reported dates of disease onset ranged from 20 June to 10 November with a peak in week 40. However, when an estimation of the date of onset for the cases without a known date of illness onset was also taken into account, the peak shifted to week 39.

For the 1,079 outbreak cases for which sex was known, 696 (65%) were female (Table 1). Age information was available for all cases (n=1,149) and the median age was 45 years (range: 0–95 years). For comparison, the median age of the other 1,624 cases of salmonellosis reported to the RIVM in 2012 was 29 years, and 53% were female. Strikingly, five outbreak cases were younger than six months and thus most likely not eating solid foods yet, three of them had family members with a confirmed infection. The youngest case was two days-old when tested, and was most likely infected during birth as the mother also tested positive. The mother of another baby who had become ill at the age of one month, had been positive two days before giving birth. A three-month-old baby had a 23-month-old sister who was ill. Another nine family clusters could be identified among the 1,149 confirmed cases: mother–child (n=3), siblings (n=3) and partners (n=3). In the extensive questionnaire, 7% (8/112) reported to have had contact with a person with diarrhoea in the week before falling ill. In the supplemental study, this question was added later with seven cases of 27 having had such contact.

The extensive questionnaire was completed by 112 respondents (response 63%). As expected, diarrhoea was the most important symptom (108/112, 96%; Table 2) with a median duration of seven days (range: 1–21 days). About one-quarter of the cases (29/104) recorded blood in their stool. Fever (›38.0˚C) was mentioned by 63 (62%) of 101 cases, and 39 of 112 cases (35%) were hospitalised for a median of four days (range: 1–15 days). The median age of the hospitalised cases was 70 years (range: 7–91 years).

Figure

Number of *Salmonella* Thompson outbreak cases according to reported and estimated day of disease onset , the Netherlands, 18 June–30 December (n=1,027)[a]



[a] Although a total of 1,149 laboratory-confirmed cases were part of the outbreak, only 1,027 are depicted in this Figure because for 122 cases the day of disease onset could not be determined due to missing information.

For cases with known dates of illness onset and sampling, the delay between date of onset and sampling for diagnostics appeared to increase during the outbreak, especially toward the end (Table 3). The time between sampling and arrival of the isolate at the RIVM remained stable with a median of seven days, and 98% (967/983) within 14 days. Consecutive second and third positive isolates were sent in at a later point in time for 43 cases and three cases, respectively. When excluding the cases with the second sampling on the same day as the first sampling, median number of days between both sampling moments was nine days, with a range of 1 to 76 days (n=30 cases). Twenty-one cases of which repeated samples were submitted were 55 years of age or older. Information on hospitalisation was available for seven of these cases, and all seven were admitted to hospital.

Case–control study

Every time completed questionnaires were returned, the case–control statistical analysis was repeated, and results of possible outbreak sources were communicated with the NVWA. Analyses indicated several potential sources, namely minced meat (10 September), ready-to-eat raw vegetables (17 September), ice cream (18 September) and smoked fish (24 September). Another result of the analyses was that cases more frequently reported to shop at certain supermarket chains affiliated with one purchasing coordinating organisation. The odds ratio (OR) for smoked fish was 6.4 (95% confidence interval (CI): 3.3–12.5) in the final multivariate analysis with all available questionnaires (108 cases and 198 controls). Fifty-seven per cent of the cases reported consumption of smoked fish (62/108), mostly smoked salmon, compared to 52 of 198 (26%) of the controls. Other risk factors were buying at supermarket chains affiliated with the purchasing organisation (OR: 3.5; 95% CI: 1.9–6.8), buying at supermarket chain A (OR: 2.3; 95% CI: 1.2–4.5), and consumption of ready-to-eat raw vegetables (OR: 3.3; 95% CI: 1.3–8.4) and ice cream (OR: 2.3; 95% CI: 1.2–4.7). No significant interaction terms were found between the supermarkets and the food products.

