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Avian influenza A(H10N7) virus involvement in mass mortality of harbour seals (*Phoca vitulina*) in Sweden, March through October 2014

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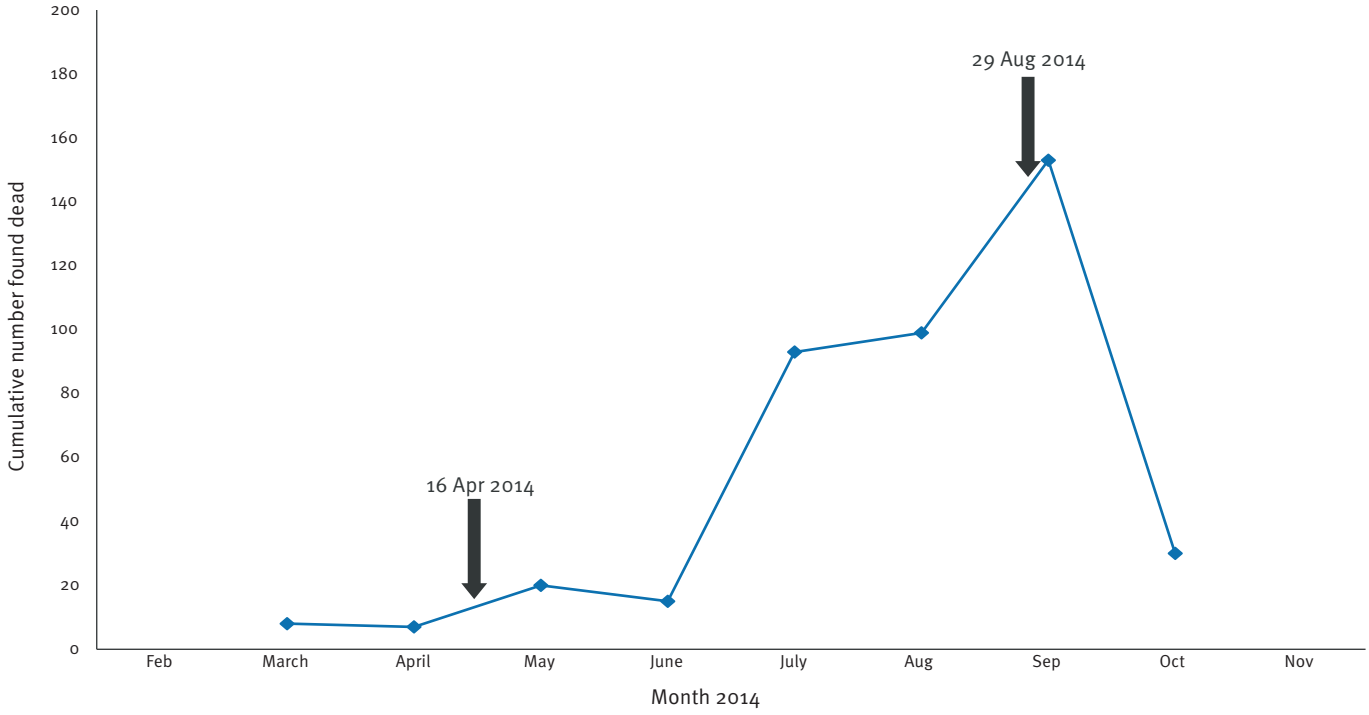
We provide the first scientific report of influenza A virus involvement in a mass mortality event among harbour seals (*Phoca vitulina*) off the west coast of Sweden. Avian influenza A (H10N7) virus was detected in the lungs of two affected animals. This subtype has not been reported in seals to date, nor has influenza A-associated mortality been reported in seals in Europe. Circulation of avian influenza viruses in mammals may have implications for public health.

Background

Increased numbers of dead harbour seals (*Phoca vitulina*) from the west coast of Sweden were first noted in March 2014. From March through October, 425 carcasses were detected in several seal colonies in the Kattegat and the Skagerrak seas (Figure 1). This unusually high mortality contrasted with the typical annual number of 30 to 40 dead seals reported from this area. Although most seals were too decomposed for examination, influenza A virus (IAV) subtype H10N7

Figure 1

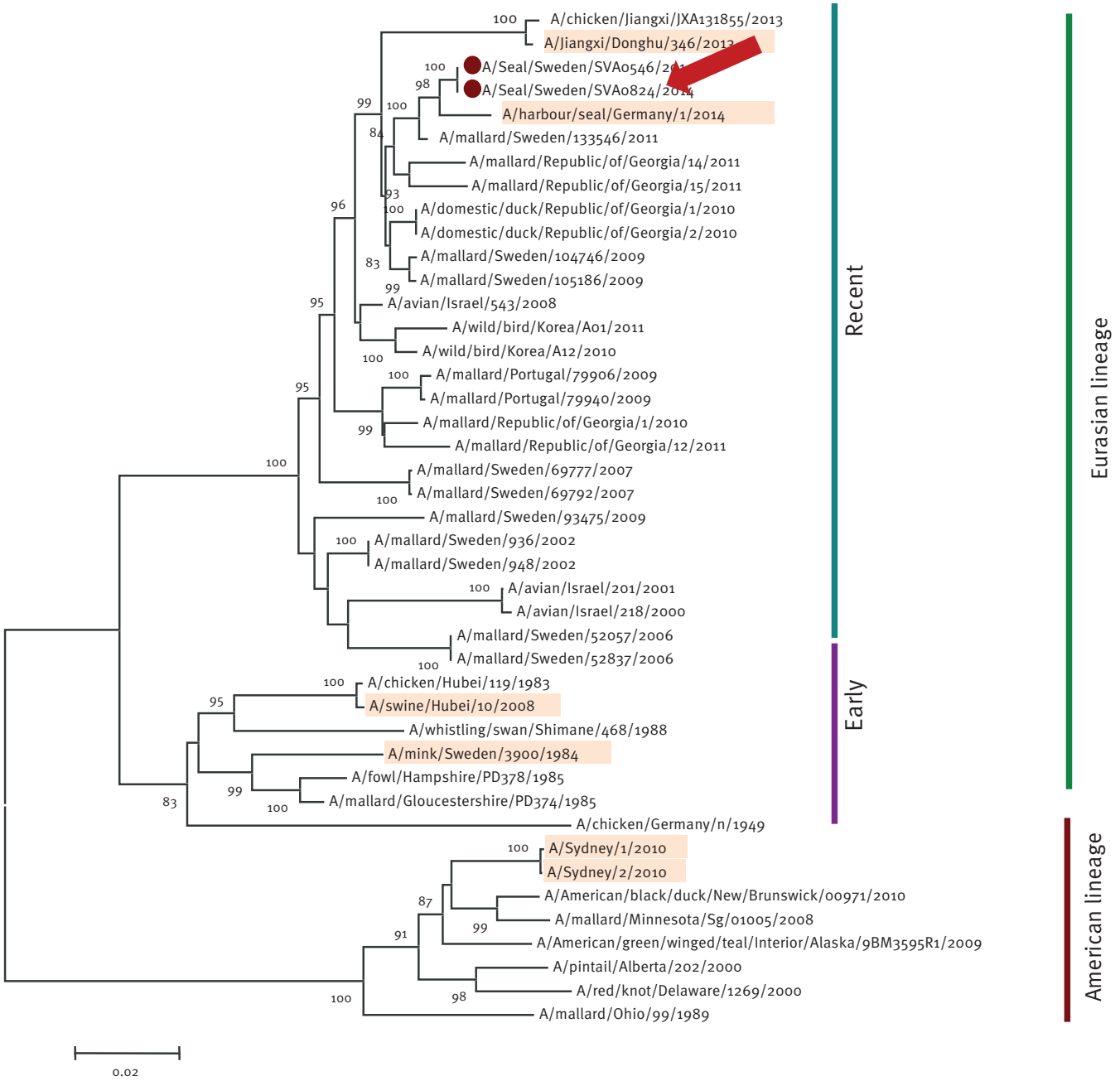
Cumulative numbers of dead stranded harbour seals (Phoca vitulina) along the west coast of Sweden, March–October 2014 (n=425)



The arrows indicate the dates of sampling of the inuenza A positive seals.

Figure 2

Phylogenetic relationship between haemagglutinin genes of H10 of influenza A virus subtypes



The protein coding region tree was generated by neighbour-joining analysis with the Tamura-Nei γ-model, using MEGA 6.0. Numbers below key nodes indicate the percentage of bootstrap values of 2,000 replicates. Isolates sequenced in this study are indicated by a red dot. Taxons leading to A(H10) subtype viruses detected in mammalian species are highlighted in pink. The nucleotide sequences obtained in the present study are available in the Global Initiative on Sharing All Influenza Data (GISAID) under HA gene accession numbers EPI545212 (Seal 1) and EPI547696 (Seal2). For the phylogenetic analysis, relevant sequences were obtained from the influenza database of the National Center for Biotechnology Information (NCBI), and the HA and NA genes of A/harbour seal/Germany/1/2014/H10N7 were obtained from GISAID's EpiFlu database.

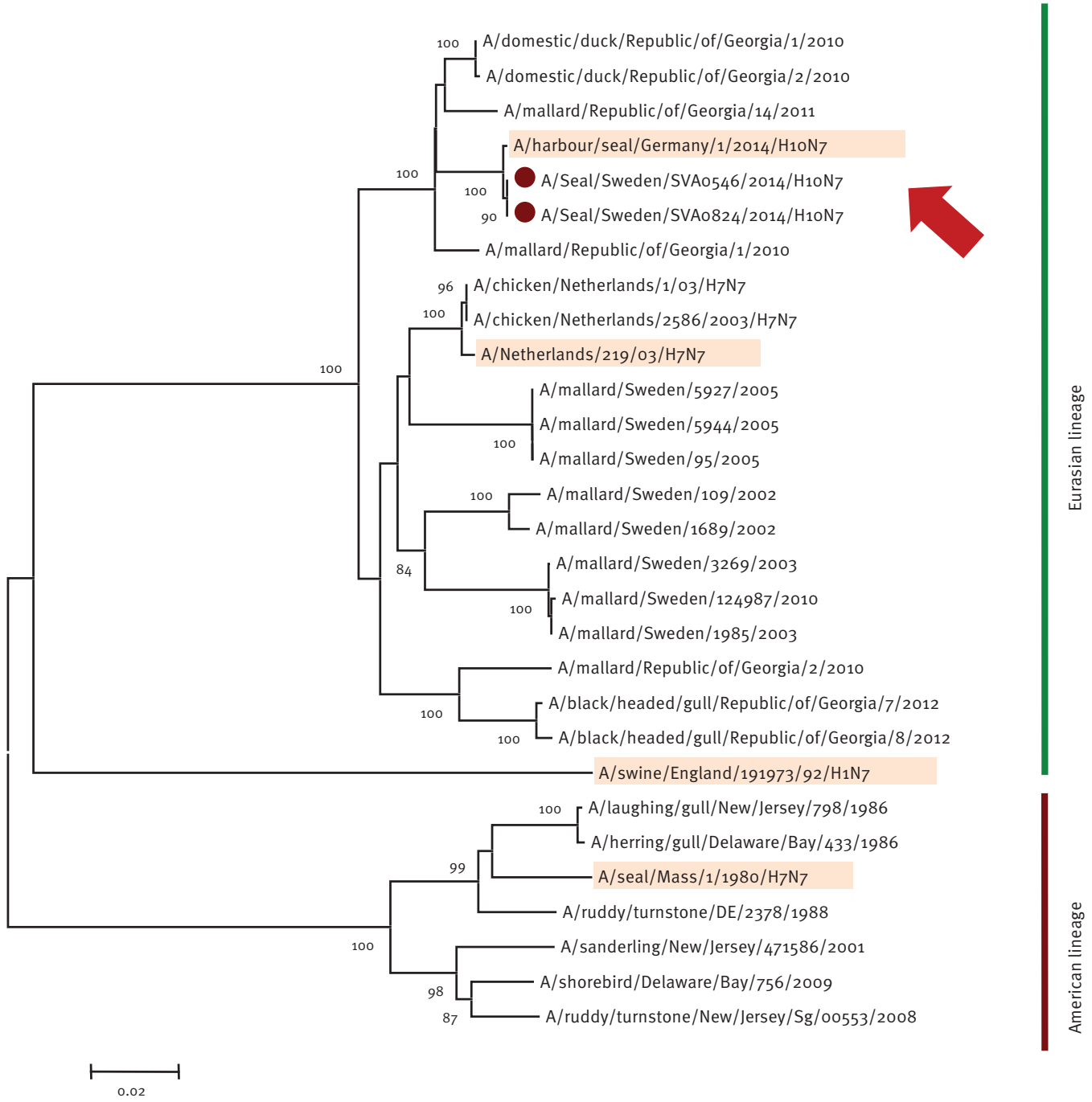
was detected in the lungs of two animals. According to media reports [1], H10N7 virus has recently been detected in dead seals in Denmark, Germany and the Netherlands in association with die-offs of seals first observed in July in Denmark and currently ongoing in Germany and the Netherlands [2,3].

Case descriptions

Seal 1 was observed suffering from buoyancy problems and respiratory distress off the coast of Gate Klova, Halland province. It was euthanised on 16 April, 2014 and examined by necropsy at the Swedish Museum of Natural History. Fresh and formalin-fixed tissues were submitted to the Swedish National Veterinary Institute (SVA) for further examination. Seal 2 was found dead on 29 August, 2014 in Beateberg, Västra Götaland

Figure 3

Phylogenetic relationship between neuraminidase genes of N7 influenza A virus subtypes



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province, sampled in the field, and lung tissue was submitted to SVA.

Pathological findings

Formalin-fixed tissues from both seals were processed by routine histological examination [4]. Seal 1 was a 12 year-old adult male in slightly impaired nutritional condition. Widespread emphysema was observed within the mediastinum and thoracic musculature, and bronchial and mediastinal lymph nodes were enlarged and oedematous. Lungs were diffusely firmer than normal, consistent with interstitial pneumonia, and the spleen was enlarged. Severe acute necrosuppurative pneumonia with widespread effacement of

Table

Haemagglutination inhibition and neuraminidase inhibition tests of one influenza virus isolated from a seal using monospecific reference antisera, Sweden, August 2014

A/Seal/Sweden/0546/2014			
Reference Antisera	Heamagglutination inhibition		
	H1N2	A/DK/HONG KONG/ 196/77	<1:2
	H2N3	A/DUCK/GERM/1215/73	<1:2
	H3N2	A/Turkey/Eng/69	<1:2
	H4N6	A/DK/CZECH/56	<1:2
	H5N1	A/CK/SCOT/59	<1:2
	H6N8	A/TURKEY/CANADA/63	<1:2
	H7N7	A/TKY/ENG/647/77	1:32
	H8N4	A/TK/ONT/6118/68	<1:2
	H9N2	A/CKKOR/99029/99	<1:2
	H10N9	A/S.AFRICA/EG. GOOSE/238/98	1:2,048
	H11N6	A/DUCK/ENG/56	<1:2
	H12N5	A/DK/ALBERTA/60/76	<1:2
	H13N6	A/GULL/MARYLAND/704/77	<1:2
	H14N6	A/MALL/GURG/244/82	<1:2
	H15N8	A/Duck/Australia/341/83	<1:2
	H16N3	A/GULL/DK/68110/02	<1:2
	H7N1	A/African Starling/Eng/983/79	<1:2
	Neuraminidase inhibition		
	H7N1	A/African Starling/Eng/983/79	Negative
	H1N2	A/DK/HONG KONG/ 196/77	Negative
	H2N3	A/DUCK/GERM/1215/73	Negative
	H8N4	A/TK/ONT/6118/68	Negative
	H12N5	A/DK/ALBERTA/60/76	Negative
	H11N6	A/DUCK/ENG/56	Negative
	H7N7	A/TKY/ENG/647/77	Positive
	H15N8	A/Duck/Australia/341/83	Negative
	H11N9	A/Mallard/Sweden/F1205/05	Negative

normal architecture was seen microscopically. Routine bacterial culture of the lung, bronchial lymph node and spleen yielded moderate to abundant growth of *Escherichia coli* in almost pure culture.

Seal 2 was severely decomposed and only a small sample of lung was available for examination. Despite the loss of cellular detail from autolysis, alveolar wall thickening supportive of an interstitial pneumonia was detected microscopically. Autolysis and limited material precluded investigation of concurrent bacterial pneumonia.

Virology

RNA was extracted from lung tissues and tested initially by real-time reverse-transcription polymerase chain reaction (rRT-PCR) targeting the matrix protein gene of avian influenza A viruses (AIV) [5] and the haemagglutinin gene of phocine distemper virus (PDV) [6]. AIV RNA was detected in lung tissue of both Seals 1 and 2, while PDV was not detected. RT-PCR for detection of the haemagglutinin (HA) and neuraminidase (NA) genes of IAV was performed using segment-specific but subtype-universal primers as previously described [7]. The nucleotide sequence A/Seal/Sweden/SVA0546/2014 (Seal 1) and A/Seal/Sweden/SVA0824/2014 (Seal 2), which were detected 4.5 months apart, possessed almost identical HA and NA genes (99% identity). The HA and NA genes clustered within the Eurasian avian lineage (Figure 2 and 3) showing 99% nucleotide similarity to the HA and NA genes of a German seal isolate from September 2014, A/harbour seal/Germany/1/2014/H10N7, using the BLAST programme of the Global Initiative on Sharing All Influenza Data (GISAID)s EpiFlu Database (http://www.gisaid.org). These sequences were obtained from GISAID's EpiFlu database (details are given at the end of the article). The amino acid sequence at the cleavage site in the HA molecule was PELVQGR/GLF, characteristic of low-pathogenicity AIV.

