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Note from the editors: A busy and eventful year has passed

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Last year was a busy time for us: a new influenza virus emerged and the Middle East Respiratory Syndrome coronavirus (MERS-CoV) spread further, poliovirus was identified in sewage in Israel and human cases of polio occurred in Syria. While we followed these events, the number of submissions to *Eurosurveillance* increased further, we implemented an electronic submission system and watched closely new developments in scientific publishing and the debate about the quality of open access journals.

In terms of infectious diseases, much international focus in 2013 was on viral diseases. One example is the avian influenza A(H7N9) virus that crossed the species barrier and since its detection in early 2013, had infected a total of 137 patients including 45 fatalities as of October 2013 [1]. Another example is the MERS-CoV. Cases had already emerged in 2012 but numbers rose considerably in 2013, reaching 176 cases including 74 fatalities as of 31 December 2013 [2]. While sustained human-to-human transmission has not been documented for these two viruses to date, both have created much interest among experts because of the severity of the disease, high case fatality rates, and the possible pandemic potential of the H7N9 influenza virus [3]. This interest is reflected in several hundreds of peer-reviewed publications indexed in Medline and Scopus already by the end of 2013; however, many questions remain unanswered.

Eurosurveillance has contributed to the wealth of growing evidence about influenza A(H7N9) and MERS-CoV with 13 and eight timely articles, respectively. The first article presenting the genetic analysis of the novel avian A(H7N9) influenza viruses and discussing its pandemic potential [3] was published on 11 April together with an editorial [4], shortly after the Chinese authorities had notified the occurrence of the new virus to the World Health Organization [5,6]. Two timely papers on MERS-CoV provided evidence about the infection of camels in the affected region with a MERS-like CoV [7,8] and indications that camels could play a role in the transmission of the disease. Conclusive evidence for this is, however, still missing.

From a European perspective, is also worth highlighting the occurrence in several European countries and in a similar time period, of hepatitis A cases that belonged to different multinational outbreaks caused by different strains of the virus. Investigations using traditional and molecular epidemiological methods including trace-back did not only identify berries as a new vehicle for hepatitis A virus infections but also led to highly relevant practical prevention messages and measures. These investigations were facilitated by the European Centre for Disease Prevention and Control (ECDC) and the European Food Safety Authority, and *Eurosurveillance* covered the outbreaks in several rapid communications from some of the affected countries [9-12].

Two special issues in 2013 highlighted the leishmaniasis situation in Europe and the molecular epidemiology of human pathogens – current use and future prospects.

The number of submissions to *Eurosurveillance* has increased further compared with previous years: overall in 2013, we received 872 papers, of which 257 were rapid communications, 589 regular articles, and 26 included editorials, letters and meeting reports. We accepted and published on average one of two submitted rapid communications that were within the scope of the journal and one of eight submitted regular articles. The total number of items published amounted to 249, of which 85 were rapid communications, 117 regular articles and 47 others (editorials, letters and news items). We received submissions from some 60 different countries including many non-European countries; among those, submissions from China (n=65) dominated by far. We had to reject many papers from countries outside Europe even if they were of good quality, because they did not fall into our main geographical focus on infectious disease surveillance, prevention and control with public health relevance in Europe.

Selecting papers carefully to avoid double publication and pick out the most interesting ones among the many submissions on both topics has been a challenge. We are grateful for the continued guidance and support,

often on short notice, we receive from our associate editors, editorial advisors as well as many of our colleagues at ECDC and expert friends who we are unable to name individually here. We were also greatly aided by some 500 often enthusiastic reviewers who dedicate their time and energy to providing us with helpful comments. To acknowledge their work, a list with their names is published in this issue [13]. We would also like to express our thanks to our readers and contributors for their interest in the journal and confidence in us. Last but not least, the editorial team would like to thank our publisher, ECDC, and its Director for granting us editorial freedom, trusting us, and providing sustained financial and logistic support to the journal.

The electronic submission system, set up to improve our workflows and transparency, seems to work well for authors and reviewers and we feel it has proved helpful. Although the automatised workflows put us at a slightly greater distance from authors and reviewers, we continue to enjoy a close personal interaction with all those involved throughout the process. In addition, we have since mid-2013 been submitting document identifiers (DOIs) for our articles to CrossRef to provide better services for our audiences. The planned development of our website is still in progress, and the work on technical improvements of the site will continue in 2014.

The widely accepted metrics for scientific journals that were released in mid-2013 have confirmed the placement of *Eurosurveillance* among the top 10 journals in its category [14]. We strive to remain attractive for our audience also in 2014, through publication of timely relevant papers on infectious disease events that require public health action in Europe and beyond. The rapid communications and timely provision of information will remain a particular feature of the journal in 2014. At the same time, we will attempt to shorten the turnaround time for regular papers. To tie in with recent events, a special issue focussing on poliomyelitis is in preparation. As in the past, we look forward to the collaboration of our supporters to help us reach our goals and jointly contribute our part towards prevention and control of infectious diseases.

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A community cluster of influenza A(H1N1)pdm09 virus exhibiting cross-resistance to oseltamivir and peramivir in Japan, November to December 2013

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Six influenza A(H1N1)pdm09 viruses were detected in Sapporo, Japan, between November and December 2013. All six viruses possessed an H275Y substitution in the neuraminidase protein, which confers cross-resistance to oseltamivir and peramivir. No epidemiological link among the six cases could be identified; none of them had received neuraminidase inhibitors before specimen collection. The haemagglutinin and neuraminidase genes of the six viruses were closely related to one another, suggesting clonal spread of a single resistant virus.

Detection of mutant H275Y influenza A(H1N1)pdm09 viruses

Between September and December 2013, 76 influenza A(H1N1)pdm09 viruses were detected in 20 local public health institutes in Japan and then screened by allelic discrimination [1] and/or neuraminidase (NA) gene sequencing to detect an H275Y substitution, which confers resistance to oseltamivir and peramivir [2] (Figure 1). This is part of our nationwide monitoring for antiviral-resistant influenza viruses in cooperation with 74 local public health institutes [2]: such surveillance is important for public health planning and clinical recommendations for antiviral use. We found that seven of the 76 influenza A(H1N1)pdm09 viruses possessed the H275Y substitution. Six of the seven H275Y mutant viruses were detected in Sapporo, the capital city of Hokkaido, the second-largest island in Japan. The seventh case was detected from another part of the country. In Sapporo, six influenza A(H1N1)pdm09 viruses were detected during weeks 46–50, and all six viruses possessed the H275Y substitution. Elsewhere in Hokkaido, nine influenza viruses were detected: all were influenza A(H3N2) viruses. In this article, we focus on the analysis of the six H275Y mutant viruses detected in Sapporo.