Food and trace-back investigations

The NVWA followed up each of the four possible sources identified by the case–control study. Where possible, supermarkets and patients were contacted and food samples were taken. Ready-to-eat vegetables were quickly ruled out, as they included a large variety of vegetables from many different sources and producers. The purchasing organisation informed the NVWA that ice cream and vegetables were not procured at one single producer. However, when the case–control analysis pointed toward smoked fish consumption, particularly smoked salmon, the purchasing organisation indicated that all smoked salmon originated from

Table 1

Characteristics of the reported *Salmonella* Thompson outbreak cases, the Netherlands, August–December 2012 (n=1,149)

Characteristics of cases	N (%)
Sex[a]	
Female	696 (65)[a]
Male	383 (35)[a]
Unknown	70 (6)
Age group in years[b]	
0–9	124 (11)
10–19	173 (15)
20–29	154 (13)
30–39	75 (7)
40–49	94 (8)
50–59	147 (13)
60–69	165 (14)
70–79	130 (11)
≥80	87 (8)

[a] The percentages of males and females are calculated from the 1,079 cases, for which Information on sex was available.

[b] The age of all cases was known and the median age of cases was 45 years (range: 0–95).

Table 2

Symptoms and hospitalisation as reported by *Salmonella* Thompson outbreak cases in the extensive questionnaire, the Netherlands, August–December 2012 (n=112)

Characteristic	n/N[a] (%)
Diarrhoea	108/112 (96)
Blood in stool	29/104 (28)
Nausea	67/112 (60)
Vomiting	44/111 (40)
Abdominal pain	88/111 (79)
Fever (> 38.0 °C)	63/101 (62)
Tremors	58/101 (57)
Hospitalisation	39/112 (35)

[a] Some symptoms were not reported by all the questionnaire respondents.

Table 3

Days between date of illness onset and date of sampling, *Salmonella* Thompson outbreak, the Netherlands, August–December 2012 (n=340)

Period within the outbreak	Number of cases	Median time between date of illness onset and date of sampling (range)
Week 31–37[a]	74	5 days (0–80 days)
Week 38–43[a]	234	5 days (0–92 days)
Week 44–52[a]	32	17 days (0–83 days)

[a] Refers to the week number in 2012 in which the isolate arrived at the National Institute for Public Health and the Environment (RIVM).

one Dutch producer. Supermarket chain A also bought a part of its smoked salmon at the same producer as the purchasing organisation. The day after this finding, 26 September, the NVWA visited the Dutch fish producer and collected samples from different batches of smoked salmon products, as did the fish producer. *S.* Thompson was detected by the NVWA in four of nine sampled batches. Subsequently, all smoked salmon from this producer was recalled, starting Friday 28 September (week 39) and a public warning was published. A trace-back analysis by the fish producer showed that the positive batches all were produced on certain production lines in the Greek processing plant of this company. There, the fish is processed before being transported to the Netherlands for further distribution. In week 40, other products containing possibly contaminated smoked salmon, such as ready-to-eat salads, were also recalled. Information given by the producer also indicated that smoked salmon was exported to countries in Europe, North America and Central America. An alert was sent out on 1 October via the Rapid Alert System for Food and Feed (RASFF; 2012.1382) to inform these countries of the recall and the ongoing outbreak. This notification did not lead to reports of cases. Furthermore, the Greek authorities (EFET: Helenic Food Authority) were informed about the problem in the Greek production facility of the Dutch fish producer. Based on this alert notification and additional information, EFET temporarily closed the Greek production site of the Dutch fish producer (3–11 October). After analysis of the production process in Greece, the Dutch producer concluded, that the continuing contamination of smoked salmon must have been caused by cross contamination from dishes on which the salmon was transported within the processing lines. These reusable dishes were the most recent main adaptation in the production process and where known to be porous. The dishes were introduced in the production process in Greece in February 2012. Indeed, research conducted by the fish producer showed that the inner layer of the dishes appeared to be filthy and was contaminated with bacteria, including *Salmonella*. Additional research conducted by the Netherlands Organisation for Applied Scientific Research (TNO) showed that the inner layer of the dishes was highly porous and absorbing the *Salmonella* [14]. How the dishes initially got contaminated remains unknown.