IAV was isolated from lung tissue of Seal 2 using specific pathogen-free (SPF) embryonated hens' eggs (EE) as previously described [8]. The allantoic fluid from the first passage in the EE showed haemagglunitating activity (HA › 256). The haemagglutinating agent could be further identified as influenza A(H10N7) using specific reference antisera for H1–H16 and N1–N9 in haemagglutination inhibition and neuraminidase inhibition test (Table) [9]. The virus had an intravenous pathogenicity index [8] value of 0.00, confirming the low pathogenicity of the virus for chickens.

Discussion

Although IAV infection has been reported in a variety of species of marine mammals including seals [10-12], this is, to our knowledge, the first published report of AIV isolation from seals in Europe and the first time that the H10 subtype has been detected in seals anywhere. It provides evidence that the H10N7 subtype was associated with an outbreak of seal mortality in Europe. Although we detected the virus in only two affected seals, media reports support H10N7 involvement in seal mortality events in Denmark, Germany and the Netherlands, as the virus was isolated from numerous dead seals [1-3].

As in AIV-associated mortality events in seals in the United States (US), Seal 1 suffered from a concurrent bacterial pneumonia [10]. Viral damage to physical components of the respiratory immune system is thought to allow secondary invasion of opportunistic bacteria. Limited quantity and quality of material from Seal 2 precluded investigation of bacterial infection.

Through phylogenetic analyses, we showed that this virus is genetically closely related to Eurasian AIVs from wild and domestic birds (Figure 2 and 3). IAVs are known to be circulating at high prevalence in European aquatic birds [13], supporting initial introduction of the H10 virus in seals from aquatic birds in Europe. The seals probably contracted the virus through direct or indirect contact with wild birds or their droppings. Interspecies transmission from birds to seals

requires concurrent alignment of numerous factors and although it occurs, it is not likely to occur often. There was an interval of 4.5 months between Seal 1 and Seal 2, suggesting that the virus was circulating among the seal population during this entire time.

From a public health perspective, extended circulation within a mammalian host not only demonstrates that this strain is capable of infecting and circulating in mammals, but it increases the opportunity for mutations to occur that may facilitate human infection. For example, the H3N8 strain from harbour seals in the US had recent mutations that are known to make influenza viruses more transmissible and cause more severe disease [12]. It also has the ability to target the SAα-2,6 receptor found in the human respiratory tract, an adaptation known to increase transmission and virulence in mammalian hosts [12]. In addition, some avian H10 viruses, including those isolated from farmed mink (*Mustela vison*) (H10N4), humans (H10N7, H10N8) and pigs (H10N5) (Figure 2) had the unique ability to cause severe disease in mammalian species without prior adaptation in poultry, supporting the hypothesis that these viruses in particular might pose a threat to human health [14-20].

Outbreaks of diseases among marine mammals can also involve interaction with humans and wild and domesticated animals, therefore, circulation of AIVs in mammals may have potential implications for public health. Management of dead marine mammals is challenging and especially difficult when they carry a new pathogen with unknown infectivity for humans. We lack information on the zoonotic potential of this particular strain of AIV and highlight the need for further assessment and research regarding risks for public health. Handling and disposal of carcasses may expose people to any number of potential zoonotic pathogens. This necessitates applying the precautionary principle as well as close collaboration and sharing of responsibility and resources between agencies at the local and national level for situations in which jurisdictional boundaries are often poorly defined.

Nucleotide sequences accession number

The nucleotide sequences obtained in the present study have been made available in the database of the Global Initiative on Sharing All Influenza Data (GISAID) under accession numbers EPI545212, EPI545213 (Seal 1) and EPI547696, EPI547697 (Seal 2). For the phylogenetic analysis, relevant sequences were obtained from the influenza database of the National Center for Biotechnology Information (NCBI). We also acknowledge the authors, originating and submitting laboratories for the HA and NA sequences of A/harbour seal/Germany/1/2014 with accession numbers EPI544351 and EPI544353 from GISAID's EpiFlu Database.

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Conflict of interest

None declared.

Authors' contributions

SZ and AN conceived and drafted the manuscript. SZ carried out PCR and sequencing, performed sequence analyses, alignments, phylogenies, interpretation of data and conducted the virological laboratory investigation. AN performed the histopathological examination and interpreted data. TH and CM conducted the epidemiological investigation and contributed to and revised the manuscript. JFV contributed to conception, interpretation of data, and revised the manuscript. All authors have reviewed and agreed on the content of the final manuscript.

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Segment ID	Segment	Country	Collection date	Isolate name	Originating and Submitting Laboratory	Authors
EPI544351	HA	Germany	2014-10-07	A/harbour seal/ Germany/1/2014	Erasmus Medical Center	Bodewes, Rogier
EPI544353	NA					

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Evaluation of the surveillance system for undiagnosed serious infectious illness (USII) in intensive care units, England, 2011 to 2013

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Emerging infections are a potential risk during mass gathering events due to the congregation of large numbers of international travellers. To mitigate this risk for the London 2012 Olympic and Paralympic Games, a sentinel surveillance system was developed to identify clusters of emerging infections presenting as undiagnosed serious infectious illness (USII) in intensive care units (ICUs). Following a six month pilot period, which had begun in January 2011, the surveillance was operational for a further 18 months spanning the Games. The surveillance system and reported USII cases were reviewed and evaluated after this 18 month operational period including assessment of positive predictive value (PPV), timeliness, acceptability and sensitivity of the system. Surveillance records were used to review reported cases and calculate the PPV and median reporting times of USII surveillance. Sensitivity was assessed through comparison with the pilot period. Participating clinicians completed a five-point Likert scale questionnaire about the acceptability of surveillance. Between 11 July 2011 and 10 January 2013, 34 cases were reported. Of these, 22 remained classified as USII at the time of the evaluation, none of which were still hospitalised. No clusters were identified. The 22 USII cases had no association with the Games, suggesting that they represented the background level of USII in the area covered by the surveillance. This corresponded to an annualised rate of 0.39 cases/100,000 population and a PPV of 65%. Clinicians involved in the surveillance reported high acceptability levels. The USII surveillance model could be a useful public health tool in other countries and during mass gathering events for identifying potential clusters of emerging infections.

Introduction

Mass gatherings have been described as 'a stress test for public health' by the Director General of World Health Organization (WHO) [1]; one of the challenges to public health is the potential for introduction of emerging infectious diseases due to the international movement of large numbers of people [2].

Emerging infections are a particular concern, as they can place a significant burden on public health and acute medical services within short time periods. A recent example is the Middle East respiratory syndrome coronavirus (MERS-CoV), which emerged in 2012 in the Arabian Peninsula [3]. The consecutive importation of MERS-CoV cases to the United Kingdom (UK) [4,5], Germany [6], France [7], Italy [8], Greece [9], the Netherlands [10], United States (US) [11] and Malaysia [12] required considerable public health resources with wide public health follow-up of contacts, extensive virological testing and international risk assessments.

Clinicians are accustomed to recognising and reporting specific diagnoses to public health surveillance systems. However, identification and reporting of emerging infections is problematic as these infections may not fit a recognisable clinical presentation and routine laboratory tests will not positively diagnose such cases. They are therefore less likely to be captured by traditional public health surveillance systems, instead requiring novel surveillance systems that aim to detect such cases of undiagnosed serious infectious illness (USII). The most severe emerging infections are likely to present to clinicians as USII in an acute medical setting such as intensive care units (ICUs). The ability to detect clusters of USII, related by common exposures, demographic or clinical characteristics could help to identify the first few cases of an emerging infection. This is especially important in complex health systems, where individual cases may be admitted to different hospitals.

To address these difficulties the Health Protection Agency (HPA, now part of Public Health England)

developed a new surveillance system to detect cases and clusters of USII, as part of public health planning for the London 2012 Olympic and Paralympic Games between 27 July and 9 September [13,14]. The structure of this USII surveillance system is based on a sentinel network of ICUs and has been described in detail previously [15]. A sentinel structure was chosen to develop a proactive network of ICUs which reported regularly and provided good coverage in the areas of interest for a limited period related to the London 2012 Games. It was considered that this would have the potential to detect a proportion of any related cases of an emerging infection. These ICUs were chosen to provide good coverage in the areas of interest for a limited period related to the London 2012 Games. More comprehensive coverage would be necessary to develop an ongoing USII surveillance system for the whole country. The system was initially piloted in six ICUs for a period of six months starting from January 2011. It was then progressively expanded between July 2011 and February 2012, to a total of 19 units (including 12 adult units and 7 paediatric units), as part of the preparedness for the London 2012 Games [15]. All 19 units were enrolled by 27 February 2012. The surveillance system was operational for 18 months from 11 July 2011.

Immediately after the 18 months of operation and until March 2013, the USII surveillance system was reviewed and evaluated, using the Centers for Disease Control and Prevention (CDC) guidelines for the evaluation of surveillance systems [16]. The purpose of this report is to describe the cases reported to the system and to assess this surveillance system for future mass gatherings, in terms of its sensitivity, acceptability and simplicity to participating clinicians, positive predictive value (PPV), and timeliness of case reporting.

Methods

Description of undiagnosed serious infectious illness surveillance

The USII surveillance system operated in 19 adult and paediatric ICUs (PICUs). These units were approached to participate on the basis of their proximity to games venues in the London region (13 units, of which eight were adult and five were paediatric units), or their role as major intensive care centres in the surrounding areas (4 in South East and 2 in East of England). These units participated on a voluntary basis and represented 48% and 59% of London ICU and PICU beds, respectively. The hospital represented by the units comprised a mix of large teaching hospitals and local acute hospitals, each with their own internal medical microbiology service and their own standard range of investigations. The majority of London 2012 Olympic and Paralympic Games activities were located in London and the South East regions. The USII case definition is shown in the Box. The USII diagnosis was made by clinicians in participating units on the basis of clinical opinion and hospital microbiology results; the precise microbiology tests used for cases varied between units as each participating hospital had its own specialist microbiology service which operated independently. One or more lead clinicians from each participating ICU reported cases primarily through a dedicated online reporting tool but cases could also be notified by email or telephone. Information was collected on patient demographics, clinical presentation, travel history (including travel within the UK and abroad) and other relevant exposures. If no cases were identified, clinicians were asked to provide a nil report every two weeks (or weekly during the London 2012 Games period). Participating clinicians were able to update information for reported cases, such as new alternative diagnoses, via the web-based tool, email or telephone.

Box

Case definition, surveillance system for undiagnosed serious infectious illness, England, 2011–2013

Cases were defined as any child (aged ≤ 16 years) or adult admitted to an intensive care unit with a serious illness suggestive of an infectious process, where the clinical presentation did not fit with any recognisable clinical picture or there was no improvement in response to standard therapy and initial laboratory investigations for infectious agents were negative or did not establish a diagnosis.

Evaluation of undiagnosed serious infectious illness surveillance

The evaluation involved a number of different approaches:

Retrospective analysis of cases and sensitivity of surveillance system

Data on cases reported during the 18 month period between 11 July 2011 and 10 January 2013, inclusive, were extracted from the secure web-based tool and added to a password-protected excel spreadsheet (Microsoft Excel 2007, Microsoft, Redmond, WA) which also contained data on cases reported by telephone and email. The cases which remained undiagnosed at the time of the evaluation (i.e. USII cases) were identified. The status of these cases was reviewed by the surveillance team in conjunction with the reporting clinicians, following the initial report and also during the evaluation, to ensure that they fulfilled the USII case definition. Cases were reviewed and described to provide an understanding of the role and function of the surveillance system.

The population coverage for the system was calculated as previously described [15] based on the proportion of all ICU beds in the local geographical area (as provided by each participating unit) and assuming a binomial distribution. This was used as the denominator, and the number of USII cases as the numerator, to calculate an annualised rate of USII assuming a Poisson distribution.

Table 1

Cases reported to the undiagnosed serious infectious illness surveillance system, England, 11 July 2011–10 January 2013 (n=34)

Characteristics of cases (total=34)	USII cases (total=22) n	Excluded cases (total=12) n
Age category (range: 4–69 years)		
Adult (>16 years-old)	19	11
Child (≤16 years-old)	3	1
Sex		
Male	11	3
Female	11	9
Predominant syndrome		
Respiratory	7	2
Presumed sepsis/bacteraemia	5	5
Neurological	4	2
Cardiac	3	0
Haematologic	1	0
Jaundice	1	1
Metabolic	1	1
Not stated	0	1
Possible travel exposures		
Travelled outside UK in the preceding six months	10	2
Outcome		
Death	10	5
Discharge from intensive care unit	10	4
Unknown	2	3

UK: United Kingdom; USII: undiagnosed serious infectious illness.

A 95% Bonferroni-type confidence interval (CI) was calculated to reflect the variability in the population covered over time. This was produced by calculating CIs using the lower and upper limits of the annualised rate of USII; the lowest of the lower limits and the highest of the upper limits, formed the lower and upper limits of the 95% Bonferroni-type CI, respectively.

These annualised rates were calculated overall for all ages and separately for adult and for paediatric (aged less than or equal to 16 years) cases and compared to the published rate from the pilot period [15], to assess the sensitivity of the surveillance system.

Acceptability and simplicity

This was assessed during site visits and meetings with clinicians at each participating trust by the authors (GD, BS, HK). Clinicians were asked to complete a short paper-based questionnaire using a five point Likert scale during these visits. The questionnaire asked about their understanding of the role of the USII surveillance system, the acceptability of sending a fortnightly nil report and their willingness to continue reporting USII cases in the future. To assess simplicity, participants were specifically asked how easy the case definition and the web-tool were to use. Completed paper questionnaires were double-entered using Epidata Entry v3.1 (The Epidata Association, Odense Denmark, 2008) and exported to Microsoft Excel 2007 (Microsoft, Redmond, WA) for analysis. The responses to each point of the Likert scale were summarised as counts in relation to the total number of completed questionnaires.

Positive predictive value

USII is a clinical diagnosis and there is no 'gold standard' test for this, with the diagnosis made by clinicians. Consequently cases initially reported as USII may cease to be considered cases if they receive an alternative diagnosis at a later stage. The PPV is therefore calculated as:

- Number of cases with USII as a final diagnosis/ (Number of cases with USII as a final diagnosis + Number of cases initially reported USII but later received alternative diagnosis) ×100

This was defined as the proportion of all cases reported to the surveillance system, which remained USII (i.e. which were not subsequently diagnosed) at the time of last report. Counts of cases notified to the USII surveillance system and of those which were subsequently diagnosed at the date of last report or outcome notification, were identified from the previously described password-protected excel spreadsheet holding case data.

Timeliness

The reporting time was defined as the number of days elapsed between a case being admitted to a participating unit and reported to the USII surveillance system. The following data fields were extracted from the online reporting tool for each case: date of admission and date of reporting for cases notified using the reporting tool between 11 July 2011 and 10 January 2013, inclusive. The median and range of reporting times was calculated for these cases. Timeliness was similarly calculated for those cases reported by email or telephone which had a recorded date of admission in surveillance records.

Results

Retrospective analysis of cases and sensitivity of the surveillance system

During the evaluation period, 34 cases were reported to USII surveillance by participating units (Table 1). Of these, 27 were notified online, six by email and one by telephone. A total of 12 cases were subsequently diagnosed and were no longer classified as USII cases, leaving 22 cases that remained USII (20 reported online and two by email) (Table 1 and 2).

At the time of the evaluation, none of the 22 cases that remained USII were hospitalised, as 12 had been discharged and the remainder had died. Of the 22 USII cases, 11 were male and 11 were female with ages ranging between four and 69 years. The principal presenting syndrome was respiratory illness (seven cases), followed by presumed sepsis/bacteraemia (five cases), neurological (four cases) and cardiac (three cases). Nineteen cases were adults and three were paediatric cases (less than 16 years-old). Ten of the adult cases had a history of travel outside the UK in the preceding six months. None of the paediatric cases had a travel history. There were no relevant exposures identified for four cases and no exposure information was given for the remaining five cases. Ten of the USII cases were fatal, giving a case fatality rate of 10/22 (45%).

There was no clustering of USII cases by clinical presentation, exposure or demographic characteristics identified during the evaluation period, including the period of the London 2012 Olympic and Paralympic Games (Figure). The 22 USII cases detected had no association with the Olympic or Paralympic Games.

Of the subsequently denotified cases most were female (9 cases), the age range was 11 to 77 years, and the main presenting syndrome was presumed sepsis/bacteraemia (5 cases). The infectious diagnoses for 10 cases or reasons for exclusion (one immunocompromised and one non-infectious and therefore outside the case definition) of these cases are shown in Table 2. The median time from ICU admission to denotification was 10 days (range: 8–41 days).

The annualised rate of all-age USII cases was 0.39 cases per 100,000 persons (95% CI: 0.23–0.64). The annualised rate for adult USII cases was 0.61 cases per 100,000 persons (95% CI: 0.34–1.1), while the annualised rate for paediatric cases was 0.067 per 100,000 persons (95% CI: 0.012–0.22).

The USII rate reported in this study was lower than that found during the initial USII pilot (an estimated annual rate of 1.2 per 100,000 persons, with a range of 0.4–3.1 per 100,000 [15]).