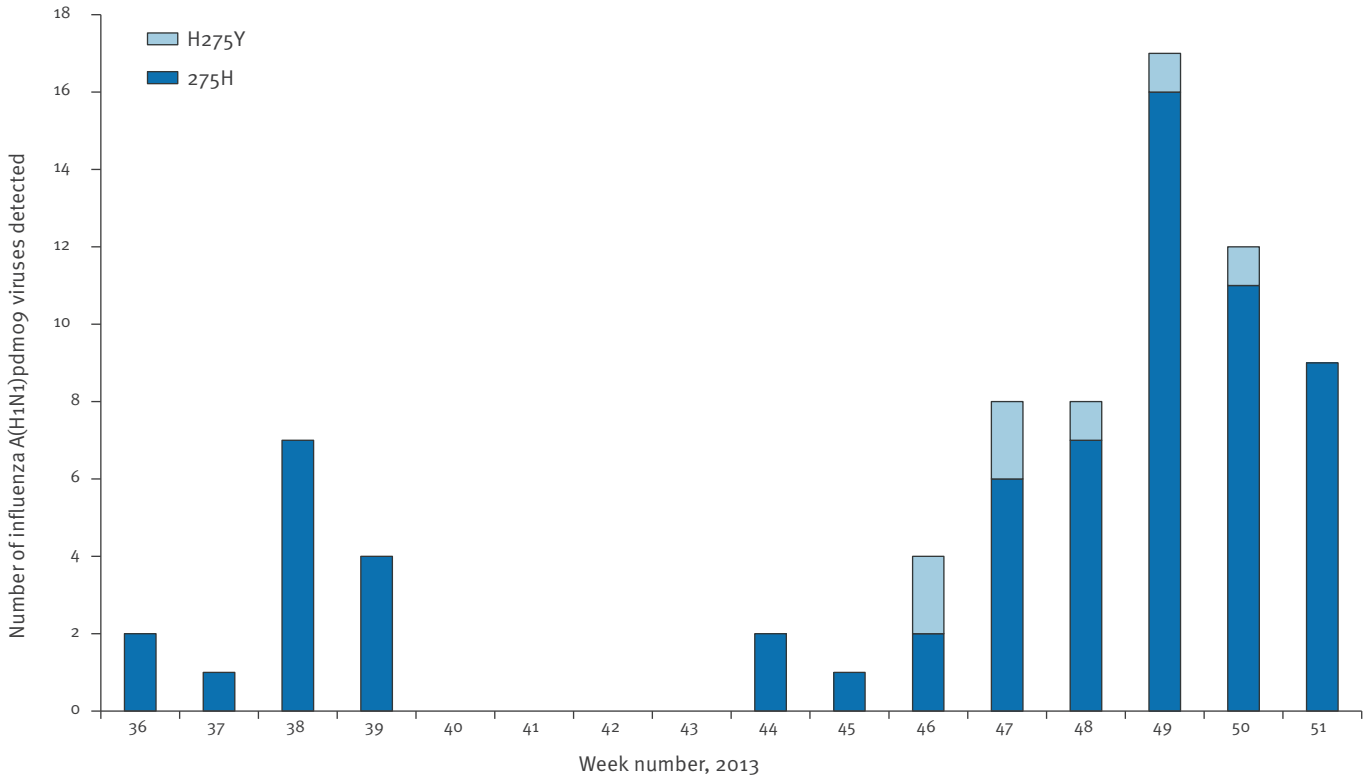
Isolate details from the six cases in Sapporo are shown in Table 1. Clinical specimens of the patients were collected in three paediatric clinics (serving outpatients only) and two general hospitals (serving outpatients and inpatients). Five of the six patients were male and four were children (aged up to 10 years). Five male patients showed mild symptoms and received only outpatient care, but a woman in her late 30s without underlying disease was hospitalised for severe pneumonia. She was admitted to an intensive-care unit because of acute respiratory distress syndrome and is currently in critical condition. All six cases occurred sporadically and no epidemiological link among them could be identified. None of them had received NA inhibitors before specimen collection. The nucleotide sequences of the haemagglutinin (HA) and NA genes of the six viruses were closely related to one another (Figure 2). These results suggest the clonal spread of a single H275Y mutant virus in Sapporo.

Antiviral susceptibility of H275Y mutant viruses

We analysed the susceptibility of five of the six H275Y mutant viruses detected in Sapporo to four NA inhibitors approved in Japan: oseltamivir, peramivir, zanamivir and laninamivir (Table 2); the sixth virus could not be cultured. A/Perth/261/2009 and A/Perth/265/2009 [3] were used as reference H275Y mutant and 275H wild-type A(H1N1)pdm09 viruses, respectively. Oseltamivir carboxylate, peramivir and zanamivir were purchased from Sequoia Research Products (Pangbourne, United Kingdom) and laninamivir was kindly provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). The susceptibility of these viruses to NA inhibitors was determined by fluorescent enzyme inhibition assay with the NA-Fluor Influenza Neuraminidase Assay Kit (Applied Biosystems, California, United States). Results were expressed as the drug concentrations required to inhibit NA activity by 50% (IC_{50}). The IC_{50} values were calculated using MikroWin 2000 software (Mikrotek Laborsysteme GmbH, Overath, Germany). To interpret the NA inhibitor susceptibility, the World Health

Figure 1

Detection of influenza A(H1N1)pdm09 viruses with H275Y substitution, September–December (weeks 36–51)[a], Japan (n=76)



Weekly reports of influenza A(H1N1)pdm09 virus isolation/detection by local public health institutes under the National Epidemiological Surveillance of Infectious Diseases.

[a] Week 36 started on 2 September 2013.

Organization criteria based on the fold change of IC_{50} values compared with reference IC_{50} values were applied [4]. For influenza A viruses, normal (<10-fold increase), reduced (10–100-fold increase) or highly reduced (>100-fold increase) inhibition were defined. All five H275Y mutant viruses showed more than 600- and 170-fold increased IC_{50} values to oseltamivir and peramivir, respectively, compared with the 275H reference virus. However, the IC_{50} values of the H275Y mutants to zanamivir and laninamivir were comparable to those of the 275H reference virus. These results indicate that the five H275Y mutant viruses tested exhibit highly reduced inhibition by oseltamivir and peramivir, but remain fully susceptible to zanamivir and laninamivir.

In the United States, the Centers for Disease Control and Prevention reported that 10 (1.3%) of 768 influenza A(H1N1)pdm09 viruses were resistant to oseltamivir in the 2013/14 season, as of week 51 2013 [5]. Five of the 10 resistant viruses were detected in Louisiana and Mississippi (Table 3), suggesting a cluster of resistant viruses. The largest cluster of influenza A(H1N1)pdm09 viruses with the H275Y substitution occurred in Newcastle, Australia, in 2011: 29 (15%) of 191 influenza A(H1N1)pdm09 viruses possessed the H275Y substitution [6].