Supplemental study

Between week 42 and 47, a total of 178 cases presented with first date of illness either after 5 October 2012 (n=14) or unknown (n=164), according to the laboratory request form. The regional public health services contacted these cases with the short questionnaire asking for date of disease onset, fish consumption and possible contact with an ill person with similar symptoms: 87 cases reported a date of illness before 5 October, 43 cases had a date of onset after 5 October, and for 48 cases it remained unknown. For 29 of the 43 cases, consumption of contaminated salmon purchased before the recall or contact with another *S.* Thompson case was known or likely. For the remaining cases, the information was insufficient or did not point in the direction of salmon or contact with another *S.* Thompson case; these cases were mostly younger than 10 years (n=6) or older than 70 years (n=5) of age. The results of this supplemental study did not indicate that any contaminated salmon remained on the market or that another source was contributing to the outbreak. Furthermore, the number of new cases per week decreased steadily during these weeks.

Microbiological investigation

The majority (93%, n=1,064) of first positive samples from cases were faeces samples. Other sources were urine (50; 4%), blood (24; 2%), and other or unknown (11; 1%). PFGE was done for isolates of 60 outbreak cases, 16 salmon samples and five non-related strains of previous years. All outbreak strains and salmon isolates presented the same pattern. This finding was confirmed by the typing laboratory of CDC in Atlanta. The strains belonged to pulse type JP6X01.0001. The non-related strains of *S.* Thompson showed a different pattern.

International inquiry

Eighteen EU Member States responded to the urgent inquiry and reported no significant increase. Three countries reported cases with a PFGE pattern matching the current outbreak strain prior to this outbreak. One of them was a Scandinavian outbreak of *S.* Thompson in 2004 caused by rucola salad [6]. Furthermore, Sweden reported one domestic case and Germany reported three domestic cases with a PFGE pattern similar to the outbreak strain and dates of illness onset during the outbreak, but without a link to the Netherlands or salmon. A Belgian truck driver with an *S.* Thompson infection who had been in the Netherlands several times in the week before falling ill, probably got infected in the Netherlands. At the same time, a cluster of *S.* Thompson infections was investigated in the United States (US) (personal communication, Dr Laura Gieraltowski and Dr Peter Gerner-Smidt, CDC, US; October 2012 and April 2013). No particular exposure was identified. First microbiological results indicated a similar PFGE pattern, but later significant differences between the strains were detected by whole genome sequencing. No connection was found between these concurrent outbreaks.

Discussion

This outbreak of *S.* Thompson is the largest outbreak of salmonellosis ever recorded in the Netherlands, with 1,149 confirmed cases. However, this number is likely to present only the tip of the iceberg. Dutch population studies provided the multipliers to estimate total numbers of *Salmonella* cases based on the cases sent in by the laboratories within the Dutch laboratory surveillance network [1,11]. Knowing that 812 outbreak cases were submitted within this network, an estimated 23,000 persons would have had acute gastroenteritis with *S.* Thompson in the general population, of which

650 would have been hospitalised and ca 24 persons may have died from this infection within two years after their infection.

Although a case–control study was started immediately after detection of the outbreak, it took several weeks before the cause could be identified. The main reason for the delay in identifying the source was the low number of cases in the beginning of the outbreak and consequently the low number of completed questionnaires, both from cases and controls, available for analysis. A number of other possible sources were suggested before the analysis led to salmon. This was most likely caused by the relatively low number of cases reporting consumption of smoked fish (62/108, 57%). This can be due to recall bias, but also because the salmon was incorporated in other products, for example pre-sale ready-to-eat salads or as part of a menu in the catering industry.