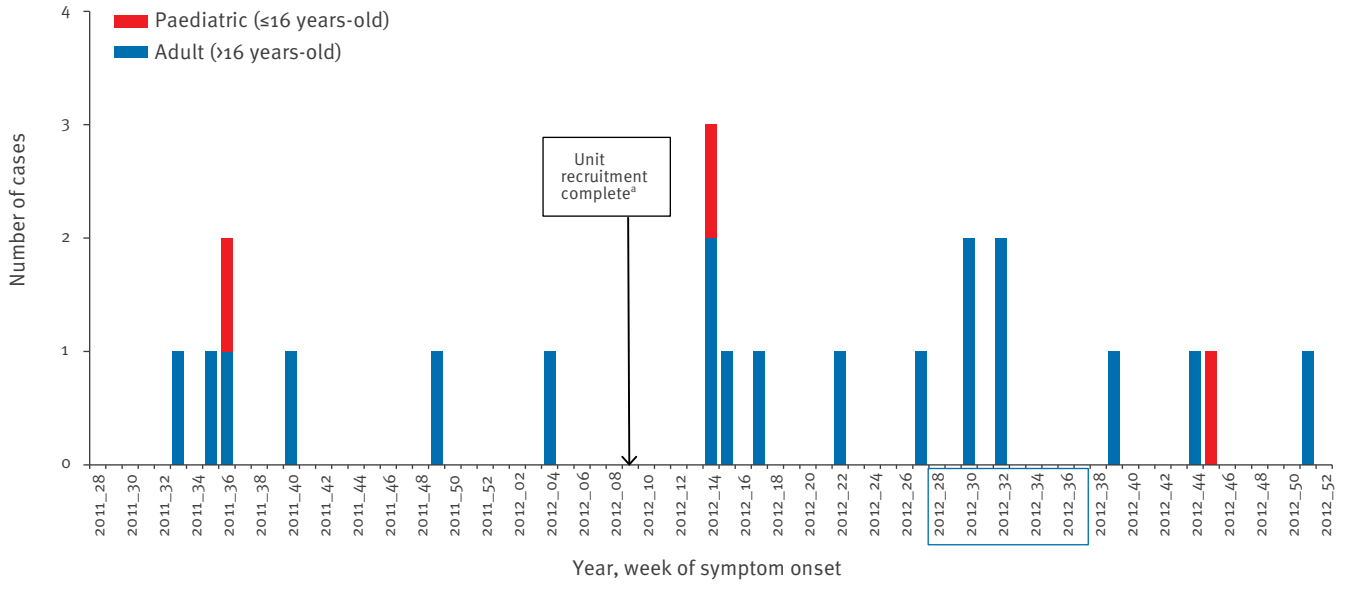
Acceptability and simplicity

Twenty-two participating clinicians completed questionnaires during site visits by the surveillance team. The majority (n=20) of respondents either agreed (n=8) or strongly agreed (n=12) that they understood the role of the USII surveillance system in identifying unknown infective syndromes. In addition 17 respondents agreed (n=10) or strongly agreed (n=7) that the request for a nil report every two weeks was convenient. Furthermore, 19 respondents stated that they would be prepared to continue reporting to the USII system.

Most respondents either strongly agreed (n=8) or agreed (n=11) with the statements that the USII case definition was easy to use and apply to cases. Approximately half of the respondents reported that

Figure

Distribution of undiagnosed serious infectious illness cases, England, 11 July 2011–10 January 2013 (n=22)



Week 32 in 2011 starts on 8 August. On the X axis label, the period corresponding to the Olympic and Paralympic Games is surrounded by a square box.

[a] Starting from January 2011, in a period lasting six months, which does not figure on the graph, a pilot sentinel surveillance of undiagnosed serious infectious illness cases, including six intensive care units (ICUs) was developed. From July 2011, the number of ICUs in the surveillance was expanded, reaching 19 units in February 2012.

Table 2

Initial cases reported to the surveillance system for undiagnosed serious infectious illness, that were subsequently denotified due to a diagnosis, England, 11 July 2011–10 January 2013 (n=12)

Final diagnoses of initial USII cases which were denotified (n=12)	Infectious disease (n=10)	Histoplasmosis Invasive aspergillosis Leptospirosis *Pseudomonas* bacteraemia Pneumococcal sepsis Disseminated tuberculosis Amp C beta-lactamase producing *E. coli* bacteraemia *Staphylococcus* bacteraemia *Haemophilus* spp. respiratory infection *Enterococcus* bacteraemia
	Non-infectious disease/ condition (n=2)	Immunocompromised Antibody-mediated encephalitis

E. coli: Escherichia coli; USII: undiagnosed serious infectious illness.

the reporting tool was easy to use (4 strongly agreed and 8 agreed).

Positive predictive value

Of the 34 cases reported to the USII surveillance system, 22 remained USII. The positive predictive value for a case reported to the surveillance system remaining a USII case was 65%.

Timeliness

Data were available to calculate median reporting time for 27 cases reported via the online reporting tool. The median reporting time for USII cases was 11 days following admission to ICU (range: 3–52 days). The median reporting time for three cases reported by email/telephone was 16 days (range: 2–49 days).

Discussion

The USII system is a unique surveillance system developed as part of a range of new and enhanced surveillance systems for the London 2012 Games. To our knowledge, there have been only two similar surveillance systems [17,18] described previously in the literature although these were not specifically developed for mass gatherings.

During 18 months of full operation a total of 22 cases of USII were identified through 19 participating ICUs. This is equivalent to a rate of 0.39 cases per 100,000 persons (95% CI: 0.23–0.64) and along with the absence of any clusters, indicates that as expected, USII cases are rare. However, these cases were associated with a case fatality of 45% which was considered to be high by many of the participating clinicians. None of the cases were associated with the London 2012 Games suggesting that observed cases represent the background level of USII in the area covered by the surveillance.

One of the difficulties in undertaking surveillance for USII is that the diagnosis is based on exclusion of known infections, and therefore depends on the extent of laboratory investigation. This may vary between different clinicians depending on local protocols and clinical experience. It is, in addition, difficult to distinguish between an unknown serious infectious illness and an unknown serious illness (which may not be infectious).

The lower USII rate reported in this study compared to a previous USII pilot was accompanied by an increase in the PPV between the evaluation (65%) and pilot periods (50%). We hypothesise that the pilot provided a period of initial learning, where clinicians were becoming accustomed to the case definition. Subsequently, the experience from the pilot period may have led to a higher threshold for reporting cases and therefore lower USII rates, during the evaluation period.

The results of the evaluation show that the USII system was acceptable to clinicians and that the system was simple to use both in terms of applying the case definition and reporting via the web-based tool. The simplicity of a reporting procedure has previously been reported as a key factor for increasing participants' willingness to report cases of infectious diseases [19]. These factors, in combination with the rarity of cases, may explain the willingness of clinicians to continue reporting.

The USII surveillance was an integral part of the surveillance initiatives introduced during the 2012 Olympics and Paralympics to monitor the potential threat of emerging infections. For instance, the HPA and European Centre for Disease Prevention and Control (ECDC) worked jointly to identify and risk assess infectious disease hazards occurring outside the UK which may have had an impact on the Olympic and Paralympic Games [14,20]. In addition, syndromic surveillance was expanded to detect signals of uncommon illnesses [13,21]. However, the USII system was unique in having the capability to collect case-level information on potential cases of new and emerging infections.

The length of the reporting times identified by this evaluation can be explained by the need to investigate suspected cases following admission, before considering a USII diagnosis. It is difficult to make an informed decision about whether these reporting times are adequate or too short, in relation to characteristics such as incubation period, as these are by definition, unknown for USII. However, this does reinforce the advantages for timeliness of using electronic reporting systems as demonstrated by other authors [22].

Data from similar surveillance have only been published from systems in Taiwan (2000–2005) and the

US (1995–1998). These show similar proportions in the presentations of reported cases. As in this study, where the majority (7/22) of USII cases had respiratory illness, surveillance from Taiwan also found that respiratory syndromes were most common, accounting for 59% of cases [17]. Respiratory syndromes were also the second most common presentation for the US system, accounting for 26% of cases, although this was closely preceded by neurological presentations (29%) [18]. The latter were less common among our cases. The USII surveillance approach may therefore be useful in addressing new and emerging respiratory infections such as influenza A(H7N9) in China [23,24].

One of the strengths of this evaluation is that we checked reported cases for diagnoses up to the end of the evaluation, maximising the length of follow-up of these cases. This allowed the exclusion of those cases which were diagnosed at later stage, which may be due to results from specialist testing, therefore retaining true USII cases only and making USII case rates more accurate.

As the study was undertaken in an acute health system in England, we anticipate that countries with similar health systems may also benefit from implementing USII surveillance during other mass gatherings. However, the challenge of emerging infections is not limited to just mass gatherings but is a persistent issue occurring in many settings, as evidenced by MERS-CoV [25] and influenza A(H7N9) in China [23,24]. We argue that USII surveillance may be useful for such rapidly evolving situations where the capability to detect emerging infections is required. This surveillance is practical to operate, requiring only half a full-time epidemiological scientist and support from a consultant epidemiologist.

The official report into public health activities during the London 2012 Games highlighted the importance of surveillance systems such as USII to public health services during mass gatherings and advocated maintaining the USII system or being able to reactivate it in the future [26]. More general, population-wide surveillance would require a remodelling of the system and its processes in terms of increasing the number and distribution of participating ICUs, to ensure sufficient coverage across England and to improve sustainability. We will investigate the feasibility of developing USII beyond its original mass gathering function, in order to address the general, ongoing threat from emerging infections.

Conclusion

Emerging infections pose a constant challenge globally and the USII surveillance model could also be a useful public health tool in other countries, seeking to identify clusters of USII. Prospective surveillance within the ICU setting is simple and acceptable to clinicians and provides a valuable opportunity for the identification of clusters of emerging infections.

The USII Collaborators

(1) The USII Steering Group: commissioned the evaluation of the system, reviewed and commented on the manuscript.
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Conflict of interest

GD, BS, HK employed by Public Health England which operates the USII system.

Authors' contributions

GD: Undertook the evaluation, wrote manuscript; BS: Contributed to the evaluation, wrote manuscript, manages USII surveillance system; HK: Contributed to the evaluation, wrote manuscript, leads USII surveillance system.

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Presence of human non-polio enterovirus and parechovirus genotypes in an Amsterdam hospital in 2007 to 2011 compared to national and international published surveillance data: a comprehensive review

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Enteroviruses (EV) and human parechoviruses (HPeV) are endemic worldwide. These infections are a constant cause of hospitalisation and severe disease, predominantly in young children and infants. Coordinated monitoring and surveillance are crucial to control these infections. We have monitored EV and HPeV epidemiology in Amsterdam from 2007 to 2011 with real-time RT-PCR and direct genotyping, facilitating highly sensitive surveillance. Moreover, we conducted a literature survey of existing surveillance data for comparison. Only 14 studies were identified. While HPeV1 was most frequently detected in Amsterdam, EV-B viruses dominated nationally and internationally. Furthermore, the top 10 strains detected differed yearly and per study. However, detection and typing methods were too varied to allow direct comparison and comprehension of the worldwide distribution and circulation patterns of the different genotypes. This limited a direct response to anticipate peaks. Uniform European monitoring programmes are essential to aid prediction of outbreaks and disease management.

Introduction

Human enteroviruses (EV) and parechoviruses (HPeV) are widespread and circulate globally. They are associated with a wide array of clinical manifestations ranging from respiratory or gastrointestinal symptoms, to neonatal sepsis and infections of the central nervous system [1-4]. Due to intensive vaccination and surveillance programmes, poliovirus has almost been eradicated. Nevertheless, outbreaks of pathogenic non-polio EV (NPEV) types remain, as illustrated by the recent outbreaks of EV71 in Asia [5-7].

EV were traditionally classified as poliovirus (PV) and the collectively named non-polio EV (NPEV), consisting of Coxsackie A and B viruses (CAV and CBV) and echoviruses (E), based on their pathogenicity in animals and cell culture. Molecular characterisation has led to their reclassification into four EV species A–D and the detection of new EV types (numbered) (http://www.picornastudygroup.com). HPeV were previously known as members of the EV genus (HPeV1 and 2) because of their similar cytopathic effect in cell culture. Based on genetic differences to EV, they were later classified as their own genus, which now comprises 16 genotypes (http://www.picornastudygroup.com).

Surveillance of EV and HPeV has traditionally been based on culturing and serotyping [3,8-10] and is primarily directed towards poliovirus eradication [11]. Nowadays, RT-PCR targeting the conserved 5'UTR is the standard method for detection followed by sequencing of the capsid genes, in particular VP1, for typing [4,8,12,13]. We have shown previously that screening by RT-PCR and direct genotyping from stool is much more sensitive than virus culture and leads to better detection of HPeV and CAV [8]. Not only does this contribute to surveillance, but it allows for a better understanding of NPEV and HPeV circulation and pathogenicity, which is of increasing importance in patient management and therapy [14-17].

Here we describe an epidemiological survey of NPEV and HPeV types detected from stool and cerebrospinal fluid (CSF) samples from patients admitted to hospital in the period from 2007 through 2011 in Amsterdam. We compare our findings with what is known from published EV surveillance data.

Methods

NPEV and HPeV positive sample cohort from patients from a tertiary hospital in Amsterdam

CSF and stool samples were obtained from 2007 through 2011 from patients (2007–2008 data published in [8]). A total of 570 patients were found positive for a NPEV (n = 339), HPeV (n = 196), or both (n = 35) by real-time RT-PCR in stool and/or CSF as described previously [18]. The median age of infection with NPEV and HPeV was 8.3 months (0–71.6 years) and 6.3 months (0–68.1 years), respectively. Positive stool samples were cultured and serotyped or were genotyped directly from stool and/or CSF (GenBank accession numbers: KC893345-KC893502, KC893504-KC893549) [19]. Sequences were analysed by Simmonics Sequence Editor (SSE) [20]. EV were characterised with the genotyping tool from the Dutch National Institute for Public Health and the Environment (RIVM) [21], and HPeV were characterised by phylogenetic analysis [8,19].

Literature survey

The goal of our literature survey was to find EV and HPeV surveillance studies giving an overview of clinical surveillance trends over the years, to enable a comparison with our data. EMBASE and PubMed searches, encompassing publications listed on 18 October 2013, were performed (Figure 1) using the terms 'enterovirus', 'parechovirus', 'surveillance or epidemiology' and 'geographic locations' (MeSH term added to expand search), while excluding animal studies and using filters for English and Dutch language. This resulted in 1,679 studies published between 1960 and 2013. After removal of duplicates, we retrieved 1,065 studies. After exclusion of studies describing a single type or fewer than 200 isolates, studies describing specific outbreaks and studies on methodology, 135 studies remained for screening of the abstract. We defined the inclusion criteria for this literature survey as follows:

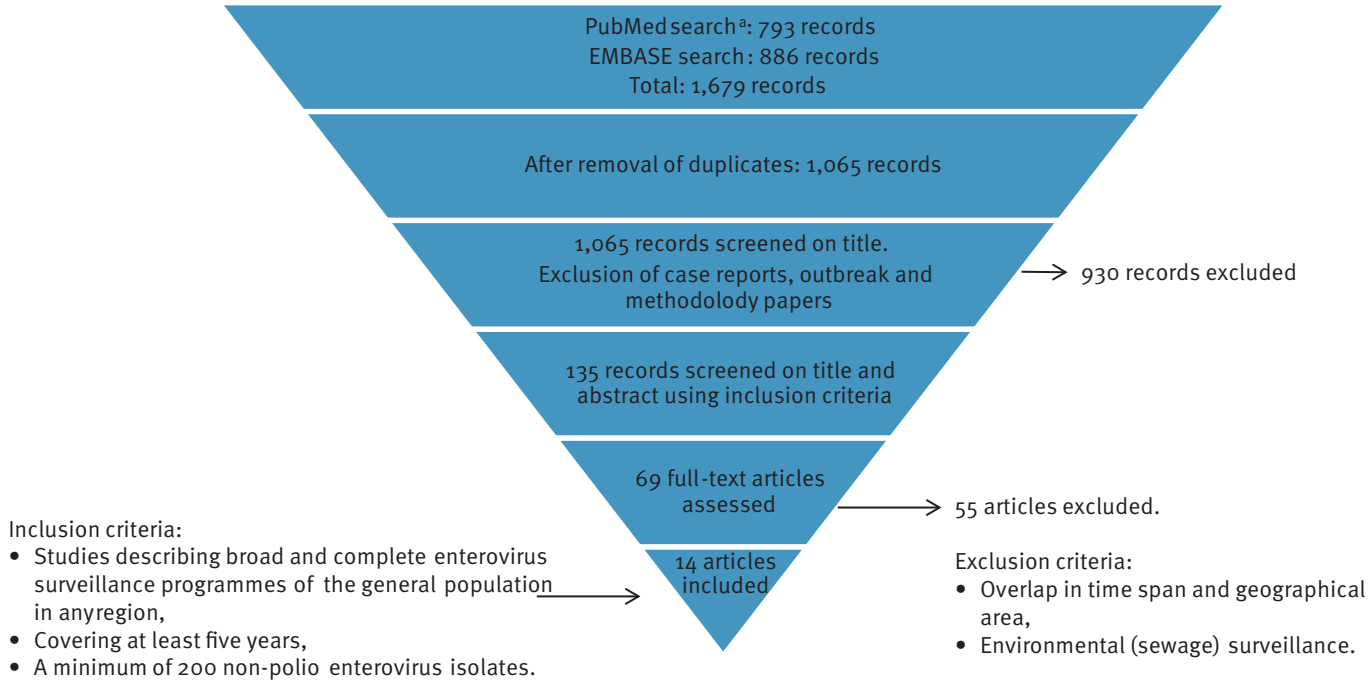
- studies describing surveillance programmes of a general but symptomatic population in any region,
- coverage of at least five years,
- description of NPEV/HPeV genotype prevalence,
- detection of at least 200 NPEV/HPeV isolates.

Based on these criteria, 69 studies were selected for full-text analysis. Two additional papers were found through cross-referencing. Studies based on environmental surveillance or on the non-symptomatic general population only were excluded. In case of overlap in time span and geographical area, the largest study was selected. Fourteen studies remained for inclusion (Figure 1).