For comparison with the six H275Y mutant viruses detected in Sapporo, HA and NA gene sequences of the H275Y mutant viruses isolated in the United States and Australia were downloaded from the EpiFlu database of the Global Initiative on Sharing All Influenza Data (GISAID) (Table 3). The HA and NA genes of the

Table 1

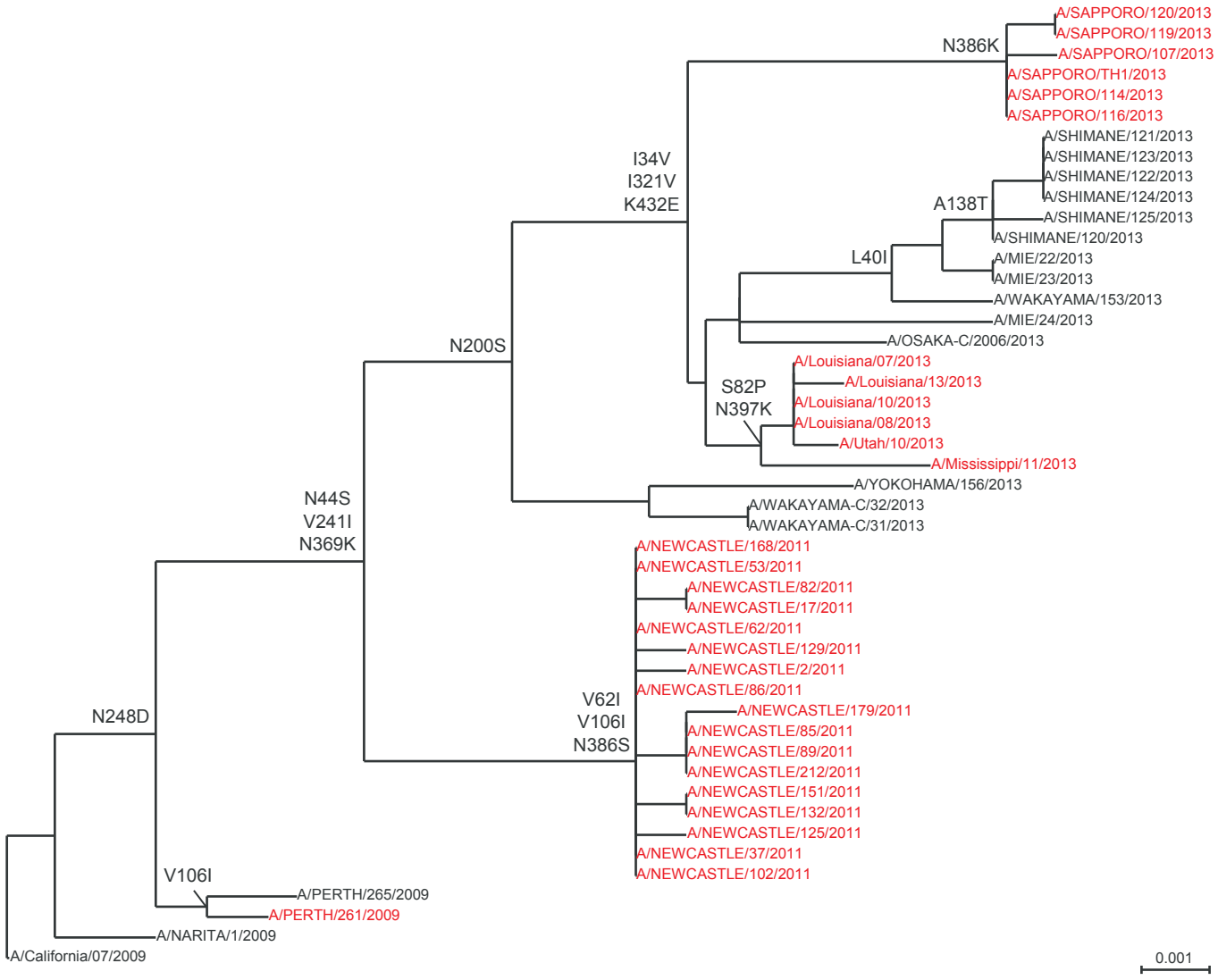
Influenza A(H1N1)pdm09 viruses with H275Y substitution detected in Sapporo, Japan, November–December 2013 (n=6)

GISAID isolate ID	Isolate name	Collection date
EPI_ISL_152910	A/Sapporo/107/2013	2013-11-15
EPI_ISL_152927	A/Sapporo/114/2013	2013-11-24
EPI_ISL_152931	A/Sapporo/TH1/2013	2013-11-25
EPI_ISL_152928	A/Sapporo/116/2013	2013-11-26
EPI_ISL_152929	A/Sapporo/119/2013	2013-12-07
EPI_ISL_152930	A/Sapporo/120/2013	2013-12-09

GISAID: Global Initiative on Sharing All Influenza Data.

Figure 2

Phylogenetic analysis of the neuraminidase gene of the six H275Y mutant influenza A(H1N1)pdm09 viruses isolated in Sapporo, Japan, and the United States in 2013 and in Australia in 2011



Multiple alignment was constructed using the CLUSTAL W algorithm. The tree was constructed using the neighbor-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. The H275Y mutant viruses are shown in red. Amino acid substitutions relative to the A/California/07/2009 virus are shown on the left of the nodes. The gene sequences of the H275Y mutant viruses isolated in the United States and Australia were downloaded from the EpiFlu database of the Global Initiative on Sharing All Influenza Data (GISAID).

Table 2

Susceptibility of five influenza A(H1N1)pdm09 viruses with H275Y substitution to neuraminidase inhibitors, detected in Sapporo, Japan, November–December 2013

Isolate name	NA substitution	IC_{50} (nM)			
		Oseltamivir	Peramivir	Zanamivir	Laninamivir
A/SAPPORO/107/2013	H275Y	240.60	35.28	0.50	0.81
A/SAPPORO/114/2013	H275Y	193.05	22.86	0.50	0.63
A/SAPPORO/116/2013	H275Y	257.10	23.97	0.43	0.53
A/SAPPORO/119/2013	H275Y	189.25	23.19	0.43	0.58
A/SAPPORO/120/2013	H275Y	192.44	22.35	0.45	0.54
Reference isolates[a]					
A/PERTH/261/2009	H275Y	257.88	34.30	0.30	0.35
A/PERTH/265/2009	275H	0.31	0.13	0.30	0.29

IC_{50}: drug concentrations required to inhibit NA activity by 50%; NA: neuraminidase.

[a] A/PERTH/261/2009 is the H275Y reference virus. A/PERTH/265/2009 is the 275H wild-type influenza A(H1N1)pdm09 reference virus.

Table 3

Accession numbers of the H275Y mutant influenza A(H1N1)pdm09 viruses detected in the United States in 2013 and in Australia in 2011

GISAID Isolate ID	Isolate name	Collection date	Originating laboratory	Submitting laboratory
EPI_ISL_150042	A/Louisiana/07/2013	2013-10-07	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention
EPI_ISL_150043	A/Louisiana/08/2013	2013-10-09	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention
EPI_ISL_150298	A/Louisiana/10/2013	2013-10-21	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention
EPI_ISL_151839	A/Mississippi/11/2013	2013-11-04	Mississippi Public Health Laboratory	Centers for Disease Control and Prevention
EPI_ISL_151838	A/Louisiana/13/2013	2013-11-11	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention
EPI_ISL_95596	A/Newcastle/2/2011	2011-05-31	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95595	A/Newcastle/17/2011	2011-06-20	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95597	A/Newcastle/37/2011	2011-06-23	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101526	A/Newcastle/125/2011	2011-06-30	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95598	A/Newcastle/53/2011	2011-06-30	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95600	A/Newcastle/82/2011	2011-07-01	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95599	A/Newcastle/62/2011	2011-07-01	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101511	A/Newcastle/86/2011	2011-07-02	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101527	A/Newcastle/129/2011	2011-07-03	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95602	A/Newcastle/89/2011	2011-07-04	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95601	A/Newcastle/85/2011	2011-07-04	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101525	A/Newcastle/102/2011	2011-07-05	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95593	A/Newcastle/132/2011	2011-07-10	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95594	A/Newcastle/151/2011	2011-07-11	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101540	A/Newcastle/168/2011	2011-07-12	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101564	A/Newcastle/179/2011	2011-07-15	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101565	A/Newcastle/212/2011	2011-08-02	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza

GISAID: Global Initiative on Sharing All Influenza Data.