The incriminated producer had a large market share for smoked salmon. Based upon information provided by randomly selected respondents in a survey conducted among the general population done by the Epidemiology and Surveillance Unit of the RIVM, 47% of the Dutch population consumed smoked salmon in a four weeks period (data not shown). Considering a market share of 50 to 80% for the company, 3.9 to 6.2 million Dutch residents would have been possibly exposed to contaminated smoked salmon in four weeks' time. The reach of one product of one producer with a high market share is huge with consequently large possible implications for the 16 million Dutch population.

Research into the specific origin of the contamination within the processing site in this outbreak proved to be difficult. In the Netherlands, the NVWA is the legal body to perform such source investigation, and is authorised to take active measures in case a source is detected. However, their mandate is limited to the Netherlands and the contaminated production site was in Greece. Via the RASFF system, the NVWA informed the Greek authorities about the problem in the Greek production site of the Dutch fish producer. However, information from the Greek investigation was scarcely available during the Dutch outbreak investigation, thus it was a challenge for the NVWA to quickly reconstruct in an objective way what had gone wrong in the production process in Greece. No objective information was available on whether the contamination possibly had spread through (other) production processes in Greece or to the Dutch production site. The NVWA mostly relied on information provided by the fish producer. Many important details have been published in a study of the Dutch safety board (Onderzoeksraad voor Veiligheid [14]) and are included in this paper. However, it must be noted that some of the information known to the NVWA cannot be disclosed due to judicial restrictions, and thus cannot be presented here.

The smoked salmon in the production line had been continuously contaminated through reusable dishes in the production process, which turned out to be porous and became loaded with bacteria, as the cleaning and disinfecting regime turned out to be not sufficient to kill bacteria on the inside of the plates. In the report of the Dutch safety board [14] data are presented on the trend lines of the number of *Enterobacteriaceae* in the production process in the Greek production facility, as measured by the fish producer. These data show a small, but not disquieting increase in *Enterobacteriaceae* levels after implementing the reusable dishes; between June and the end of September, when the source of the outbreak was found, the trend line started to strongly fluctuate. The fluctuation was caused by the alternating growth of bacteria in the production process followed by extra cleaning procedures. Circumstances in Greece were favourable for *Salmonella* to proliferate. First, the bacteria were present in the inside of the porous reusable dishes, out of range of the cleaning and disinfection procedures. Second, the temperature in the non-refrigerated storing room was running up during the hot Greek summer, reaching values favourable for outgrowth of *Salmonella*. However, how the first dish got contaminated remains unknown. The reused dishes were introduced in the production process in Greece in February 2012, and were immediately replaced by single-use dishes after being identified as the source of the contamination. A remarkable feature in the epidemic curve is the relative stable number of cases falling ill up to week 35, before the steep increase. This supports the hypothesis that the level of contamination at the production site was low at the onset, increasing over time and at a certain point in time increased exponentially. However, the outbreak received more attention as it progressed. Before the recall of the smoked salmon, knowledge of the outbreak was mostly limited to the professionals involved in the outbreak response, but the outbreak received a considerable amount of media attention after the recall. This probably led to more patients visiting their physician, physicians requesting more laboratory testing and more laboratories apart from the surveillance laboratories submitting strains, which all could have affected the epidemic curve. The increase of time lag between date of onset and sampling date at the end of the outbreak is supportive for this.

The peak of cases, based upon date of onset, was in week 39 with a rapid decline in the number of cases afterwards. As the recall of smoked salmon started at the end of week 39, it is very plausible that the decline in cases is the result of this recall. As the number of cases was increasing rapidly in the weeks prior to the recall, it is very likely that it would have continued to rise if the smoked salmon had not been withdrawn from the Dutch market. Nevertheless, the outbreak did not stop immediately after week 39. Possible reasons are the recall starting at the end of week 39, and an additional recall for products containing smoked salmon starting in week 40. Furthermore, not everyone

may have noticed the recall immediately or would have thrown away the smoked salmon already bought. Finally, because of the high number of cases person-to-person transmission could have prolonged the outbreak. Before the start of the outbreak, zero to seven infections with *S.* Thompson per year were confirmed at the RIVM. Comparison of the PFGE of isolates prior to the outbreak revealed a different pattern compared to the outbreak strain. Although the strain appeared to be new in the Netherlands, several European countries had previously reported cases affected by a strain of similar pattern, including an outbreak in Norway and Sweden in 2004 [6]. Therefore, the strain does not appear to be new, even though it is unknown how well PFGE discriminates between the different *S.* Thompson strains.