Tables with NPEV/HPeV genotype prevalence were extracted from the publications. The percentages of each individual genotype rather than absolute numbers were used to form a top 10 list of most prevalent types during the entire period from 2007 to 2011,

Figure 1

Inclusion criteria for literature search on enterovirus and human parechovirus surveillance

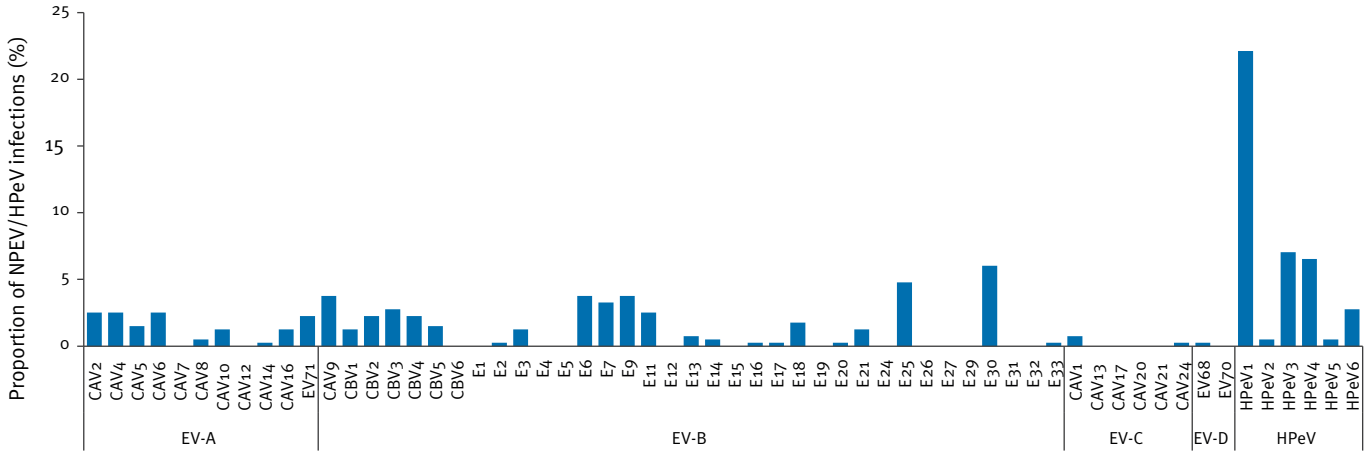


[a] enterovir*[ti] OR parechovirus*[ti] AND (surveillance[ti] OR epidemiol*[ti] OR "Geographic Locations"[Mesh]) AND (english[la] OR dutch[la]) NOT (animals NOT (animals and humans)).

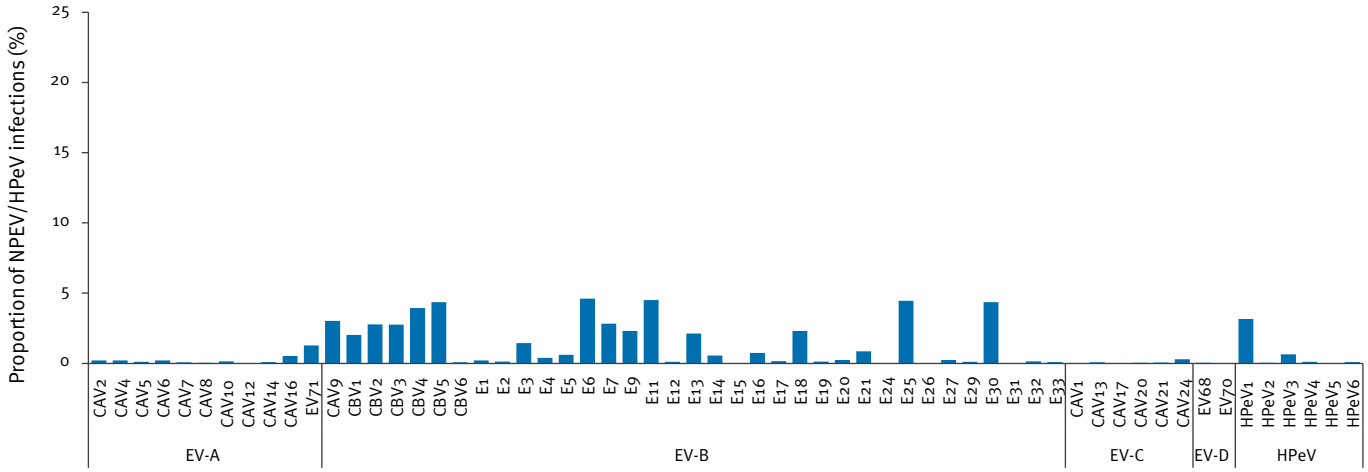
Figure 2

Non-polio enteroviruses within species EV-A to EV-D and human parechovirus genotypes in (A) Amsterdam 2007–11 (n=241 and n=157, respectively) and (B) the Netherlands, 1996–2011 (n=8,396)

A. Viruses detected in this study (n=398)



B. Viruses detected in Van der Sanden et al. [11] (n=8,396)



CAV: Coxsackie A virus; CBV: Coxsackie B virus; E: echovirus; EV: enterovirus; HPeV: human parechovirus.

thereby ruling out any bias by over-representation of studies with a large sample size.

Results

Distribution of NPEV and HPeV types in patients from a tertiary hospital in Amsterdam

In total, 241 NPEV and 157 HPeV could be typed (64.4% and 68%, respectively) from 374 EV-positive and 231 HPeV-positive stool and/or CSF samples. Typing was more successful in stool samples (69%) than in CSF (35%). Overall, we detected 22 different EV-B types, accounting for 55 CBV strains and 123 echovirus strains (Figure 2A). In CSF, only NPEV strains from species EV-B were found. EV-B strains accounted for 178 (74%) of the NPEV strains found overall, and seven of them ranked among the top 10 NPEV/HPeV types found (E30, E25, CAV9, E6, E9, E7 and CBV3, Table 1). Over the five years from 2007 to 2011, circulation of these types varied, with different types co-circulating each year (data not shown). Viruses of the EV-A species comprised 58 strains (nine types), while only four strains could be characterised as EV-C (CAV1 (n=3) and CAV24 (n=1)) and one virus as EV-D (EV68).

Overall, the different EV types were frequently (n=206, 85.5%) detected in children under the age of three years, with 46.8% (n=96) of those under the age of three months (Figure 3A). There was a clear difference in the distribution of the species. Members of species B were predominantly identified in children under the age of three months, while members of species A were identified in children aged six to 12 months.

HPeV1 was the dominant HPeV type (n=88, 56%) and ranked first place, accounting for almost a quarter of all combined NPEV/HPeV strains (22%) (Table 1). HPeV3 was the second dominant strain (n=28, 12%) followed

Table 1

Top 10 non-polio enteroviruses and human parechovirus in Amsterdam 2007–11, the Netherlands 1996-2011 and in the international literature, 1967–2010

	Amsterdam (%) (this study)	The Netherlands (%)[11]	International (mean %; range)[a]
1	HPeV1 (22.1)	E6 (7.6)	E30 (12.32; 0.00–35.76)
2	HPeV3 (7.0)	E11 (7.5)	E6 (6.70; 0.00–18.57)
3	HPeV4 (6.5)	E25 (7.4)	E9 (6.55; 0.00–20.36)
4	E30 (6.0)	E30 (7.3)	CBV5 (5.23; 1.81–9.17)
5	E25 (4.8)	CBV5 (7.2)	E11 (5.16; 0.00–12.42)
6	CAV9 (3.8)	CBV4 (6.5)	E4 (4.69; 0.00–38.90)
7	E6 (3.8)	HPeV1 (5.2)	CBV3 (4.43; 0.00–15.68)
8	E9 (3.8)	CAV9 (5.0)	CAV16 (4.27; 0.00–19.92)
9	E7 (3.3)	E7 (4.7)	CAV9 (3.46; 0.00–9.44)
10	CBV3 (2.8)	CBV2 (4.6)	E13 (3.36; 0.00–16.14)

CAV: Coxsackie A virus; CBV: Coxsackie B virus; E: echovirus; HPeV: human parechovirus.

Percentages are based on the number of samples typed; Amsterdam n=398, the Netherlands n=8,398, international n=180,995.

[a] The percentages of each individual genotype rather than absolute numbers were in each study were added up and used to form a top 10 list for the international studies, thereby ruling out any bias by over-representation of studies with a large sample size.

by HPeV4 (n=26, 11%) (Table 1). HPeV3 was the only type found in CSF while HPeV1 was much more prevalent in stool. While HPeV1 and HPeV4 circulated every year, HPeV3 only circulated in the even years, predominantly in the summer and almost exclusively in children younger than three months (Figure 3B).

Comparison of Amsterdam data with national surveillance data

A recently published paper by Van der Sanden et al. presents an overview of Dutch NPEV/HPeV surveillance data over the years 1996-2011 [11]. The standard method for the majority of the data was virus culture and serotyping from stool samples. When analysing this set of national data, (Figure 2B), it was observed that the top 10 isolated viruses were similar to our data (Figure 2A), with six of 10 types found in both lists (Table 1). The contribution of the Amsterdam data to the nation-wide data is ca 5–10%. An untyped HPeV outbreak occurred in the Netherlands in 2010. Based on the seasonal and biannually distribution known for HPeV3, the untyped HPeV most probably represented HPeV3 [11]. The detection rates for EV in that study were low, with 2.87% EV-A, 0.49% EV-C and 0.06% EV-D (Figure 2B) [11].

Overall, our results were in line with the national data. More EV-A and HPeV strains could be detected and typed by the use of supplementary molecular methods.

Trends in published NPEV and HPeV surveillance data derived from a systematic literature survey and comparison with the Amsterdam data

Fourteen studies were selected describing NPEV surveillance from 1967 to 2011 in several European countries, Japan, South Africa, Taiwan, Tunisia and the United States (US) (Table 2) [2,22-34]. Across all studies, a total of 186,930 NPEV/HPeV were found, of which 180,995 NPEV and HPeV isolates were typed. The studies differed with respect to data collection, sample types and isolation methods, varying from virus culture to real-time RT-PCR and direct genotyping. Therefore, no significant conclusions could be drawn, but several trends were observed.

Age distribution

Percentages of 30–74% of all isolates were derived from children younger than five years, with up to 47% coming from patients younger than three months [2]. In our study, the vast majority of infections occurred in children under the age of three years with, respectively, 25% and 37% of NPEV and HPeV isolates found in children younger than three months. Moreover, one study found a significant association between neonatal (age under one month) enteroviral infection and higher mortality (11.5% vs 2.5–5.1%), specifically related to CBV4 and E9 [35]. A difference in the predominant EV types among neonates and older children was also observed: E11, CBV2 and CBV5 were most prevalent in neonates, while E30, E9 and E11 were most prevalent in the older age group [28].

Sample types

The NPEV and HPeV types were predominately detected from stool and therefore indicated the circulation of types rather than a direct clinical association. Interestingly, the Asian studies show throat swabs to be the most frequent sample taken [29,33,34].

Furthermore, 56% of all echoviruses in a study in Scotland came from CSF samples but only 28 and 34% of CAV and CBV enteroviruses [25]. This is in line with our observation that echoviruses were frequently found in CSF.

NPEV circulation

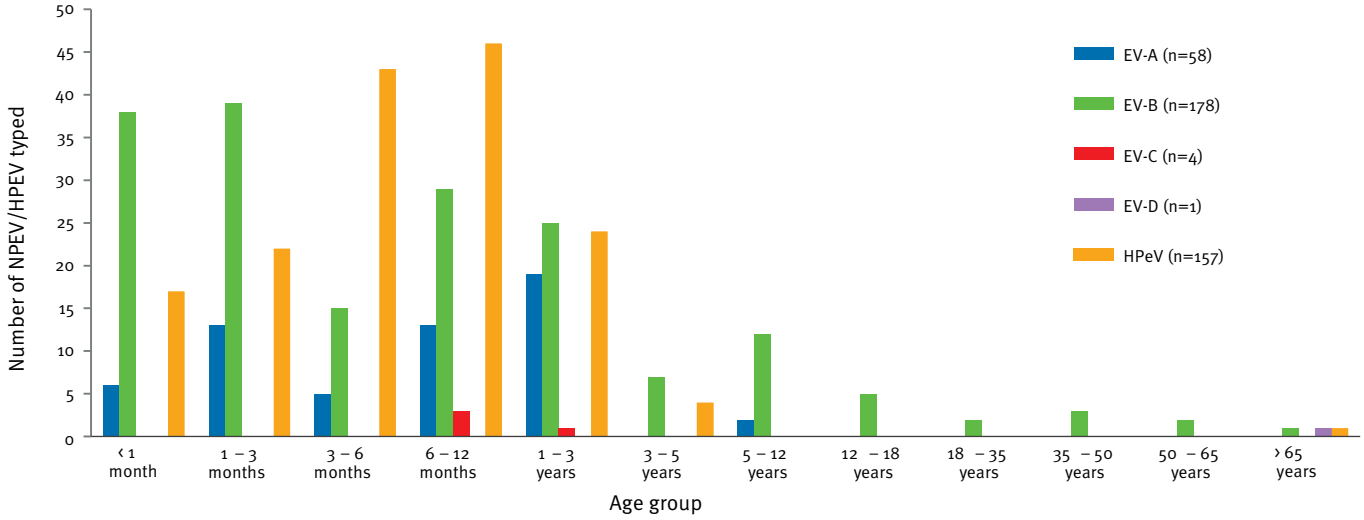
All analysed studies, including our data and national data, showed a seasonal pattern for NPEV circulation with a distinct peak in summer. The genotypes isolated in each study are summarised in Table 2, while Table 1 lists the 10 most frequently found genotypes. E30 was the most prevalent type internationally. Least prevalent were EV86, CAV12 and EV70. While most EV types were found at least once, CAV11, CAV19 and CAV22 were never seen. Similar to our data from Amsterdam, the composition of co-circulating types differed in the years studied.

Our data were similar to international data in that EV-B viruses, in particular echoviruses, were most frequently

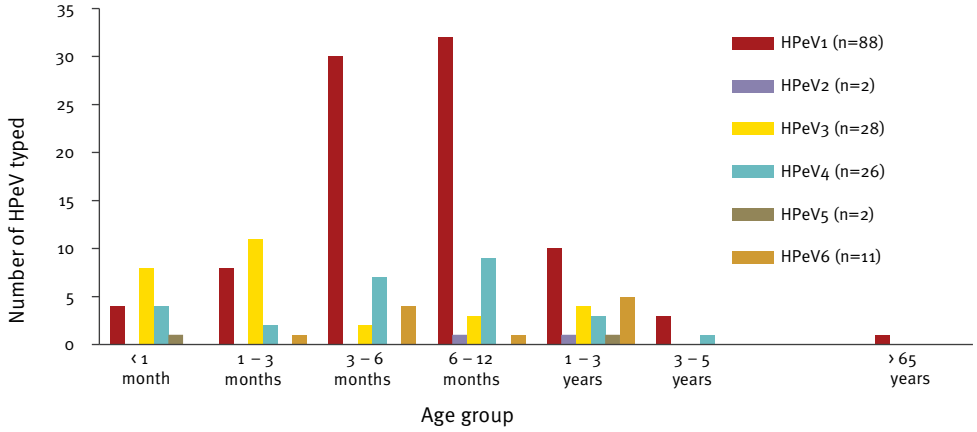
Figure 3

Age distribution of non-polio enterovirus species EV-A to EV-D and human parechovirus genotypes, Amsterdam, 2007-2011 (n=398)

A. Age distribution for the four EV species and HPeV identified in this study (n=398)



B. Age distribution for the six HPeV genotypes identified in this study (n=157)



EV: enterovirus; HPeV: human parechovirus.

detected (n=140,078; 74.9% of total EV/HPeV) (Table 2) [2,22,23,25-28,30,32]. Similarly, 10–30% of all virus isolates were CBV strains [19,23,26,27,32]. E30, among the top 10 types in our data, was observed as being the most prevalent type in the analysed literature. While six of our top 10 NPEV strains were also represented in the top 10 internationally, none of the 14 studies showed E25 dominance (Table 1), which is in sharp contrast to the E25 dominance observed in our data. This type came 16th in the international ranking. The literature survey further showed that EV-B viruses were frequently responsible for outbreaks throughout the years. E30 activity in the US between 1975 and 2005 was always associated with a new genetic lineage [28]. E6, E13 and E30 caused widespread outbreaks in 2000 and 2001 in Austria, Germany, Iceland, Kosovo, the Netherlands and the United Kingdom, [23,36,37]. Continued E13 outbreaks were seen in 2004 and 2006 in several European countries and the US, causing severe meningitis [11,24,32,38]. E13 had rarely detected been in England, Wales and the US before the large outbreak in 2000 [24]. Other viruses of the EV-B species responsible for outbreaks throughout the years were CAV9, E4, E6, E9, E11 CBV4 and CBV5 [11,23,24,32,38].