H275Y mutant viruses in Sapporo, the United States and Australia were distinct from one another (Table 4).

Discussion

During the 2007/08 influenza season, an oseltamivir-resistant former seasonal influenza A(H1N1) virus emerged in Europe and became the majority of A(H1N1) viruses within a year [7]. This oseltamivir-resistant A(H1N1) virus possessed the H275Y substitution; some additional amino acid substitutions were also reported for the virus that could make the mutant virus biologically stable [8,9]. Since the H275Y substitution would destabilise the mutant virus, the oseltamivir-resistant A(H1N1) virus probably acquired the capacity for efficient human-to-human transmission through these stabilising substitutions.

Table 4

Characteristic amino acids of H275Y mutant influenza A(H1N1)pdm09 viruses isolated in Sapporo, Japan, in 2013 (n=6) and the United States in 2013 and in Australia in 2011

HA gene of isolated viruses	Amino acid position										
	15	97	112	143	163	197	222	223	256	283	499
Reference virus A/PERTH/265/2009	T	D	E	S	K	A	D	Q	A	K	E
Sapporo, Japan, 2013	S	N	E	S	Q	A	D	Q	T	D	K
United States, 2013	T	N	E	S	Q	A	D	Q	T	E	K
Australia, 2011	T	D	K	G	K	T	D	Q	A	K	E

NA gene of isolated viruses	Amino acid position										
	34	62	82	106	200	241	275	321	369	386	397	432
Reference virus A/PERTH/265/2009	I	V	S	I	N	V	H	I	N	N	N	K
Sapporo, Japan, 2013	V	V	S	V	S	I	Y	V	K	K	N	E
United States, 2013	V	V	P	V	S	I	Y	V	K	N	K	E
Australia, 2011	I	I	S	I	N	I	Y	I	K	S	N	K

HA: haemagglutinin; NA: neuraminidase.

In the case of H275Y mutants of influenza A(H1N1) pdm09 viruses, three substitutions (V241I, N369K and N386S) in the NA protein may offset the destabilising effect of the H275Y mutation [6]. The influenza A(H1N1)pdm09 virus that appeared in 2009 as a pandemic virus had none of these stabilising substitutions, whereas the A(H1N1)pdm09 viruses that have been circulating since 2011 to date have acquired two of the three substitutions. The H275Y mutant viruses detected in a community cluster in 2011 in Newcastle, Australia, contained these three substitutions [6]. Furthermore, the same substitutions were detected in H275Y mutant viruses isolated from Dutch travellers returning from Spain in 2012 [10]. In our study, all H275Y mutant viruses detected in Sapporo possessed V241I, N369K and N386K substitutions (Table 4); H275Y mutant viruses found in the United States possessed only V241I and N369K substitutions (Table 4). Before the 2013/14 influenza season, we had not detected any H275Y mutant viruses with V241I, N369K and N386K substitutions in Japan. The effect of the N386K substitution – at the same position but with an amino acid residue that differs from N386S previously reported for H275Y mutant viruses – remains to be clarified.

D222G and Q223R substitutions in the HA protein of influenza A(H1N1)pdm09 viruses are known to cause a switch in receptor-binding preference from human-type α-2,6 to avian-type α-2,3 sialic acid [11-13]. All H275Y mutant viruses detected in Sapporo and the United States in the 2013/14 season did not contain these substitutions that would be associated with increased pathogenicity (Table 4). The reason why the patient in her late 30s in Sapporo developed severe pneumonia has yet to be studied.

It has been shown that oseltamivir-resistant influenza A(H1N1) virus infection reduced the effectiveness of oseltamivir and this tendency was more apparent in children 0 to 6 years old [14-16]. Among patients from whom oseltamivir- and peramivir-resistant A(H1N1) pdm09 viruses have been detected in Japan, the percentage with no known exposure to NA inhibitors has increased significantly, from 16% during the pandemic period to 44% during the post-pandemic period [2]. These observations may suggest that human-to-human transmission with H275Y mutant viruses has increased gradually in the post-pandemic period. Consequently, surveillance of antiviral-resistant influenza viruses should be continued and strengthened, particularly for the choice of antiviral drugs.

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Conflict of interest

None declared.

Authors' contributions

Designed the analyses: ET, AO, HN, TO, MT. Analysed and interpreted data: ET, ME, RI, MM, AO, HN, TO, MT. Drafted the article: ET. Revised the article: TO, MT.

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An outbreak of *Salmonella* Newport associated with mung bean sprouts in Germany and the Netherlands, October to November 2011

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The largest *Salmonella enterica* serovar Newport outbreak (n=106) ever reported in Germany occurred in October and November 2011. Twenty associated cases were reported in the Netherlands. The outbreak investigation included an analytical epidemiological study, molecular typing of human and food isolates and food traceback investigations. Unspecified *Salmonella* had been detected in samples of mung bean sprouts at a sprout producer (producer A) in the Netherlands and mung bean sprouts contaminated with *S.* Newport had been found during routine sampling at a sprout distributor in Germany. Therefore, we tested the hypothesis of sprouts being the infection vehicle. In a case–control study, we compared 50 notified adult *S.* Newport cases with 45 *Salmonella enterica* serovar Enteritidis cases regarding their food consumption in the three days before illness. In multivariable logistic regression analysis, only sprout consumption was significantly associated with *S.* Newport infection (odds ratio: 18.4; 95% confidence interval: 2.2–150.2). Molecular typing patterns of human isolates were indistinguishable from a mung bean sprouts isolate. Traceback of sprouts led to distributors and producer A in the Netherlands. Since sprouts are frequently contaminated with microorganisms, consumers need to be aware that consumption of raw or insufficiently cooked sprouts may pose a health risk.

Introduction

Foodborne *Salmonella* infections are a significant public health problem in many countries, including Germany and the Netherlands. *Salmonella enterica* serovar Newport has been an uncommon cause of acute gastroenteritis in Germany with a mean of 113 notified cases per year in the time period 2001 to 2010. In 2010, a total of 25,310 cases of *Salmonella* infections were notified, of which only 83 (0.3%) were caused by *S.* Newport [1]. In comparison, 22 (1.5%) of the 1,466 reported *Salmonella* infections were *S.* Newport infections in the Netherlands in 2010. Twenty-four outbreaks of *S.* Newport were reported in Germany from 2001 to 2010. The number of respective outbreak-associated cases only ranged from two to nine. Notification data did not include information on possible sources of these outbreaks. *S.* Newport outbreaks in other European countries and the United States (US) were linked to the consumption of various food items such as ground beef [2], horse meat [3], cheese [4], tomatoes [5], lettuce [6,7], ready-to-eat salad vegetables [8] and alfalfa sprouts [9]. Mung bean sprouts were associated with outbreaks of *Salmonella* serovars other than Newport [10-13], but to our knowledge, mung bean sprouts have not been described as the infection vehicle in a *S.* Newport outbreak to date.