Salmon is a rare cause of foodborne outbreaks of salmonellosis. Two outbreaks of *S.* Montevideo occurred in 1984 in a restaurant in the United Kingdom due to salmon, most likely contaminated by personnel after the cooking process [15]. Further, two different outbreaks of *S.* Enteritidis occurred after consumptions of salmon in 1999 in Denmark and in 2000 in the US, however in both outbreaks salmon dishes also contained eggs which could have been the cause of the outbreaks [16,17]. Reports of outbreaks due to *S.* Thompson are also scarce, and have not been linked to salmon before [5-9]. The cause of the current outbreak of *S.* Thompson was determined to be smoked salmon, based on the epidemiological case–control analyses, food investigation and product tracing, and microbiological confirmation. It is the largest outbreak of salmonellosis ever recorded in the Netherlands. The temporary closure of the Greek production site and recall of the smoked salmon prevented further cases. This outbreak showed that close collaboration between diagnostic laboratories, regional public health services, the national institute for public health and the food safety authorities is essential in outbreak investigations. Furthermore, outbreak investigations should start with an extensive questionnaire, as an outbreak can have an unexpected source.

Acknowledgements

We acknowledge the cases and controls, the officers from the public health services, physicians and microbiologists whose collaboration made this investigation possible. We thank Henny Maas, Anjo Verbruggen, Thijs Bosch and Kim van der Zwaluw for their technical assistance in the laboratory. Furthermore, we thank the colleagues of the NVWA involved in the recall (both laboratory and inspectors), and Corien Swaan, Harald Wychgel and Rody Zuidema (Centre for Infectious Disease Control, RIVM, Bilthoven) for their assistance in managing the outbreak. We thank Eva Gort and the colleagues of the Centre for Infectious Disease Control for their assistance in calling laboratories and regional public health services. Finally, we thank Eija Trees (Enteric Diseases Laboratory Branch, DFWED, NCEZID, CDC, Atlanta) for performing confirmatory PFGE on the Dutch *S.* Thompson.

Conflict of interest

None declared.

Authors' contributions

IF and AH coordinated the epidemiological part of the outbreak investigation, and collected, analysed and interpreted the data. IF drafted the manuscript. AdJ, and KN coordinated the food investigation part, and collected and interpreted the food-related data. DH and GvV carried out the food investigation. MH and DN coordinated the microbiological part of the outbreak investigation, and collected and analysed the *Salmonella* isolates. RdJ participated as advisor in the investigation. PvB and HvdK coordinated the internal and external communication about the outbreak and the investigation. WvP supervised the outbreak investigation and participated in the interpretation of the data. All authors participated in editing the manuscript, and read and approved the final manuscript.