The second dominant EV species in our data set and internationally was EV-A (13.1% of all NPEV/HPeV). However, EV-A was the most dominant species in two of three Asian studies (35.4–71.0% of all NPEV/HPeV) [29,33,34] where major outbreaks were primarily caused by CAV16 and EV71, types known to cause hand, foot and mouth disease. The most notable EV71 outbreak occurred in 1998 in Asia, where EV71 was implicated in a large number of fatal cases of encephalitis [39]. Most EV-A types were seen to circulate at low rates throughout the years both in our data and Dutch

Table 2

Number of isolated enteroviruses and human parechoviruses, percentages of typed isolates and characteristics of studies included in the literature review

Location	Time span	Isolation method	Sample type[a]	Total (n) NPEV/ HPeV	EV-A (%)	EV-B (%)	EV-C (%)	EV-D (%)	HPeV (%)	Reference
Amsterdam	2007–2011	PCR genotyping	Stool	398	14.6	41.7	1.1	0.5	41.7	This study
The Netherlands	1996–2011	Virus culture / serotyping (since 2007 also PCR genotyping)	Stool	13,952	2.9	52.8	0.5	0.1	2.9	Van der Sanden [11]
Scotland	2005–2010	PCR genotyping	CSF	232	2.1	60.1	0	0	13.7	Harvala [2]
Japan	2004–2008	Virus culture / serotyping and PCR genotyping	Throat swab	241	71.0	14.1	0	0	ND	Momoki [30]
Spain	1998–2007	Virus culture / serotyping and PCR genotyping	CSF	2,572	1.4	93.8	0.7	0	0[b]	Trallero [33]
United States	1970–2005	Virus culture / serotyping and PCR genotyping	No data	49,637	3.1	94.8	0.3	0.1	0[b]	Khetsuriani [29]
Taiwan	2000–2005	Virus culture / serotyping and PCR genotyping	Throat swab	12,052	48.1	22.0	0.7	0	ND	Tseng [34]
Germany	2000–2005	Virus culture / serotyping and PCR genotyping	Stool	674	5.6	83.8	0.3	0	0	Roth [31]
France	2000–2004	Virus culture / serotyping and PCR genotyping	Stool	2,754	0.7	95.0	0.4	0	0.6	Antona [24]
Tunisia	1992–2003	Virus culture / serotyping	Stool	236	0	90.3	8.1	0	0.8	Bahri [27]
Spain	1988–1997	Virus culture / serotyping	CSF	727	1.0	98.6	0	0	0.4	Trallero [32]
Belgium	1980–1994	Virus culture / serotyping	Stool	3,333	4.0	89.5	1.3	0	5.2	Druyts-Voets [28]
England and Wales	1975–1994	Virus culture / serotyping	Stool	40,364	3.5	87.8	0.3	0	8.4	Maguire [25]
Japan	1981–1991	No data	Throat swab	28,570	35.0	62.0	0	1.0	1.0	Yamashita [35]
South Africa	1981–1989	Virus culture / serotyping	CSF	3,098[c]	ND	66.9	ND	ND	ND	McIntyre [23]
Scotland	1967–1974	Virus culture / serotyping	No data	42,440	8.1	89.7	0	0	0.6	Grist [26]

CSF: cerebrospinal fluid; EV: enterovirus; HPeV: human parechovirus; ND: not done; NPEV: non-polio enterovirus.

[a] Predominant sample type.

[b] Without rounding 0.04%.

[c] Enteroviruses untyped: 33%.

national data. In addition, no other major type-specific outbreaks of EV-A were seen.

Circulation of viruses of the EV-C and EV-D species was low in all studies, which is consistent with our data. On average, they accounted for less than 1% of the total detected NPEV/HPeV in the analysed studies.

HPeV circulation

Despite HPeV dominance in our population, HPeV were only the third most common type after EV-B and EV-A internationally (2.4% of all isolated EV/HPeV). However, two of three studies using RT-PCR genotyping as the main detection method, ranked HPeV types in their top five of most frequently isolated types [2,19].

HPeV circulates endemically in the States [28]. A seasonal pattern was identified for HPeV in Amsterdam and Scotland [2].

Discussion

This study described the clinical epidemiology of NPEV and HPeV types over a five-year period in an academic hospital in Amsterdam as identified by real-time RT-PCR and direct genotyping. We compared our

data with clinical surveillance data published across Europe, Japan, South Africa, Taiwan, Tunisia and the US, providing an overview of the complex circulation pattern of different NPEV and HPeV types over several decades [2,11,22-34]. Conclusions could not be drawn due to the heterogeneity of the available data, but several trends were observed.

In all studies including our own, NPEV/HPeV infections predominantly affected neonates (under the age of one month), with up to half of all infections identified in neonates. In addition, a higher mortality has been reported, in some cases related to specific types frequently found in neonates [28,35]. These comprise mostly EV-B types [19].

With respect to circulation of the NPEV/HPeV viruses, our study was representative for what is found nationally and internationally (Table 2). However, there were differences in the frequency of HPeV and CAV. While large outbreaks in one region can influence the distribution of viruses in other regions and the studied time periods differed, it seems likely that these differences are attributable to the difficulty of culturing these viruses on standard EV cell lines, since most international studies rely on data from virus culture. Furthermore, RT-PCR for HPeV is not routinely performed and therefore some studies do not detect HPeV (Table 2). Among the top 10 virus types identified in our study, the most common were EV-A viruses and HPeV. Studies using RT-PCR and direct genotyping generally detect a larger proportion of HPeV and EV-A viruses, arguing that these types may have been underreported in other studies based on cell culture and serotyping [2,19]. These observations do not apply for the Asian studies, where CAV16 and EV71 are the dominant types. The more pathogenic Asian subgenogroups have already been described in Europe [40-42]. Although more pathogenic genotypes may be overrepresented because diagnostics are more likely to be requested for severe cases, EV71 is not routinely monitored in Western countries leaving a void in the international surveillance of this type.

Interestingly, E25 was observed as one of the dominant NPEV types in our population and was among the top 10 types nationally. Before 2011, however, E25 had been seen less frequently in literature [26]. Most studies included here cover time periods earlier than our own data, which could explain the lack of E25 detection. The recent peak in E25 detection could be related to a genetic divergence in this type to which the population is not immune, as has been shown for other EV types [40,43].

Recently the US Centers for Disease and Control and Prevention reported an increase in EV-D68 cases with severe respiratory disease [44]. The US outbreak has led to increased awareness of the virus in European countries. EV-D68 was rare in our study between 2007 and 2011 (one case in 2010) and nationally (n=5, 0.1%) between 1996 and 2011 [11]. However, EV-D68 did cause an increase in severe respiratory infections in the fall of 2010 in the Netherlands [45]. Because of the acid-sensitive phenotype, EV-D68 is rarely detected in stool, which could explain the lack of the virus in both studies which were for the most part based on stool isolations. Currently, an increase in the number of EV-D68 severe respiratory cases is reported through the general practitioner surveillance which monitors influenza-like illness and other acute respiratory infections and through the national enterovirus surveillance [46].

Data on yearly circulation patterns may be helpful in predicting such peaks in the future. However, the different time periods covered in the two studies prevented us from studying yearly prevalence and co-circulation of NPEV and HPeV.

Conclusion

This is the first study that has compared local data with national and international clinical surveillance data. Comparison and comprehension of the data proved difficult due to differences in techniques, samples collected and years studied. Coordination of data collection and standardisation of methods, as well as the design of easy-to-use databases for the collection of sequence data in combination with epidemiological data [47], are essential in order to elucidate epidemiology, calculate disease burden and improve outbreak management of individual types. First efforts to create such a coordinated environment have been made in the Netherlands with the creation of VIRO-TypeNed [47]. Uniform international data collection using of similar techniques would be a next step in international coordination.

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Conflict of interest

None declared.

Authors' contributions

V. Janes: literature survey and draft of the manuscript; R. Minnaar: PCR and genotyping experiments; G. Koen, H. van Eijk, and K. Dijkman-de Haan: culture and serotyping experiments; D. Pajkrt: critical review of the manuscript; K. Wolthers: draft and critical review of the manuscript; K. Benschop: data maintenance and analysis, draft and critical review of the manuscript.

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Predictive performance of telenursing complaints in influenza surveillance: a prospective cohort study in Sweden

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Syndromic data sources have been sought to improve the timely detection of increased influenza transmission. This study set out to examine the prospective performance of telenursing chief complaints in predicting influenza activity. Data from two influenza seasons (2007/08 and 2008/09) were collected in a Swedish county (population 427,000) to retrospectively determine which grouping of telenursing chief complaints had the largest correlation with influenza case rates. This grouping was prospectively evaluated in the three subsequent seasons. The best performing telenursing complaint grouping in the retrospective algorithm calibration was fever (child, adult) and syncope (r=0.66; p<0.001). In the prospective evaluation, the performance of 14-day predictions was acceptable for the part of the evaluation period including the 2009 influenza pandemic (area under the curve (AUC)=0.84; positive predictive value (PPV)=0.58), while it was strong (AUC=0.89; PPV=0.93) for the remaining evaluation period including only influenza winter seasons. We recommend the use of telenursing complaints for predicting winter influenza seasons. The method requires adjustments when used during pandemics.

Background

Data source alternatives to mandatory reporting by microbiological laboratories and sentinel physician practices have been sought to improve the timely detection of influenza outbreaks [1,2]. Telenursing is defined as call centres staffed by registered nurses who perform counselling and patient triage as a means of augmenting self-care support and regulating patient access to medical services. This strategy is rapidly expanding in many countries, with prominent examples in Sweden, the United Kingdom (UK) and Canada, [3] and telenursing call data have been regarded as a promising source of syndromic surveillance [4–6]. However, a study published in 2009 reported low validity of current telenursing data for monitoring and predicting influenza outbreaks [7]. Several possible reasons for this shortcoming were identified, such as a lack of specificity due to broad definitions of influenza-like illness (ILI) and the use of suboptimal evaluation methods. Cough and high fever with rapid onset have been known as the symptoms best discriminating influenza infection [8], but recently it has been observed that when aggregated at the population level, the incidence of influenza symptoms may differ between age groups, status (hospitalised or outpatient), or (sub) type of influenza virus [9]. In a patient cohort in the United States (US) with confirmed influenza A(pH1N1) pdm09 virus infection, the most common symptoms were fever (94%) and cough (92%), followed by sore throat (66%), diarrhoea (25%), and vomiting (25%) [9]. In contrast with a parallel Singapore cohort of A(pH1N1) pdm09 virus -infected patients, similar proportions with cough (88%), fever (79%), and sore throat (54%) were reported, but fewer patients described vomiting (1.1%), and diarrhoea (0.7%) [10]. In the latter study it was also reported that there were differences in symptom patterns between patients presenting with seasonal influenza (H3N2, H1N1, and B) and the pandemic A pH1N1 influenza.

This study examines the use of data from calls to telenursing services as population-level predictors of influenza season activity and its progression. It employs data from the Swedish telenursing service Healthcare Direct/1177 and an electronic health data repository covering an entire county population [11]. The

electronic repository collects data from all calls made by the county residents to the nation-wide telenursing service, and data from all healthcare episodes provided in the county at primary and secondary levels. Specifically, the aim of the study is to examine the prospective performance of chief complaints documented during telenursing calls, hereafter referred to as telenursing chief complaints, in predicting influenza activity on a daily and weekly basis, respectively, during a three-year period.

Methods

This observational study uses an open cohort design based on the total population in a Swedish county. A detection algorithm was calibrated using retrospective data from two years (covering influenza winter seasons 2007/08 and 2008/09) and then prospectively evaluated during a three-year period (covering the pandemic 2009/10 and influenza winter seasons 2010/2011 and 2011/12). The study was based on administrative public health databases established for the purpose of systematically and continuously developing the quality of service. In accordance with Swedish legislation (SFS 2008:355), personal identifiers were removed from the records. The study design was approved by the Regional Research Ethics Board in Linköping (2012/104-31).

The study was performed in Östergötland (population 427,000) located in south-eastern Sweden. The daily and weekly rates of clinical cases are used as measures of influenza activity. An account of the age-stratified influenza activity in the county has previously been reported [12]. Annual aggregated data on the sex, age, and residence of the population were collected from Statistics Sweden [13]. Data from Östergötland residents who had contacted the telenursing service or had been clinically diagnosed with influenza were identified from the electronic health data repository associated with the county-wide electronic health record systems at the County Council. Data from the clinical laboratories, however, were only collected for this study during the period from 1 January 2009 to 15 September 2010. Influenza cases were identified from the electronic health data repository by the International Classification of Diseases version 10 (ICD-10) codes for influenza (J10.0, J10.1, J10.8, J11.0, J11.1, J11.8) [14]. For individuals having received an influenza diagnosis at both primary and secondary levels of care, the diagnosis code recorded at the first contact was used for the analyses. If the codes were recorded at the same day, only the secondary-level diagnosis code was used. ILI-related telenursing call cases were identified by the chief complaint codes associated with influenza symptoms (dyspnoea, fever (child, adult), cough (child, adult), sore throat, lethargy, syncope, dizziness, and headache (child, adult)) from the fixed-field terminology register.

Case data validation

The influenza case data defined by clinical diagnoses were validated against case data from the microbiological laboratories for the period 1 January 2009 to 15 September 2010. In these analyses, both data sets were separately adjusted for weekday effects on care resource utilization. The correlations between the number of cases reported each day in the clinical and laboratory data were analysed with 0–6 day lag. The results showed a strong correlation between the number of clinically diagnosed influenza cases per day and the corresponding number of cases verified daily by microbiological analyses during the validation period. The correlation with largest strength ($r=0.625$; $p<0.001$) was observed between the clinically and the microbiologically verified cases with a two-day lag.

Retrospective algorithm calibration

A calibration procedure [15] was used for determining the telenursing chief complaint grouping that retrospectively demonstrated the best predictive accuracy with regard to the influenza case rates. Data from two influenza seasons (2007/08 to 2008/09) were collected and used to determine the influenza activity prediction (IAP) grouping having the strongest correlation with case rates and the best-performing threshold for alerts. Initial calibrations using correlations have been suggested to complement the application of a threshold or scan statistic in the analyses of surveillance performance [16]. Correlations between chief complaints documented during telenursing calls and influenza case rates were, therefore, first examined for all possible combinations of complaints with a preceding time lag to physician diagnosis starting from 0 days and until the correlations started to decay, but at least up to 14 days. The three groupings of chief complaints with the strongest correlation to the influenza case rate for each time lag were listed. The chief complaint grouping with the largest correlation strength was chosen as the influenza activity prediction (IAP) grouping to be used in the following analyses of predictive performance.

In the second calibration step, a detection algorithm was used to examine the prospective performance of the selected IAP grouping. A Shewhart-type algorithm [17], where the signal decisions depend on the observed measure of activity from the current time period was used. The baseline temporal trend of calls to Healthcare Direct due to complaints included in the IAP grouping was estimated in the retrospective data set using the formula b_0+b_1t, where b_0 is the intercept, b_1 the slope, and t is time (day). The actual number of calls to Healthcare Direct was then identified for each day and the estimated baseline level value for that day was subtracted from the recorded number. If the difference was positive, the value was saved, and if it was negative it was set to 0. This transformation yielded an adjusted set of telenursing data. To detect outbreaks on a daily basis we calculated a moving average for the adjusted data set (the value for day 8 is the average number of calls for days 1-7, the value for

Table 1

Numbers of daily influenza cases and telenursing influenza-like illness calls per 100,000, Östergötland country, Sweden, winter influenza seasons including the 2009 pandemic, and intermittent periods 2007–2012

Mean daily numbers per 100,000									
	Influenza B and A(H1) 2007/08	May–November 2008	Influenza A(H3N2) 2008-09	April–July 2009	Influenza A(pH1N1) pdm09 2009/10	January–November 2010	Influenza B and A(pH1N1) pdm09 2010/11	May–December 2011	Influenza A(H3N2) 2011/12
Influenza cases	1.12	0.07	1.63	0.11	1.34	0.09	1.22	0.08	1.89
Telenursing calls (%)									
Total ILI complaints	20.0 (100)	15.4 (100)	22.4 (100)	16.7 (100)	22.5 (100)	18.6 (100)	27.3 (100)	21.1 (100)	27.9 (100)
Dyspnea	1.67 (8.3)	1.48 (9.6)	1.81 (8.1)	1.42 (8.5)	2.08 (9.2)	1.56 (8.4)	2.09 (7.6)	1.88 (8.9)	2.35 (8.4)
Fever (child)	4.84 (24.3)	3.11 (20.1)	5.95 (26.6)	3.52 (21.1)	5.52 (24.6)	4.04 (21.8)	7.33 (26.9)	4.44 (21.1)	7.38 (26.4)
Fever (adult)	1.48 (7.4)	0.95 (6.1)	2.09 (9.3)	1.36 (8.2)	2.58 (11.5)	1.23 (6.6)	2.51 (9.2)	1.63 (7.7)	2.66 (9.5)
Cough (child)	2.79 (14.0)	1.40 (9.1)	2.64 (11.8)	1.38 (8.3)	2.11 (9.4)	2.38 (12.8)	3.57 (13.1)	2.10 (9.9)	3.54 (12.7)
Cough (adult)	1.70 (8.5)	1.38 (8.9)	2.60 (11.6)	1.45 (8.7)	2.37 (10.6)	1.73 (9.3)	2.58 (9.5)	2.30 (10.9)	3.21 (11.5)
Sore throat	4.09 (20.5)	3.59 (23.3)	3.82 (17.0)	4.04 (24.2)	3.93 (17.5)	3.84 (20.7)	4.49 (16.4)	4.02 (19.1)	4.11 (14.7)
Dizziness	1.22 (6.1)	1.23 (8.0)	1.21 (5.4)	1.31 (7.9)	1.24 (5.5)	1.22 (6.6)	1.55 (5.7)	1.61 (7.6)	1.64 (5.9)
Lethargia	0.46 (2.3)	0.51 (3.3)	0.57 (2.6)	0.58 (3.5)	0.57 (2.5)	0.68 (3.7)	0.82 (3.0)	0.66 (3.1)	0.70 (2.5)
Syncope	0.20 (1.0)	0.19 (1.3)	0.20 (0.9)	0.21 (1.2)	0.22 (1.0)	0.25 (1.4)	0.36 (1.3)	0.30 (1.4)	0.27 (1.0)
Headache (child)	0.28 (1.4)	0.36 (2.3)	0.26 (1.2)	0.27 (1.6)	0.37 (1.6)	0.29 (1.6)	0.42 (1.5)	0.42 (2.0)	0.38 (1.4)
Headache (adult)	1.23 (6.1)	1.25 (8.1)	1.25 (5.6)	1.13 (6.8)	1.47 (6.6)	1.33 (7.2)	1.59 (5.8)	1.72 (8.1)	1.70 (6.1)

ILI: influenza-like illness.

day 9 is the average number of calls for days 2-8, etc.). The threshold levels for signalling an alert were determined using Receiver Operating Characteristic (ROC) curves. The area under the ROC curve (AUC) calculated from plots of the sensitivity and 1-specificity of the outbreak predictions and the positive predictive value (PPV) of these predictions on a daily and weekly basis, respectively, were used as performance indicators [18]. The limit for start and end time of influenza outbreaks was set to 1.8 cases/100,000 during a floating seven-day period [19].