In Germany, in November 2011, the National Reference Centre for *Salmonella* and other bacterial enteric pathogens (NRC) at the Robert Koch Institute (RKI) observed an increase of *S.* Newport isolates originating from patients who had developed gastroenteric symptoms during a stay at a rehabilitation clinic in northern Germany, which indicated an outbreak situation. An increase of *S.* Newport isolates was also reported by the Institute of Hygiene and the Environment in Hamburg, a large diagnostic laboratory. Furthermore, analysis of

data from the national surveillance database of notifiable infectious diseases at the RKI revealed a substantial increase of notified *S.* Newport cases from an annual average of two or three cases per week in 2001 to 2010, to eight in week 43 and 39 in week 44 of 2011. By 21 November 2011, *S.* Newport infections had been recorded in 15 of the 16 federal states in Germany since the end of October 2011.

In the Dutch laboratory surveillance network for gastroenteric pathogens, 16 regional public health laboratories send *Salmonella* isolates from patients to the National Institute for Public Health and the Environment (RIVM) for confirmation and further typing [14]. This surveillance network was established in 1987, and has been estimated to cover approximately 64% of the population. An unusual increase of *S.* Newport isolates in October and November 2011 was noticed and communicated on 21 November 2011 via the Epidemic Intelligence Information System (EPIS), located at the European Centre for Disease Prevention and Control (ECDC). In addition, Germany and the Netherlands informed other European countries through the European Early Warning and Response System (EWRS) on 21 and 22 November 2011, respectively.

Mung bean sprouts were suspected as the vehicle in the outbreak because two lots of mung bean sprouts contaminated with an unspecified serovar of *Salmonella* had been found at sprout producer A in the Netherlands, sampled during the company's own testing of production batches on 18 and 21 October 2011, and *S.* Newport had been detected in mung bean sprouts taken during routine sampling at a sprout distributor in northern Germany on 19 October 2011. The mung bean sprouts originated from one of the contaminated lots produced in the Netherlands. Sprouts had been delivered to the distributor from producer A on 18 October 2011. These findings were notified to European food safety and public health authorities through the Rapid Alert System for Food and Feed (RASFF) of the European Commission on 17 November and, as a first follow-up, on 8 December 2011, roughly coinciding with the beginning of the outbreak investigation.

Here we describe the outbreak investigation launched to identify the source of the outbreak, including an epidemiological study, microbiological analyses and traceback investigations.

Methods

On 21 November 2011, the RKI, the national public health authority in Germany, was invited by state health authorities in one of the affected federal states to support the outbreak investigation. The outbreak investigation was coordinated by RKI and the Federal Institute for Risk Assessment (BfR) in close collaboration with the local and state human health and food safety authorities as well as the Federal Office of Consumer Protection and Food Safety (BVL). Information on the respective outbreak investigations was exchanged between RKI and RIVM. Traceback investigations were conducted by local and state food safety authorities and coordinated by the BfR on the federal level. The BVL cooperated with the corresponding Dutch Food Safety Authority (NVWA) to investigate supply chains in the Netherlands.

In the Netherlands, the RIVM requested the regional public health services to contact the cases within their region. Simultaneously, the NVWA was informed that an outbreak investigation was started. The RIVM and the NVWA exchanged information on the progress of the investigation on a regular basis.

Case definition of outbreak cases

In Germany, a case was defined as a laboratory-confirmed *S.* Newport infection notified to the public health authorities with at least one symptom of acute gastroenteritis (diarrhoea or stomach pain or vomiting or fever) and onset of symptoms between 20 October and 8 November 2011. Illnesses were not considered as outbreak-related if case patients reported travelling outside of Germany in the three days before onset of symptoms, or if molecular subtyping of the *S.* Newport isolate by pulsed-field gel electrophoresis (PFGE) revealed a pattern different from the outbreak strain. If the date of onset of symptoms was not available from notification data, it was estimated by subtracting from the reported notification date the average time interval between date of disease onset and date of notification available from notified cases with reported date of disease onset (11 days).

In the Netherlands, a case was defined as a *S.* Newport infection laboratory-confirmed at the RIVM in October or November 2011. Cases were excluded as outbreak cases, if PFGE revealed a pattern different from the outbreak strain and/or the case had been abroad in the seven days before disease onset.

Case–control study in Germany

We conducted a case–control study to test the hypothesis that consumption of mung bean sprouts was associated with illness. *S.* Newport case patients 18 years or older were compared with a control group of *S.* Enteritidis patients regarding frequency of exposure to suspected risk factors (case–case design). Controls were defined as laboratory-confirmed *S.* Enteritidis infections in adults (18 years or older) notified to the public health authorities with at least one symptom of acute gastroenteritis and onset of symptoms between 14 November and 11 December 2011. Controls were excluded if they reported travelling outside of Germany in the three days before onset of symptoms. If the date of onset of symptoms was missing from notification data, it was estimated based on *S.* Enteritidis controls with available data as described above for case patients (average time interval between disease onset and notification: 10 days).

Cases and controls were frequency-matched by age groups (18–31 years, 32–48 years, 49–88 years). All case patients and controls were recruited by local health authorities in their county of residence. Informed consent from participants was obtained through local health authorities before the interview. Staff from RKI and from state health authorities (Bavaria and Baden-Wuerttemberg) conducted the interviews by telephone using a standardised questionnaire. Questions referred to the three days before disease onset and were focused on the consumption of sprouts and food items that often contain mung bean sprouts, such as wok dishes, Asian rolls, and salads. Furthermore, the questionnaire queried about eating in restaurants offering Asian food or in other restaurants, about consumption of food items that were already known to be a potential source for *Salmonella* infections, such as raw pork, about symptoms, duration of illness, and basic demographics.

Case patient interviews in the Netherlands

Fifteen of 20 case patients were interviewed using a standardised questionnaire. This trawling questionnaire covered consumption of different meats, fish, dairy products, vegetables and fruits, snacks, establishments where food was purchased and contact with animals, in the seven days before onset of illness. Furthermore, information about the symptoms, onset of illness and hospitalisation was obtained. The Dutch case patients were not included in the case–control study.

Statistical analyses

Univariate and multivariable logistic regression analyses of data acquired for the case–control study were performed using Stata version 12 (Stata Corporation, College Station, US). Exposure-specific odds ratios (OR) and 95% confidence intervals (CI) were calculated. All exposure variables with a p value ‹0.1 in univariate analysis were included in the multivariable analysis. Regression models were built using forward elimination of variables with a cut-off p value of 0.05, and adjusted for age group and sex. Rank sum or t-tests were used for comparison of continuous variables.

As the investigation of the large outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 in Germany in 2011 showed that consumption of sprouts is difficult to remember [15], a variable was created to describe ‘probable sprout consumption’. This variable was defined as affirmed sprout consumption or having eaten in an Asian restaurant or having stayed in the rehabilitation clinic in northern Germany, where sprouts had been served at the salad bar.

In the Netherlands, the questionnaires were entered in Questback (Questback, Oslo, Norway). Frequency tables were generated using the export function. A variable for ‘probable sprout consumption’ was created by combining variables for reported affirmed and possible sprout consumption and reported meals that typically or possibly contained sprouts, for example Asian meals.