References

1. Havelaar AH, Haagsma JA, Mangen MJ, Kemmeren JM, Verhoef LPB, Vijgen SMC, et al. Disease burden of foodborne pathogens in the Netherlands, 2009. Int J Food Microbiol. 2012;156(3):231-8. http://dx.doi.org/10.1016/j.ijfoodmicro.2012.03.029
2. Graveland H, Roest HJ, Stenvers O, Valkenburgh S, Friesema I, Van der Giessen J, et al. Staat van zoönosen 2012 [State of zoonoses]. Bilthoven: National Institute for Public Health and the Environment (RIVM); 2013. Dutch.
3. van Pelt W, Mevius D, Stoelhorst HG, Kovats S, van de Giessen AW, Wannet W, et al. A large increase of Salmonella infections in 2003 in The Netherlands: hot summer or side effect of the avian influenza outbreak? Euro Surveill. 2004;9(7):pii=473.
4. Van Duynhoven YT, Isken LD, Borgen K, Besselse M, Soethoudt K, Haitsma O, et al. A prolonged outbreak of Salmonella Typhimurium infection related to an uncommon vehicle: hard cheese made from raw milk. Epidemiol Infect. 2009;137(11):1548-57. http://dx.doi.org/10.1017/S0950268809002337
5. Campbell JV, Mohle-Boetani J, Reporter R, Abbott S, Farrar J, Brandl M, et al. An outbreak of Salmonella serotype Thompson associated with fresh cilantro. J Infect Dis. 2001;183(6):984-7. http://dx.doi.org/10.1086/319254
6. Nygard K, Lassen J, Vold L, Andersson Y, Fisher I, Löfdahl S, et al. Outbreak of Salmonella Thompson infections linked to imported rucola lettuce. Foodborne Pathog Dis. 2008;5(2):165-73. http://dx.doi.org/10.1089/fpd.2007.0053
7. Kimura AC, Palumbo MS, Meyers H, Abbott S, Rodriguez R, Werner SB. A multi-state outbreak of Salmonella serotype Thompson infection from commercially distributed bread contaminated by an ill food handler. Epidemiol Infect. 2005;133(5):823-8. http://dx.doi.org/10.1017/S0950268805004127
8. Shapiro R, Ackers ML, Lance S, Rabbani M, Schaefer L, Daugherty J, et al. Salmonella Thompson associated with improper handling of roast beef at a restaurant in Sioux Falls, South Dakota. J Food Prot. 1999;62(2):118-22.
9. Wright HA, Norval J, Orr A. Salmonella Thompson gastro-enteritis; report of two outbreaks. BMJ. 1957;2(5036):69-71. http://dx.doi.org/10.1136/bmj.2.5036.69
10. Friesema I, de Jong A, Fitz James I, Heck M, van den Kerkhof J, Notermans D, et al. Outbreak of Salmonella Thompson in the Netherlands since July 2012. Euro Surveill. 2012;17(43):pii=20303.
11. van Pelt W, de Wit MA, Wannet WJ, Ligtvoet EJ, Widdowson MA, van Duynhoven YT. Laboratory surveillance of bacterial gastroenteric pathogens in The Netherlands, 1991-2001. Epidemiol Infect. 2003;130(3):431-41.
12. World Health Organization collaborating centre for reference and research on Salmonella. Antigenic formulae of the Salmonella serovars. Paris: Institut Pasteur; 2007.
13. International P. One-Day (24-28 h) Standardized Laboratory Protocol for Molecular Subtyping of Escherichia coli O157:H7, Salmonella serotypes, Shigella sonnei, and Shigella flexneri by Pulsed Field Gel Electrophoresis (PFGE). Atlanta: Centers for Disease Prevention and Control; 2009. [Accessed 12 Oct

2012]. Available from: http://www.pulsenetinternational.org/protocols/

14. Onderzoeksraad voor Veiligheid. Salmonella in gerookte zalm. [Salmonella in smoked salmon]. Den Haag: Onderzoeksraad voor Veiligheid; 2013. Dutch. Available from: http://www.onderzoeksraad.nl/uploads/phase-docs/450/4e4564b46952rapport-salmonella-nl-web.pdf
15. Cartwright KA, Evans BG. Salmon as a food-poisoning vehicle--two successive Salmonella outbreaks. Epidemiol Infect. 1988;101(2):249-57. http://dx.doi.org/10.1017/S0950268800054169
16. Locht H, Mølbak K, Krogfelt KA. High frequency of reactive joint symptoms after an outbreak of Salmonella enteritidis. J Rheumatol. 2002;29(4):767-71.
17. Jones RC, Gerber SI, Diaz PS, Williams LL, Dennis SB, Parish ES, et al. Intensive investigation of bacterial foodborne disease outbreaks: proposed guidelines and tools for the collection of dose-response data by local health departments. J Food Prot. 2004;67(3):616-23.