Statistical data analysis

The IAP grouping and threshold were prospectively evaluated using data from three subsequent seasons (2009/10 to 2011-12). During this period, the telenursing data were adjusted to the baseline temporal trend with the same methods as used in the retrospective algorithm calibration. For the evaluation, correlations with influenza case rates were calculated and estimates of the AUC and PPV computed as performance indicators. The level of statistical significance was set to $p<0.05$. To denote the strength of correlations, limit values were applied as suggested by the Cohen Scale [20]. This scale defines small, medium and large effect sizes as 0.10, 0.30, and 0.50 respectively. The limits for interpreting the AUC (or c-statistic) were set to 0.90, 0.80, and 0.70, denoting very strong (outstanding), strong (excellent), and acceptable discriminatory performance, respectively [21]. The analyses were performed using SPSS version 19, R Statistical Software version 2.15.2, and Minitab Statistical Software version 16.1.1.

Results

The highest incidence of influenza cases during the study period was recorded for the influenza winter season 2011/12 in Östergötland county (1.9 cases/day/100,000; 8.2 cases/day in the county) (Table 1). The average number of telenursing calls recorded during an influenza winter season or pandemic with a chief complaint in the ILI category increased from 20.0 calls/day/100,000 (84.4 calls/day) during the B and A H1 influenza winter season in 2007/08 to 27.9 calls/day/100,000 (120.4 calls/day) during the H3N2 influenza winter season in 2011/12. Correspondingly, the calls with a chief complaint in the ILI category during the intermittent periods increased from 15.4 calls/day/100,000 (65.3 calls/day) in May–November 2008 to 21.1 calls/day/100,000 (90.9 calls/day) in May–December 2011.

Retrospective calibration

The grouping of chief complaints with the largest correlation strength on a daily basis ($r=0.66$; $p<0.001$) and longest lead time (14 days) to influenza case rates in the retrospective data was fever (child, adult) and syncope (Table 2). On a weekly basis, the strength of the correlation was larger ($r=0.91$; $p<0.001$), while the lead time remained at two weeks. The chief complaints cough (child, adult), lethargy, dizziness, and

Table 2

Best performing telenursing complaint groupings in retrospective analysis displayed by lead time to physicians' diagnosis of influenza, Östergötland, Sweden, winter influenza seasons including the 2009 pandemic and intermittent periods 2007–2012

Telenursing chief complaint grouping		
Lead time (days)		Correlation (r)
0	Fever (child), fever (adult), syncope, headache (child)	0.491
	Fever (child), fever (adult), syncope	0.490
	Fever (child), fever (adult), headache (child)	0.489
1	Fever (child), fever (adult), syncope	0.513
	Fever (child), fever (adult)	0.512
	Fever (child), fever (adult), lethargia, syncope	0.511
2	Fever (child), fever (adult), syncope	0.549
	Fever (child), fever (adult), syncope headache (child)	0.548
	Fever (child), fever (adult)	0.547
3	Fever (child), fever (adult), dizziness	0.598
	Fever (child), fever (adult)	0.598
	Fever (child), fever (adult), headache (child)	0.595
4	Fever (child), fever (adult)	0.575
	Fever (child), fever (adult), syncope	0.572
	Fever (child), fever (adult), headache (child)	0.572
5	Fever (child), fever (adult)	0.581
	Fever (child), fever (adult), syncope	0.579
	Fever (child), fever (adult), headache (child)	0.573
6	Fever (child), fever (adult)	0.586
	Fever (child), fever (adult), syncope	0.582
	Fever (child), fever (adult), headache (child)	0.582
7	Fever (child), fever (adult)	0.615
	Fever (child), fever (adult), syncope	0.614
	Fever (child), fever (adult), dizziness	0.606
8	Fever (child), fever (adult)	0.627
	Fever (child), fever (adult), headache (child)	0.626
	Fever (child), fever (adult), cough (adult)	0.623
9	Fever (child), fever (adult)	0.638
	Fever (child),fever (adult), syncope	0.638
	Fever (child), fever (adult), headache (child)	0.633
10	Fever (child), fever (adult)	0.627
	Fever (child), fever (adult), syncope	0.622
	Fever (child), fever (adult), headache (child)	0.619
11	Fever (child), fever (adult)	0.658
	Fever (child), fever (adult), headache (child)	0.657
	Fever (child), fever (adult), syncope	0.656
12	Fever (child), fever (adult), syncope	0.640
	Fever (child), fever (adult)	0.638
	Fever (child), fever (adult), syncope, headache (child)	0.635
13	Fever (child), fever (adult), cough (adult)	0.630
	Fever (child), fever (adult)	0.630
	Fever (child), fever (adult), cough (child), cough (adult)	0.629
14	Fever (child), fever (adult), syncope	0.661
	Fever (child), fever (adult)	0.660
	Fever (child), fever (adult), cough (child), syncope	0.654

Influenza-like illness (ILI) complaints included in the analysis were dyspnoea, fever (child), fever (adult), cough (child), cough (adult), sore throat, dizziness, lethargia, syncope, headache (child), and headache (adult).

P<0.001 for all correlations.

headache (child) were included in groupings showing large correlations strength (r>0.5) with influenza case rates. The chief complaints not included in any grouping reaching the level of statistical significance were sore throat and common cold. Based on these observations, fever (child, adult) and syncope were chosen as the IAP complaint grouping for use in alerts. The alerting threshold was determined to a moving average of 0.9 calls/day/100,000 above the baseline level (Figure 1). The performance of alerts on a daily basis (14 days lead time) was very strong (AUC=0.94; PPV=0.92); the specificity was 0.94 and the sensitivity was 0.85. For alerts on a weekly basis, the threshold was determined to 4.7 calls/week/100,000 above the baseline level. Also for the weekly alerts (two weeks lead time), the retrospective performance was very strong (AUC=0.93; PPV=0.96); the specificity was 0.97 and the sensitivity 0.87.

Prospective evaluation

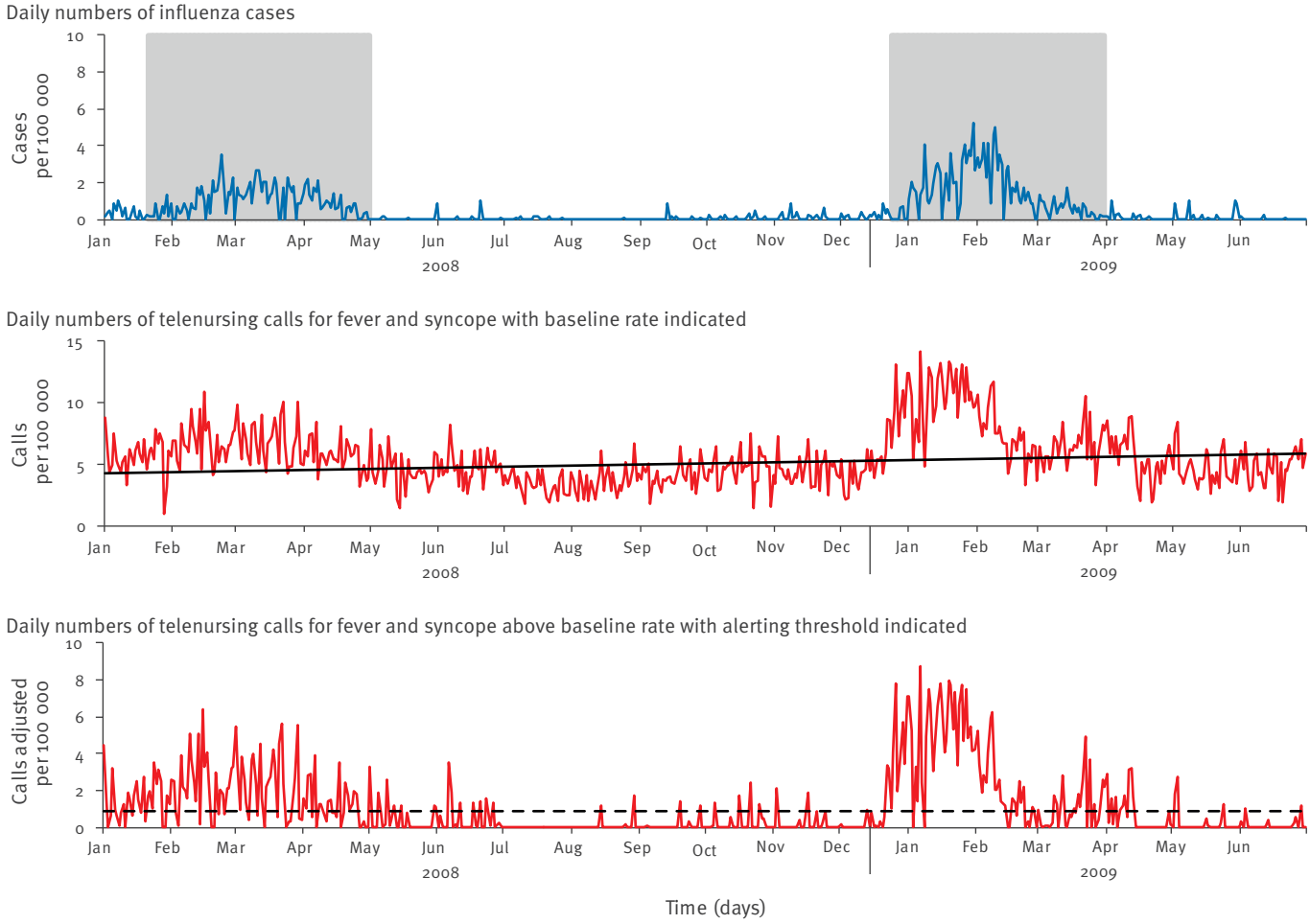
The strength of the correlation between telenursing call rates for the IAP grouping and influenza case rates was, on a daily basis, slightly smaller than observed in the retrospective analysis but still large (r=0.59; p<0.001). The correlation strength was smaller during the first part of the period (July 2009 to June 2010) including the 2009 influenza pandemic (r=0.56; p<0.001) than in the second part of the period (July 2010 to April 2012) including only winter influenza seasons (r=0.64; p<0.001) (Figure 2). Similarly, the weekly correlation strength was smaller than observed from the retrospective data (r=0.80; p<0.001). Here it was also smaller during the first part of the evaluation period (r=0.76; p<0.001) than in the later part including only influenza winter seasons (r=0.86; p<0.001). The AUC for the 14-day predictions on a daily basis was 0.87 (PPV=0.75) for the entire prospective evaluation period; the specificity was 0.88 and the sensitivity was 0.67 (Figure 3). The performance was acceptable for the part of the evaluation period including the 2009 influenza pandemic (AUC=0.84; PPV=0.58), while it was strong (AUC=0.89; PPV=0.93) for the remaining period including only influenza winter seasons. On a weekly basis, the AUC was strong 0.81 (PPV=0.90) for the entire prospective evaluation period; the specificity was 0.94 and the sensitivity was 0.68. Also on a weekly basis, the performance of predictions was acceptable for the pandemic outbreak (AUC=0.78; PPV=0.79) and strong for the influenza winter seasons (AUC=0.83; PPV=1.00).

Discussion

This is the first study of the predictive performance of telenursing data in influenza surveillance based on recommended standard statistical outcome measures for evaluations of methods for forecasting infectious disease activity [22]. The complaint grouping found in retrospective analyses of two consecutive influenza winter seasons to have the longest lead time and strongest correlation to variations in influenza case rates was fever (child, adult) and syncope.

Figure 1

Retrospective data used for algorithm calibration from Östergötland County, Sweden, January 2008–June 2009

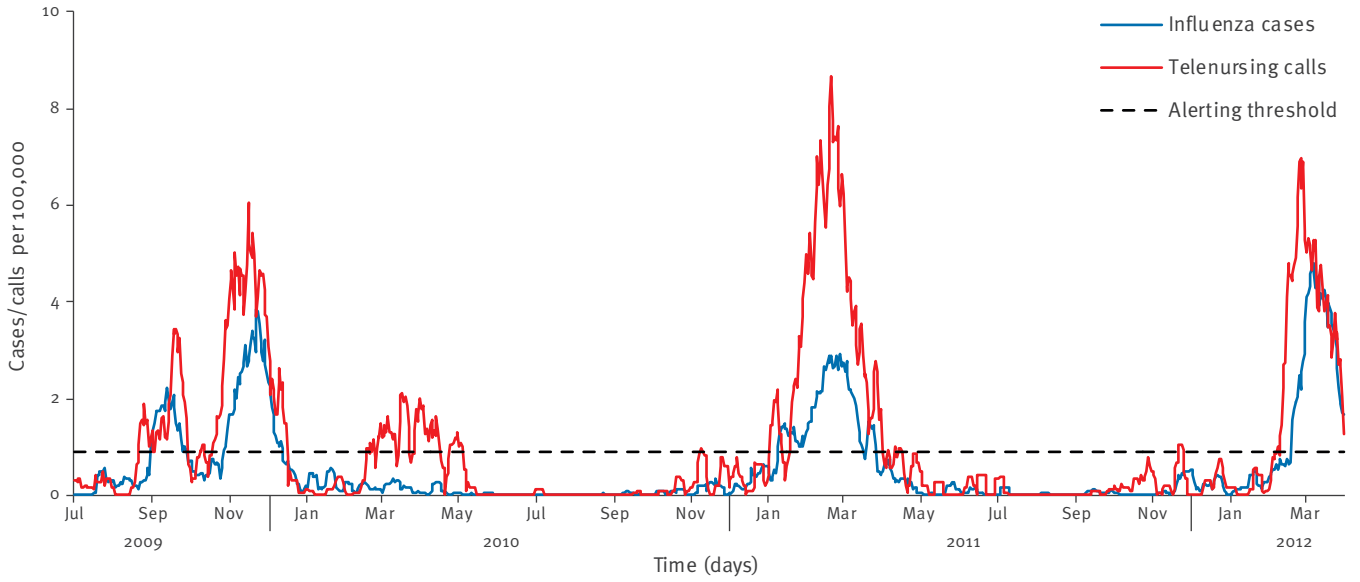


The prospective correlation with weekly influenza case rates over three consecutive influenza seasons was found to have slightly greater strength (r=0.80; p<0.001) than the retrospective median correlation (r=0.74 (range 0.34–0.89)) reported from a state-level US study [7]. The latter study did not include optimisation with regard to alternative chief complaint groupings or prospective evaluation, but it reported that the correlation between influenza case rates and viral isolate data was strong. In our prospective evaluation, the performance of daily telenursing complaint data in 14-day predictions of influenza case rates was found to be strong (AUC=0.87; PPV=0.75). The performance was poorer during the first part of the prospective evaluation period including the 2009 influenza pandemic (AUC=0.84; PPV=0.58) than during the remaining period, including only influenza winter seasons (AUC=0.83; PPV=1.00). The poorer performance can be explained both by the fact that the symptom patterns during pandemic influenza outbreaks differ from the corresponding patterns of seasonal influenza and also by differences in healthcare utilisation and health seeking behaviour between pandemic outbreaks and winter seasons [23,24]. This implies that predictions based on telenursing data from influenza winter seasons can be assumed to be less accurate when applied during pandemic s. Our results also confirm exploratory findings reported from other settings. In a study performed within the NHS Direct telenursing service in the United Kingdom, alerting thresholds defined as 9% fever complaints in the age group 5–14 years and 1.2% 'cold/flu' complaints of all complaints were derived using Poisson regression modelling [25]. In a pragmatic prospective evaluation, the thresholds were found to provide up to 14 days advance warning of seasonal influenza activity. Similarly, a retrospective study from Canada using data on total call rates from the Telehealth Ontario telenursing service and case rate data on respiratory illnesses showed strong correlations and indicated that, if threshold levels had been set for the start of outbreaks, it would have been possible to provide up to 15 days advance warnings of emergency department visits [26]. No prospective evaluation was reported from the Canadian telenursing setting.