Microbiological methods

In Germany, for human isolates of *S.* Newport cases, the NRC performed PFGE using *Xba*I restriction enzyme following the PulseNet CDC-Protocol [16]. For comparison of PFGE patterns, additional *S.* Newport isolates were provided to the NRC by the Hamburg Institute for Hygiene and the Environment (human and animal isolates), by the National Reference Laboratory for *Salmonella* at the BfR (NRL-Salm) (isolates from turkey and mung bean sprouts) and by the Technical University of Denmark, Copenhagen (isolates from turkey meat originating from Germany). In addition, the NRL-Salm analysed 33 *S.* Newport isolates that had been isolated between 2009 and 2011 from food items (turkey and chicken), reptiles and other animal and environmental sources. Furthermore, human isolates, including the outbreak strain, were provided to the NRL by the NRC for comparison. The NRL-Salm performed *Xba*I-PFGE and an in-house multiple-locus variable number tandem repeat analysis (MLVA) method for *Salmonella enterica* isolates comprising the determination of eight repetitive loci. The MLVA method was performed by capillary electrophoresis according to Malorny et al. [17] using the following loci: STTR9, STTR5, STTR3, STTR11 [18,19] Salo2, Salo6, Sal20 [20] and SE-7 [21]. Antimicrobial susceptibility of strains was tested against 14 antimicrobial drugs or drug combinations by determining the minimum inhibitory concentration (MIC) using the Clinical and Laboratory Standards Institute’s broth micro dilution method [22] in combination with the semi-automatic Sensititre system (TREK Diagnostic Systems, Cleveland, US). Cut-off values (mg/L) to determine susceptibility to 10 antimicrobials were applied as described in the Commission Decision on a harmonised monitoring of antimicrobial resistance in poultry and pigs [23], namely cefotaxime (FOT, ›0.5), nalidixic acid (NAL, ›16), ciprofloxacin (CIP, ›0.06), ampicillin (AMP, ›4), tetracycline (TET, ›8), chloramphenicol (CHL, ›16), gentamicin (GEN, ›2), streptomycin (STR, ›32), trimethoprim (TMP, ›2) and sulfamethoxazole (SMX, ›256). Cut-off values for the remaining four antimicrobials were adopted from the European Committee on Antimicrobial Susceptibility Testing [24] namely colistin (COL, ›2), florfenicol (FFN, ›16), kanamycin (KAN, ›32) and ceftazidime (TAZ, ›2).

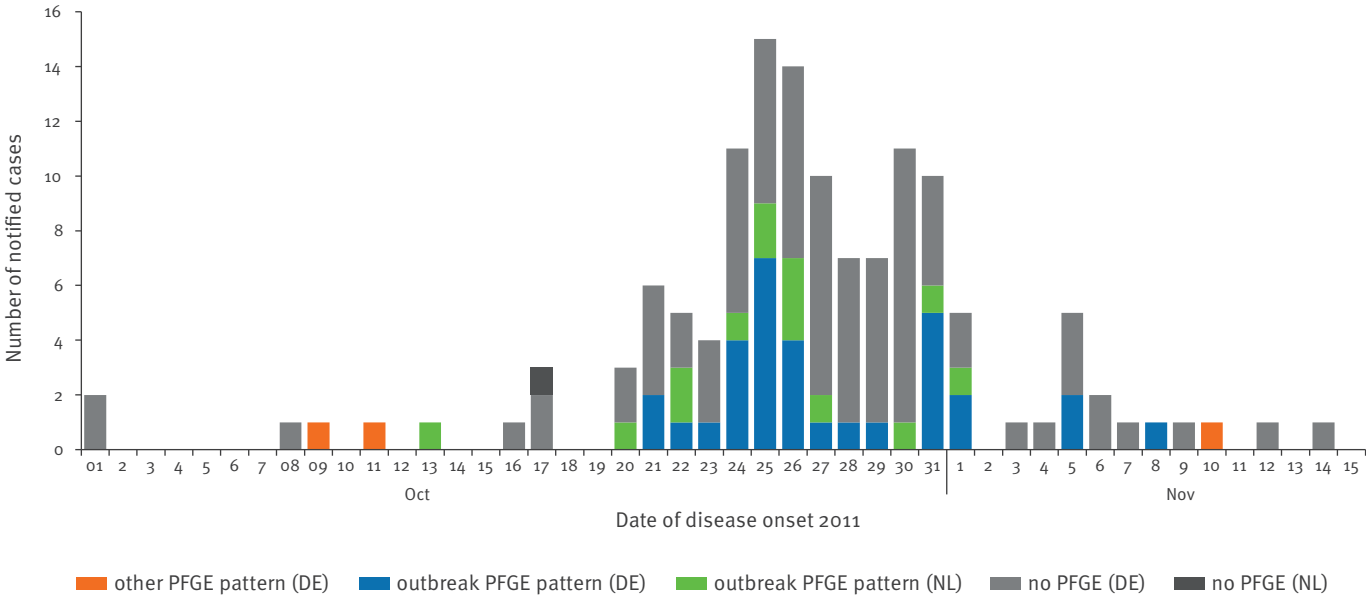
In the Netherlands, human isolates from *S.* Newport cases were compared using PFGE as described above.

Traceback and environmental investigations

Starting from the rehabilitation clinic and from Asian restaurants where *S.* Newport cases reported to have eaten in the three days before disease onset, mung bean sprouts were traced back. Traceback investigations were only initiated if the information provided by case patients regarding the restaurant and the date they had eaten there was considered sufficiently specific.

Figure 1

Notified *Salmonella* Newport cases by date of disease onset, Germany and the Netherlands, 1 October–15 November 2011 (n=133)



106 notified *S.* Newport cases were associated with the *S.* Newport outbreak in Germany (disease onset from 20 October– 08 November 2011, outbreak period in Germany). Twenty *S.* Newport cases were notified in the Netherlands, of whom 15 with disease onset from 13 October to 1 November 2011 (outbreak period in the Netherlands) were interviewed and are included in the epidemic curve.

Local food safety authorities also collected information in these restaurants on how mung bean sprouts were prepared and served. In the rehabilitation clinic in northern Germany, samples from leftover food items and stool samples from kitchen personnel were taken.

In the Netherlands, sprouts served in a hospital where two cases were hospitalised during the incubation period were traced back.

Results

Descriptive epidemiology

In total, the outbreak in Germany comprised 106 cases (Figure 1). Median age was 38 years (range: 0–91 years). Fifty-two per cent of cases were female. Hospitalisation due to *S.* Newport infection was reported for 28% of the cases. No deaths were reported.

In the Netherlands, a total of 20 outbreak-related *S.* Newport cases were reported. The onset of illness, known for 15 of the Dutch outbreak-related case patients, was between 13 October and 1 November 2011 (Figure 1). Median age was 37 years (range: 10–89 years), 15 cases were female. Two cases were already hospitalised when developing gastrointestinal symptoms, and for three cases no information on hospitalisation was available. Seven of the remaining 15 cases were hospitalised. Four of 15 interviewed case patients reported having eaten or possibly having eaten sprouts in the seven days before disease onset, and another nine case patients reported having eaten meals in which sprouts are typically used or could be used. For the remaining two case patients, no link to possible sprout consumption was found.

Case–control study in Germany

Fifty cases and 45 controls were included in the case–control study. The remaining 56 cases could not be contacted, were not willing to be interviewed, or did not meet the inclusion criteria for study participation because they were younger than 18 years or had travelled in the three days before disease onset. *S.* Newport case patients participating in the case–control study differed from non-participating adult case patients (n=47) with respect to age (median age 44 years versus 34 years) and sex (48% versus 51 % female). More than 60% of contacted *S.* Enteritidis cases agreed to participate in our study as control group, and contact data of 56% of these controls were forwarded to the RKI within two working days after the request was made to the local health authorities. The median time interval between disease onset and interview was 51 days (interquartile range (IQR): 48–53 days) for *S.* Newport case patients and 35 days (IQR: 24–42 days) for *S.* Enteritidis controls. Case patients were slightly younger than controls (median age: 44 years versus 50 years). Twenty-four of 50 case patients and 22 of 45 control patients were female. The most frequently reported symptoms in *S.* Newport case patients were

Table

Risk factors for infection: results of univariate and multivariable logistic regression analysis of case–control study, *Salmonella* Newport outbreak, Germany, 20 October–8 November 2011 (n=50 cases, 45 controls)

Exposure (Food items/restaurant visit)	Cases	Controls	Odds Ratio [95% CI]	p value[a]
	Exposed/total (%)	Exposed/total (%)		
Univariate				
Probable sprout consumption	21/50 (42)	1/45 (2)	31.9 [4.5–1,346]	<0.001
Eating out (Asian restaurant)	13/47 (28)	0/45 (0)	23.1 [3.6–∞][b]	<0.001
Affirmed sprout consumption	14/43 (33)	1/45 (2)	21.2 [2.9–918]	<0.001
Asian vegetables	8/47 (17)	0/43 (0)	11.7 [1.7–∞][b]	0.008
Turkey	16/41 (39)	5/39 (12)	4.4 [1.3–17.0]	0.008
Eating out (non-Asian restaurant)	34/49 (69)	19/45 (42)	3.1 [1.2–7.9]	0.008
Salad	12 /45 (27)	14/37 (38)	2.7 [1.0–7.1]	0.036
Ready-made sandwiches	8/50 (16)	9/45 (20)	2.3 [0.9–5.7]	0.049
Raw egg products	8/45 (18)	14/41 (34)	0.5 [0.1–1.3]	0.082
Multivariable model 1[c]				
Probable sprout consumption			34.6 [4.3–279]	0.001
Multivariable model 2[c]				
Affirmed sprout consumption			18.4 [2.2–150]	0.007

CI: confidence interval.

[a] Exposure variables with a p value <0.1 in univariate analysis were included in the multivariable analysis.
[b] Exact logistic regression.
[c] Controlled for age group and sex.

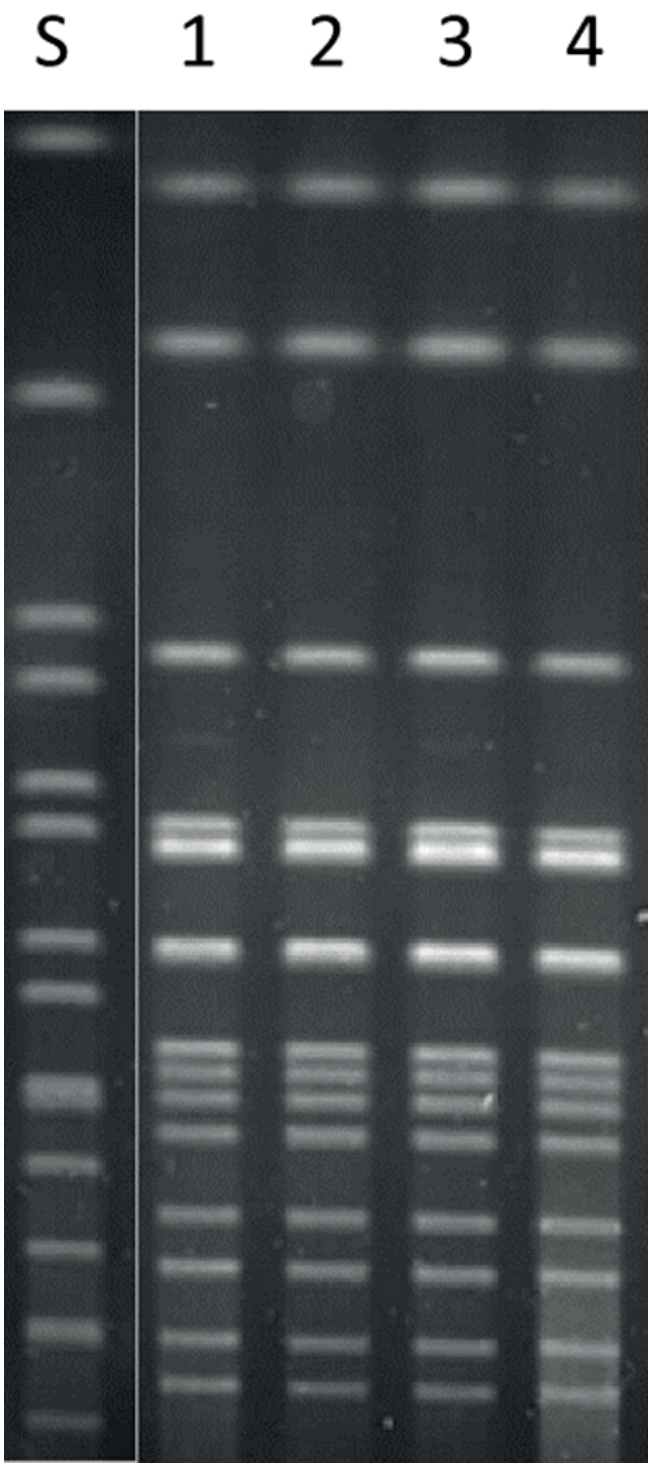
diarrhoea (50/50) and abdominal pain (34/50). Median duration of symptoms was six days (range: 1–28 days). Ten case patients and 15 controls reported hospitalisation associated with their *Salmonella* infection. Interviews with case-patients and controls showed that 14 of 43 case-patients that provided information on sprout consumption and only one person out of the 45 controls recalled having eaten sprouts in the three days before onset of symptoms. Of the 14 case-patients who recalled sprout consumption, eight could not name the kind of sprouts they had eaten, one named mung bean sprouts, four named soybean sprouts, and one named other sprouts. Ten case-patients remembered that the consumed sprouts had been long and white, the kind of sprouts typically served in Asian restaurants, and eight recalled that the sprouts had been raw (n=5) or only briefly heated (n=3). In univariate analysis, probable sprout consumption was associated strongest with *S.* Newport infection (Table). Statistically significant association with *S.* Newport disease was also found for eating in Asian restaurants, affirmed sprout consumption, consumption of Asian vegetables, consumption of turkey, eating in (non-Asian) restaurants, consumption of salad and ready-to-eat sandwiches (Table). In multivariable analysis, controlled for age group and sex, only probable sprout consumption and affirmed sprout consumption remained significantly associated with *S.* Newport infection (Table).