In previous studies involving telenursing service users and clinical outpatients, fever has been found to be an

Figure 2

Daily numbers of influenza cases and telenursing calls for fever and syncope above the baseline temporal trend for calls to the telenursing service in Östergötland County, Sweden, during the prospective evaluation July 2009–April 2012

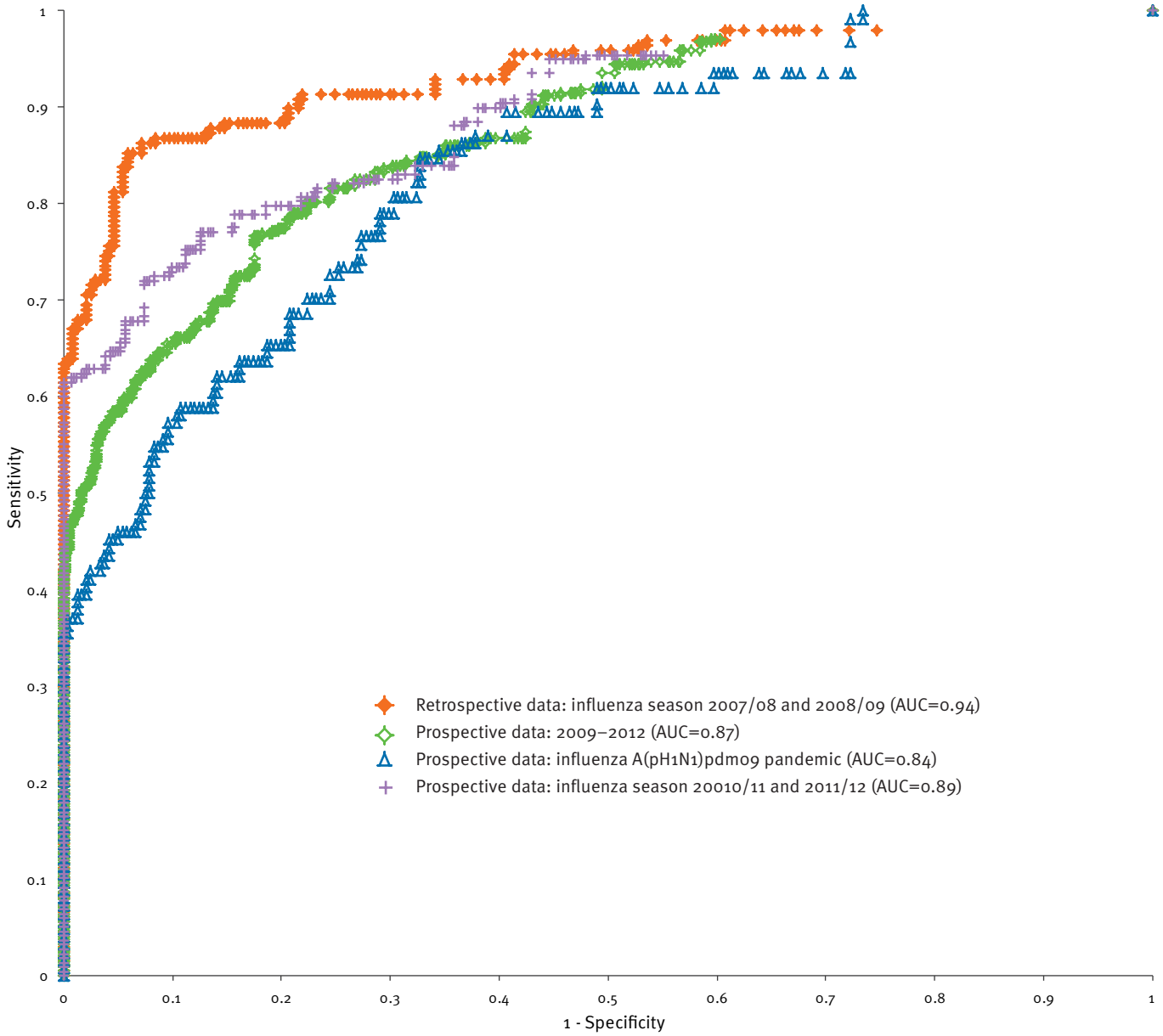


early correlate to influenza case rates [27,28]. Requiring fever as part of the case definition has been shown to increase the specificity of influenza diagnosis among clinical ILI cases [29,30]. One explanation of the predictive performance of fever in influenza surveillance could be that the symptom mediates timely outbreak detection particularly when the predominant circulating influenza strain does not initially cause significant levels of illness among those infected, while only later and different complaints and complications necessitate medical care. Cough was not included in the IAP grouping in this study, although this symptom has commonly been reported from studies of the clinical presentation of influenza [8¬–10]. Cough is among the most common reason for seeking medical care, and most episodes result from a self-limited acute viral upper respiratory tract infection [31]. However, particularly among older adults, the symptom can also be caused by a number of other disorders, such as gastro-oesophageal reflux disease, upper airway cough syndrome, and asthma [32]. In our study, cough was included in several groupings of chief complaints showing large correlations with influenza case rates. Nonetheless, unlike for fever, in some seasons, cough was mainly reported from children and during other seasons from adults. Variations were also found in the age distribution of clinical case rates as indicated by the relative illness ratio (unpublished data). It is thus reasonable to assume that the predictive performance of telenursing chief complaint groupings in influenza surveillance, as these groupings provide a selective representation of those infected with symptoms, is both population- and season-dependent. Therefore, fever appears to be a common denominator among the chief complaints with regard to predictive performance. More research on the clinical presentation of influenza as well as the grouping of telenursing chief complaints for prospective use in influenza surveillance is warranted.

This study has several limitations that should be considered when interpreting the results. First, influenza cases were defined by clinical diagnosis, and microbiological validation was restricted to a limited period of the study. However, the strength of the correlation between the microbiological and clinical diagnosis rates was large during the validation period, and similar findings have also been reported from other settings [30,33]. Second, the telenursing data were based on chief complaint codes defined for Sweden. Some complaints, such as fever and cough, were coded as age-specific syndromes, while other complaints had an age-neutral coding. Internationally standardised telenursing complaint codes would facilitate valid and reliable recording and comparisons between systems. The World Health Organization framework for influenza preparedness [34] could provide a forum for implementing such a process. Moreover, the epidemiological context for interpreting telenursing data has not been established. The majority of calls to telenursing systems are about infections, such as colds, influenza or diarrhoea [25,35]. A study of the Telehealth Ontario telenursing service in Canada showed that the call volume was weighted for the 0–4 years age group (49%), while the outpatient visits during the same period were mainly from those 18–64 years old (44%) [26]. An early Swedish study reported that about every second call to telenursing service is made by a third party on behalf of the ill person, mostly by a spouse or parents of preschool-aged children, and that another large group of callers is young adults living independently [36]. According to a recent Canadian study, the overrepresentation of younger age groups among telenursing

Figure 3

Receiver operating characteristic (ROC) curves based on retrospective and prospective data for prediction of influenza case rates from telenursing calls for fever and syncope, Östergötland County, Sweden, January 2008–April 2012



AUC: area under the curve.

callers can be explained by both epidemiological and social factors, that is, the incidence of acute respiratory infections is high among young people and that first-time parents without previous parenting experience make more calls for their children [37]. In order to further develop the performance of telenursing data in infectious disease surveillance, the biases associated with using these data in epidemiological analyses have to be better understood. Schemes such as the Behavioral Risk Factor Surveillance System (BRFSS) supplied by the Centers for Disease Control and Prevention in the US are needed for longitudinal collection and analysis of standardised data on health behaviours during and between seasons [38].

Conclusions

In this first prospective study based on standardised outcome measures, the telenursing complaints fever and syncope were found to be strongly correlated to influenza case rates and the complaints grouping showed strong performance in predicting winter influenza seasons. The method performed poorer during the 2009 pandemic outbreak when health behaviours did not follow anticipated patterns. This paper has presented data from Sweden, but the results have international relevance, as telenursing services are rapidly expanding worldwide [3]. We recommend the use of telenursing data in surveillance of seasonal influenza. The relationship between the utilisation of the service in population subgroups during winter influenza

seasons and pandemic outbreaks and the herd immunity associated with different influenza types warrants further study.

Author contributions

TT, OE, AS and EAG conceived and designed the study. OE, AS and ÖD analysed the data. TT, ÖD, EH and MS contributed materials and analysis tools. TT and AS wrote the paper. OE, EAG, MS, EH, JE, ÖD, JH, JMN and HE revised the manuscript and provided intellectual content. TT, AS, OE, ÖD, EAG, MS, EH, JE, JH, JMN and HE gave final approval of the version to be published. TT is guarantor of the content.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Genetic diversity and evolutionary relationships among *Legionella pneumophila* clinical isolates, Portugal, 1987 to 2012

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The genetic diversity of 89 clinical *Legionella* isolates, collected between 1987 and 2012, in 22 hospitals from the five regions of Portugal, was analysed in this study using monoclonal antibodies (MAbs) of the Dresden panel and the sequence-based typing (SBT) protocol. The eBURST algorithm was used to infer levels of relatedness between isolates. All isolates collected were *Legionella pneumophila*, which were further characterised into four subgroups by MAbs, and 30 sequence types (STs) by SBT. Twelve of the STs were unique to Portugal; one of them (ST100) was represented by 32 epidemiologically related isolates. The ST44 was the profile with the highest number of epidemiologically unrelated isolates. The eBURST analyses indicate that, within the group formed by the 30 STs identified in this study, 17 STs were genetically close to at least another ST in the group. The comparison between the eBURST diagrams obtained with the STs from this study and the entire SBT database of the European Working Group for *Legionella*, showed that 24 (seven of them unique to Portugal) of our 30 STs were related with STs identified in others countries. These results suggest that the population of *L. pneumophila* clinical strains in Portugal includes both worldwide and local strains.

Introduction

Legionellaceae are ubiquitous in the environment, being particularly prevalent in man-made habitats, such as cooling towers and domestic hot and cold water distribution systems. This family consists of a single genus, *Legionella*, but contains 56 species/subspecies belonging to over 70 serogroups [1]. *Legionella* are the causative agents of Legionnaires' disease (LD), a severe pneumonia that is transmitted through inhalation of contaminated aerosols. The most common species to cause disease is *L. pneumophila*, which has 16 serogroups, but the majority of human disease (84% worldwide, 95% in Europe) is caused by *L. pneumophila* serogroup (sg) 1 [2,3].

When a case of LD occurs, it is essential that public health authorities are able to detect the source of infection promptly by comparing clinical and environmental isolates, so that decontamination measures can prevent further cases. For this comparison, the sequence-based typing (SBT) scheme and monoclonal antibodies (MAbs) of the Dresden panel are the typing methods widely used by the members of the European Working Group for *Legionella* Infections (EWGLI), renamed to European Study Group for *Legionella* Infections (ESGLI-ESCMID), since September 2012.

In 1999, LD was included in the Portuguese system of mandatory notifications of infectious diseases. In 2004, an integrated programme of epidemiological surveillance was implemented in order to improve reporting, diagnosis and investigation of cases, through the inclusion of obligatory laboratory notification and communication of guidelines to the professionals involved.

In Portugal the number of reported cases per year between 2004 and 2012 ranged from 61 to 140. The peak in the number of cases is usually between August and November and most of the notifications come from the North region. From 2004 to 2012, a total of 868 cases were notified (crude annual reporting rate of 0.91/100,000 inhabitants) with 50 fatalities (case fatality ratio of 6%). The majority of cases (672/868, 77%) were male and the most affected age groups were the 50 to 59 (217 cases, 25%) and 40 to 49 year-olds (196 cases, 23%) [4].

As partner of this surveillance scheme, the *Legionella* Laboratory of the Microbiology Department of the Faculdade de Ciências Médicas (Lisbon) is responsible for the characterisation of the clinical strains isolated

in Portugal. In a previous study, the *L. pneumophila* clinical strains isolated in Portugal were characterised by MAbs and SBT methodologies, which allowed the construction of a database for use in epidemiological surveillance efforts [5].

The aim of the current study was to assess the genetic diversity and evolutionary relationships among *L. pneumophila* clinical isolates collected in Portugal during the period from 1987 to 2012, and to compare these results with the available data submitted to EWGLI-SBT database until 10 April 2013.

Methods

Origin and epidemiological characteristics of isolates

In total, 89 clinical *Legionella* isolates were analysed. Forty-one clinical isolates were obtained from the 868 cases reported to the integrated programme of epidemiological surveillance and 48 clinical isolates came from the collection of the Microbiology Laboratory of Hospital Egas Moniz-CHLO (1987 to 2003). These 89 clinical isolates, all from patients requiring hospitalisation, were sent by 22 hospitals from five regions of Portugal: 60 from Lisbon, 25 from the North, two from the Centre, one from Alentejo and one from Algarve. Thirty-six of the 60 strains from Lisbon were isolated at the same hospital (hospital A) between 1987 and 2007.

Among the 89 total isolates, 36 of the isolates were collected from patients with nosocomial infections and 39 from community-acquired infections. The remaining 14 isolates had an unknown origin. All but one of the nosocomial isolates came from hospital A; the other strain was isolated during a small outbreak in hospital B in the North region. Only five of the isolates from community-acquired infections were related cases and came from two outbreaks, one in 2009 and the other in 2012, in two cities of the North region of Portugal.

The median ages of patients (63 males and 16 females; 10 unknown) were 55 and 58 years-old for males and females, respectively (range: 13–80 years; 16 patients with unknown age).

Phenotype, genotype and diversity of isolates

The *L. pneumophila* strains were phenotyped at the Microbiology Laboratory of Hospital Egas Moniz-CHLO with MAbs of the Dresden panel [6]. Genotyping was performed at the *Legionella* Laboratory of the Microbiology Department of the Faculdade de Ciências Médicas using a seven-allele standard SBT scheme [7–9]. Briefly, the Dresden panel identifies 15 of the 16 *L. pneumophila* sgs using sg-specific MAbs. In addition, for the sg1 of *L. pneumophila*, the Dresden panel uses five MAbs plus the Mab 3 of the International Panel obtained from the American Type Culture Collection [10]. With this scheme of six MAbs, it is possible to differentiate the sg1 strains into nine subgroups (Philadelphia, Allentown/France, Knoxville, Olda, Benidorm, Oxford, Bellingham, Heysham and Camperdown) [6]. Both panels were performed by direct immunofluorescence and are based on the reactivity of the surface lipopolysaccharide epitopes.

Within the SBT scheme, part of the genes *flaA, pilE, asd, mip, mompS, proA*, and *neuA*, were sequenced. For each gene sequence a distinct allele number is assigned through the EWGLI-SBT database for *L. pneumophila* (available at: http://www.hpa-bioinformatics.org.uk/legionella/legionella_sbt/php/sbt_homepage.php). The combination of seven allele numbers defines an allelic profile to which a sequence type (ST) is attributed. In order to assign a ST, when the standard *neuA* primers failed to amplify, we used the novel primer set, specifically designed for the *neuA* homolog (*neuAh*) [11]. This *neuAh* is present in some non-sg1 strains and is functionally equivalent to the *neuA* gene of the strain Philadelphia-1 [12].

Clinical isolates diversity was assessed by calculating Hunter and Gaston's modification of Simpson's index of diversity (IOD) [13], using the V-Dice application (available at: http://www.hpa-bioinformatics.org.uk/cgi-bin/DICI/DICI.pl).

Assessing relationships between isolates

For establishing the possible evolutionary relationships between isolates, we applied the eBURST algorithm v3 (available at: http://eburst.mlst.net). The eBURST group was obtained with a less stringent definition, in which STs are included within the same group only if they share identical alleles at five or six of the seven SBT loci with at least one other ST. STs that cannot be assigned to any group are called singletons. The statistical confidences for the primary founders were assessed using 1,000 bootstrap re-samplings [14].

Results

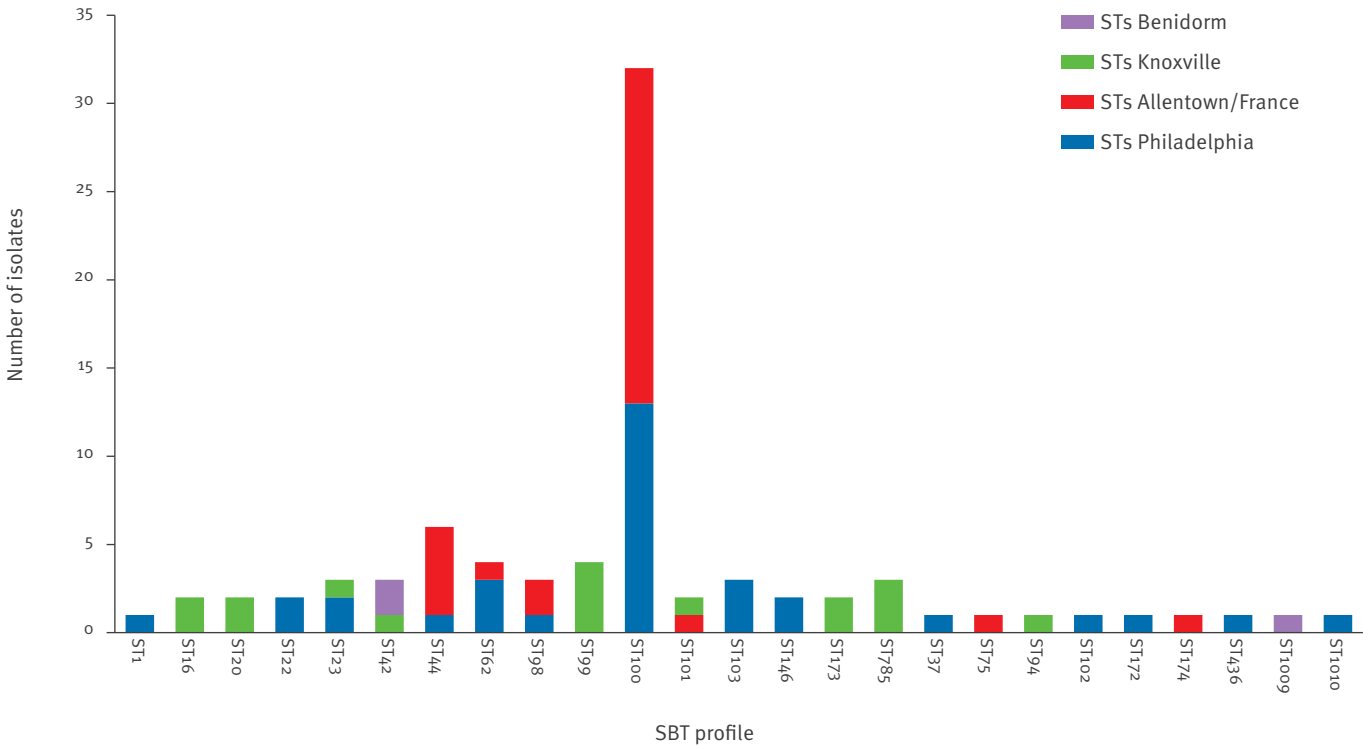
Characteristics and diversity of isolates

All the 89 clinical isolates were *L. pneumophila* and 84 belonged to sg1, the other five were serotyped as sg8, 10, 12 and 14. Of the 84 sg1 isolates, 83 had the virulence-associated epitope recognised by MAb3/1; 33 belonged to the Philadelphia subgroup, 30 to the Allentown/France, 17 to the Knoxville and three to the Benidorm. The only isolate MAb3/1 negative was characterised as OLDA. The Knoxville was the most widespread subgroup in our country, being present in four of five regions investigated (North, Centre, Lisbon and Algarve).

With regard to the category of the infection, the strains from community-acquired cases showed greater diversity (four subgroups: Philadelphia, Allentown/France, Knoxville and Benidorm) than the nosocomial cases (two subgroups: Philadelphia and Allentown/France).

Figure 1

Sequence-based typing of clinical *Legionella pneumophila* isolates, all MAb3/1 positive, from 22 hospitals located in five regions[a] of Portugal, 1987–2012 (n=83)



SBT: sequence-based typing; ST: sequence type.

[a] The five regions were: Lisbon, the North, the Centre, Alentejo and Algarve.

All the strains from the sg1 and one from sg12 were genotyped by standard SBT. Figure 1 shows the repartition per ST of the 83 isolates positive for MAb3/1, as well as the sg1 subgroups within each ST. The *neuA* primers failed to amplify four isolates, all of them non-sg1 (sg8, 10, 14). As described in the methods section, in these cases we used the *neuAh* primers, which improved the quality of the sequences and allowed us to assign a ST to these isolates.

Of the 89 *L. pneumophila* clinical isolates available for SBT analysis, 52 were from single clinical cases (unrelated strains, comprising the 14 of unknown origin), the remaining isolates were associated to outbreaks (related strains: five strains were recovered from the two community outbreaks in 2009 and 2012, and 32 were isolated in hospital A from nosocomial infections). After inclusion of one isolate for each group of epidemiologically related strains, 55 isolates (these three isolates plus the 52 unrelated strains) were included in our SBT analysis. These 55 isolates were found to include 30 STs (IOD = 0.972; 95% confidence interval (CI): 0.960–0.985), with the *mompS* gene being the most discriminative (IOD = 0.893) and the gene *proA* the less discriminative (IOD = 0.748).

Fourteen STs consisted of groups containing between two and five unrelated isolates and the remaining STs accounted for only one single isolate each. Twelve of the 30 STs were unique to Portugal, according to data submitted to the EWGLI-SBT database at the time of writing: nine STs from *L. pneumophila* sg1 (ST98, ST100, ST101, ST102, ST173, ST174, ST785, ST1009, ST1010) and three STs from *L. pneumophila* non-sg1 (ST1343, ST1383 and ST1384). Altogether, these new STs comprised 16 of the 55 isolates included in this study. In addition, eight new allele numbers were assigned by the EWGLI-SBT database curators after our data were submitted to the database (22 and 29 for the *mip* gene, 24 and 37 for the *pilE* gene and 20, 34, 23 and 219 for the *asd*, *mompS*, *proA* and *neuAh* genes, respectively). It is interesting to note that six of these new allele numbers were detected only in *L. pneumophila* non-sg1 strains.

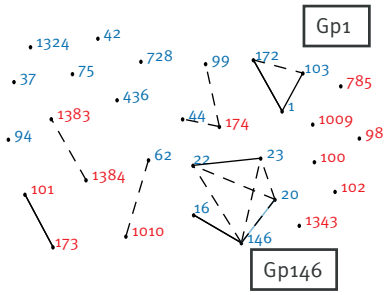
The ST100, one of the profiles exclusively found in our country, was restricted to hospital A, with 32 isolates. Another ST specific to our country was the ST785; this profile, which was represented by three isolates in our study, was only found in the 2009 community outbreak involving eight cases in the North region.

The ST44, found in five strains, was the profile with the highest number of clinical unrelated isolates. All of them were recovered from community-acquired infections. This ST was also associated with the community

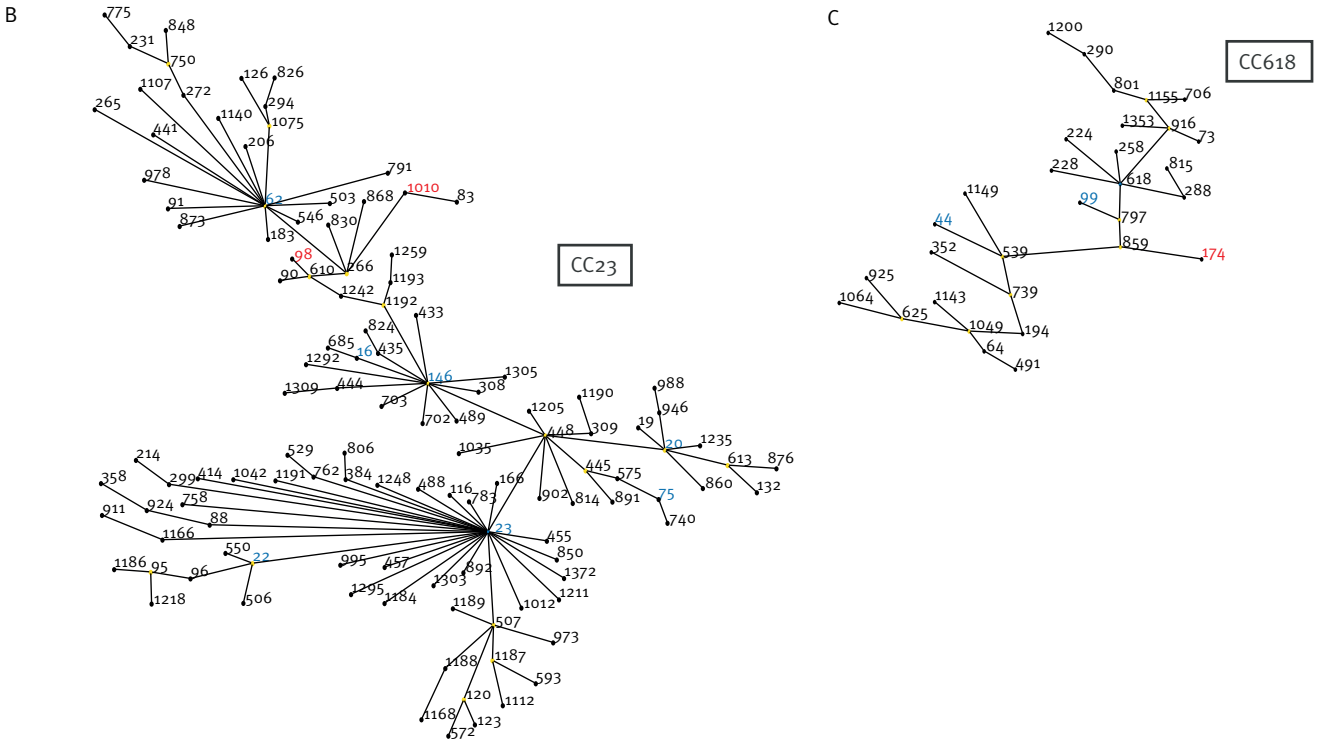
Figure 2

eBURST analysis of *Legionella pneumophila* sequence types obtained from clinical isolates, Portugal, 1987–2012

A. Relationships between the 30 *L. pneumophila* sequence types observed in Portugal during the study



B, C. Two of the 24 clonal complexes obtained by applying the eBURST algorithm on 1,451 sequence types reported to the EWGLI-SBT database



CC: clonal complex; EWGLI-SBT: European Working Group for Legionella Infections-sequence-based typing.

In red were the STs unique to Portugal and in blue the STs from Portugal, which can also be found in other countries. Full lines link single-locus variants and broken lines link double-locus variants. Blue spots represent founders and yellow spots represent sub-founders.

outbreak in 2012, in the North region, from which two isolates were analysed here.

The ST62 and ST99 were found in four clinical isolates each. Two of the ST62 strains were associated with nosocomial infections, one recovered from a single case in hospital A, in 1990; and the other was associated with a small outbreak, involving three cases, in hospital B, in 2011. Two other STs, ST172 and ST1384, were also detected in single isolates from hospital A. The ST99 was retrieved, between 2000 and 2009, from community-acquired infections in the North region, Centre and Algarve.

The ST1 was found only twice among our strains, and both isolates were obtained from sporadic cases in 1995, in the Lisbon region.

The combination of the two methodologies, MAbs and SBT, showed that the Philadelphia subgroup was the most heterogeneous as it was divided into 14 different STs (Figure 1).

Evolutionary relationships among isolates

The 30 STs generated in this study were subdivided by eBURST into six groups (Gp) and 13 singletons using the less stringent definition (Figure 2A). The six groups include 17 STs and 38 of the 55 unrelated clinical isolates studied. The Gp146 was the most representative

group, containing five STs (ST16, ST20, ST22, ST23 and ST146) and 11 isolates, all of them recovered from sporadic cases of community-acquired infections. The ST146 was the hypothetic founder of the group, from which derived one single-locus variant (SLV) and three double-locus variants (DLVs). The ST146 comprised only two strains, isolated in 2007 and 2008, in the North region. The Gp1 is composed by ST1, the predicted founder of the group, from which derived two SLVs, ST103 and ST172. All of the six strains included in this group were isolated in the Lisbon region between 1991 and 1999.

All but two of the 13 singletons STs were represented by a single strain each; the ST42 and ST98 were associated with three strains each.

According to the results of our eBURST analysis, six of the twelve STs unique to Portugal were singletons (ST98, ST100, ST102, ST785, ST1009 and ST1343). Of the remaining STs found exclusively in our country, the ST101 and ST173 were related, diverging only in *neuA*. The ST1383 and ST1384 were slightly more distant from each other, being DLV. The ST174 was a DLV with ST44 and ST99, which were the STs with highest number of clinical unrelated isolates in this study.

Using the eBURST algorithm on the entire EWGLI-SBT database, 24 clonal complexes (CC) were generated. These CCs were obtained with the most stringent definition, i.e. all the STs are SLVs. With this method, 24 of the STs from our country were assigned into eight of these CC. The other six STs cannot be linked with any other ST of the database, being singletons (ST100, ST436, ST785, ST1343, ST1383 and ST1384).

The CC59 (ST59 was the predicted founder with bootstrap support of 100%), included three STs identified in Portugal (ST101, ST173 and ST728). Five STs that circulated in our country (ST1, ST37, ST103, ST172 and ST1009) were associated to CC1. Two of these STs (ST37 and ST1009) were singletons in the first eBURST analysis. The CC23 included our ST62, ST1010, Gp146 (that enclosed ST16, ST20, ST22, ST23 and ST146), and two other STs (ST75 and ST98), that in the previous eBURST diagram were singletons (Figure 2A and Figure 2B). In this CC the ST62, ST146, and ST20, emerged as subfounders (bootstrap subgroup value of 100%, 98% and 79%, respectively). These STs diversified from the founder and generated their own SLVs (i.e. had at least two links to other STs previously unassigned, and the link to the progenitor). Two of the most represented STs in our collection (ST44 and ST99) were grouped in CC618 (Figure 2C).

An overview of the eBURST diagram using the entire EWGLI-SBT database showed that nine of 13 ST that were singletons in the first eBURST analysis were now associated with a CC; among these, it should be highlighted that the ST42 and ST94 were predicted founders of two CCs. Five of the 12 STs specific to Portugal were assigned as singletons in this analysis (ST100, ST785, ST1343, ST1383 and ST1384).

Discussion

In Portugal, like other countries, most of the diagnoses of LD are currently made by antigen detection in urine, due to the simplicity, rapidity and specificity of this test for *L. pneumophila* sg1. Culture is not a methodology widely used and, therefore, *Legionella* clinical isolates are not available for the majority of LD cases, which limits epidemiological studies [15,16]. Actually, this was observed in the current study, since only 41 clinical isolates were obtained from 868 cases reported to the integrated programme of epidemiological surveillance.

Another limitation of the present study is that our population of isolates represents the more severe cases of disease, as all isolates were from patients requiring hospitalisation. Therefore, the present report may not entirely reflect the distribution of the *Legionella* strains responsible for LD in Portugal, because cases with less severe disease are probably underrepresented in this collection. However, this study probably provides a good representation of the circulating STs in the country.

During the 25-year study period, we gathered 89 clinical isolates, 55 of them from unrelated cases. The majority of the clinical isolates (n=63) were obtained from men, which is in accordance with the literature [17-19].

As expected, the majority (n=84) of the clinical isolates were *L. pneumophila* sg1. Using the MAbs of Dresden panel all but one of the strains possessed virulence-associated epitope recognised by MAb3/1; this is in line with previous reports suggesting that these strains are more likely to cause disease than the others not exhibiting this phenotype [6,17]. In our study, the two major subgroups were Philadelphia and Allentown/France. These two MAb patterns only differ in the MAb8/4 reactivity and we had verified that, using indirect immunofluorescence in some strains, the MAb8/4 staining was heterogenic, with only 1 to 2% of the bacteria showing a strong positive signal; in these cases, we classified them as Allentown/France.

The SBT results showed significant genetic diversity, which is in accordance with reports from other countries. The diversity of clinical isolates in our study (0.972) was similar to that respectively described previously in Japan (0.979) [17], Canada (0.964) [20], England and Wales (0.901) [21], and slightly greater than in Belgium (0.879) [18]. As in other studies, *mompS* proved to be the most discriminating locus [22].

In this study, 12 of the 30 STs detected were new to the EWGLI-SBT database. These results suggest that the population of *L. pneumophila* clinical strains circulating

in Portugal, during the last 25 years, was a combination of worldwide and local strains.

The STs of the 89 isolates analysed were uploaded to the EWGLI-SBT database, which allows comparison between countries. Over the study period, the ST44 was linked with the highest number of Portuguese clinical unrelated isolates. According to the EWGLI-SBT database, the ST44 has repeatedly been associated with clinical isolates from France, Germany, Italy, and United Kingdom, with the first one recovered in 1994. The other major STs were ST62 and ST99, both with four clinical isolates. The EWGLI-SBT data show that ST62 is found ubiquitously across Europe but ST99 was isolated only once in the Brussels region, in 2008.

The ST1 appears in the EWGLI-SBT database as the profile most frequently reported around the world, with 1,132 of the 8,300 strains reported. Interestingly, one of the strains associated with this profile was isolated in 1947, in Washington, from a patient with pneumonia during an outbreak of unknown aetiology. The reanalysis of this unclassified agent in 1979, by McDade, showed that it was the same species as the LD bacterium [23].

This report provides the evolutionary relationships among the *L. pneumophila* clinical isolates collected in Portugal during the period from 1987 to 2012 by using the eBURST algorithm. This algorithm uses STs to divide bacterial populations into groups of closely related strains based on the theory that a genotype within a population starts diversifying, by mutation or recombination, to produce variants that differ in only one or two of the seven loci.

In the eBURST analysis of STs detected in this study, the less stringent definition for groups was chosen due to the small number of isolates tested (n=89). The data generated indicate that 13 STs do not relate with any other ST, and for the remaining, nine were SLVs and 11 were DLVs. Thus, the STs involved in these links are considered to have diverged very recently.

In the diagram drawn with the 1,451 STs reported to the EWGLI-SBT database, eBURST identified four of the STs detected in Portugal (ST1, ST23, ST42 and ST94) as founder genotypes of CCs. Another six STs (ST20, ST22, ST37, ST62, ST146 and ST172) were also predicted as subgroup founders. This means that from these STs many other genotypes have diversified.

In conclusion, we applied SBT and the eBURST algorithm to examine the genetic diversity and evolutionary relationships among the *L. pneumophila* clinical isolates collected in Portugal during the period from 1987 to 2012. This study provides information about the strains circulating in Portugal and their relationship with strains from other countries. However, additional research is required in order to improve the knowledge of the geographic distribution of virulent clones.

Acknowledgments

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Conflicts of interest

None declared.

Authors's contribution

Maria-Jesus Chasqueira: designed the study and the analytical strategy, final approval of the version. Lúcia Rodrigues: implementation of molecular biology technique; interpretation of data; critical review of the manuscript; final approval of the version. Marta Nascimento: implementation of phenotypic technique; final approval of the version. Marina Ramos: analysis of data from the integrated programme of epidemiological surveillance; final approval of the version. Teresa Marques: critical review of the manuscript; final approval of the version.

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ECDC publishes 2013 surveillance data on antimicrobial resistance and antimicrobial consumption in Europe

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On the occasion of the European Antibiotic Awareness Day (EEAD) on 18 November 2014, the European Centre for Disease Prevention and Control (ECDC) has released 2013 data on antimicrobial resistance and antimicrobial consumption in Europe.

The ECDC collects data on antimicrobial resistance and antimicrobial consumption from 30 European Union (EU) and European Economic Area (EAA) countries through the European Antimicrobial Resistance Surveillance Network (EARS-Net) and the European Surveillance of Antimicrobial Consumption Network (ESAC-Net). Surveillance data for 2013 are provided through openly accessible online interactive databases [1,2], in summaries supporting the EAAD event [3] and, for EARS-Net, in the annual report on antimicrobial resistance surveillance in Europe [4].

EARS-Net data for 2013 confirmed the high percentages of invasive isolates of *Klebsiella pneumoniae* and *Escherichia coli* with resistance to third-generation cephalosporins that were reported in the previous three years. For *K. pneumoniae*, EARS-Net observed an increase, during the period 2010–2013, of the EU/EEA population-weighted mean percentage of isolates showing combined resistance to three major classes of antibiotics and of isolates with resistance to carbapenems.

ESAC-Net data for 2013 showed that overall consumption of antibiotics (ATC group J01, antibacterials for systemic use) in the hospital sector remained stable in the EU/EEA. However, the EU/EEA population-weighted mean consumption of carbapenems and of polymyxins both increased during 2009–2013. This increase may possibly reflect overuse, or may simply indicate an increased clinical need for these antibiotics in hospital settings because of an increasing prevalence of infections with multidrug-resistant Gram-negative bacteria in Europe.

For the first time, EARS-Net reported data on polymyxin resistance, showing the presence of such resistance in all Gram-negative bacteria under surveillance, especially in countries with already high levels of carbapenem resistance. This new, worrisome development means that, for patients with serious infections with such carbapenem- and polymyxin-resistant Gram-negative pathogens, only few alternatives remain available for appropriate antimicrobial treatment.

Antimicrobial resistance, especially to last-line antibiotics, is a serious threat to public health and patient safety in Europe. It would be worthwhile to link antimicrobial consumption data from ESAC-Net with antimicrobial resistance data from EARS-Net in order to gain a better understanding of the dynamic relationship between these parameters in the hospital sector in Europe. A prerequisite for such an integrated analysis would require a hospital-based reporting of antimicrobial consumption to ESAC-Net as well as use of a unique, though confidential, hospital identifier for reporting data to both EARS-Net and ESAC-Net.

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