Microbiology

From the 106 *S.* Newport case patients attributed to the outbreak in Germany, 32 isolates were available for PFGE analysis at the NRC. All human isolates, of which 14 originated from patients included in the case–control study, showed an identical PFGE pattern. The PFGE pattern was indistinguishable from the pattern of the mung bean sprout isolate, which originated from a sample taken in October 2011 during routine food sampling at a distributor in northern Germany (Figure 2). This PFGE pattern had not been registered before in the NRC database which includes 230 *S.* Newport strains analysed in the years 2000 to 2011. An additional 33 *S.* Newport isolates from food items, animals, and environmental sources were analysed at the NRL-Salm at the BfR. With the exception of the mung bean sprout isolate, all of those showed PFGE and MLVA patterns that were different from the pattern of the outbreak strain. Outbreak isolates were susceptible to all 14 of the tested antimicrobial agents. Seventeen of the isolates that differed in PFGE and MLVA pattern from the outbreak strain were resistant to at least one antimicrobial agent.

Figure 2

Pulsed-field gel electrophoresis of XbaI-digested genomic DNA from the *Salmonella* Newport outbreak strain, Germany, 20 October- 8 November 2011



Lanes 1-3: human isolates from case patients; lane 4: mung bean sprout isolate; lane S: the PulseNet universal size standard *Salmonella enterica* serovar Braenderup H9812 strain.

In the Netherlands, 18 of the 20 case patients had an identical PFGE pattern and two were without PFGE confirmation. The PFGE pattern was indistinguishable from the PFGE pattern of isolates from the German case patients and the mung bean sprout isolate.

Traceback and environmental investigations

Sprouts served in the rehabilitation clinic in Northern Germany could be traced back via a distributor to producer A in the Netherlands. Six Asian restaurants where cases had eaten before falling ill had received mung bean sprouts from sprout producer A, some of them via several distributors.

The restaurants under investigation reported that sprout preparation varied from briefly heated (addition of sprouts to the dish shortly before serving it) to well cooked (sprouts cooked with the dish). At the rehabilitation clinic, sprouts had been served uncooked at the salad bar. All stool samples taken from kitchen staff and all retained food samples collected in the clinic tested negative for *S.* Newport. However, samples of the mung bean sprouts that had been served at the clinic's salad bar were not available for testing.

In the Netherlands, sprouts served in the affected hospital were traced back via several distributors to producer A.

Discussion

We describe the largest *S.* Newport outbreak in Germany reported to date, involving 106 cases, with an additional 20 cases in the Netherlands. We conclude that a single strain of *S.* Newport caused illness in the German and Dutch cases. Combined efforts of epidemiologists, microbiologists and food safety authorities identified contaminated mung bean sprouts as the source of the outbreak.

The case–control study revealed a strong and significant association between sprout consumption and *S.* Newport infection. Patients able to recall the type of sprouts they had eaten named mung bean or soybean sprouts, which are often confused with mung bean sprouts. Some patients were unable to name the type of sprouts, but their description of the consumed sprouts was consistent with mung bean sprouts. Case patients infected with *S.* Newport showing the outbreak PFGE pattern in Germany and the Netherlands fell ill between 13 October and 8 November 2011. The epidemic curve showed a distinct peak from 21 October to 5 November 2011, which suggested an infection vehicle that was in circulation only for a limited time period, consistent with a contaminated food item with a short shelf-life, such as mung bean sprouts.

Self-reported sprout consumption could explain only one third of the cases (14/43). This is in line with other epidemiological investigations where sprouts have been identified as vehicle of infection, e.g. a

multinational *S.* Newport outbreak in the US and Canada in 1995, and the large STEC O104:H4 outbreak in Germany in 2011 [9,15]. In these outbreaks, sprout consumption was remembered by only 41% and 25% of interviewed cases, respectively [9,15]. However, when a methodology was used in the latter outbreak that relied on recipe-based data rather than on patient memory, it was confirmed that all cases had consumed sprouts [15]. Sprouts are often used as garnish or side dishes, or are served mixed with other food items in dishes such as Asian rolls, which makes them difficult to remember by patients and renders them classical 'stealth' vehicles. The long time lag between exposure period and the interview may be another reason why the proportion of cases who recalled sprout consumption was small. Furthermore, case patients may have been infected by other food items that were cross-contaminated via kitchen staff and tools. The proportion of case patients who remembered sprout consumption was higher among those with *S.* Newport isolates showing the outbreak PFGE pattern (10/14; 71%) than among all case patients (14/43). Since molecular typing could be performed only in about one third of the cases, it cannot be excluded that some of the 106 cases were misclassified as belonging to the outbreak. However, the background occurrence of *S.* Newport infections in the general population is low, and we therefore assume that the number of cases falsely attributed to the outbreak was small.

Comparative molecular typing was instrumental in detecting the outbreak vehicle, as the PFGE pattern of 32 human isolates was indistinguishable from the mung bean sprouts isolate. In addition, food traceback investigations were crucial, because sprouts served at locations where cases had eaten could be linked to sprout producer A where *Salmonella*-contaminated sprouts had been detected. It can only be speculated whether the seeds used by the producer were contaminated, as has been described for other outbreaks associated with sprouts [12,25].

In our case–control study we selected notified cases with *S.* Enteritidis infection as control group for various reasons: (i) they could be contacted in a timely manner and recruited more easily than healthy individuals because contact information was available at the local health authorities; (ii) we assumed that symptomatic individuals would be more willing to participate in an epidemiological study and would remember food consumption better than healthy individuals; (iii) we assumed that *S.* Newport case patients and *S.* Enteritidis controls would remember food items consumed before disease onset equally well since we aimed at conducting the interviews within a similar time period after disease onset in cases and controls; (iv) we assumed that consumption habits of *S.* Enteritidis controls would not differ from those of the source population for *S.* Newport cases; (v) *S.* Enteritidis infection is not typically associated with consumption of mung bean sprouts. We do not assume that our study was biased by this methodological approach, which has been described before [26-29], because we have no reason to believe that sprout consumption habits of *S.* Enteritidis patients would be different from those of the general population. Eggs and chicken meat are typical transmission vehicles of *S.* Enteritidis infections, although occasionally mung bean sprouts have been associated with *S.* Enteritidis infections in outbreaks [12].

Contaminated fresh produce has increasingly been recognised as an important source of foodborne outbreaks [30]. The outbreak caused by mung bean sprouts described here occurred shortly after the 2011 STEC O104:H4 outbreak in Germany caused by fenugreek sprouts. Neither in Germany nor in the Netherlands was a consumer warning against the consumption of the implicated lots of mung bean sprouts released. At the time when sprouts were suspected as the outbreak vehicle and when the outbreak investigation began, the shelf life of the implicated mung bean sprouts lots (26 October 2011) had already expired by about a month, and it was assumed that sprouts of these lots had already been consumed or discarded. Also, after the second lot of sprouts had tested positive for *Salmonella* at producer A (sample from 21 October 2011), the incriminated seed lots had been blocked at that site.

Only at one site of exposure (the clinic in northern Germany) had uncooked mung bean sprouts been served. Although restaurants stated that they had at least briefly cooked the sprouts, temperature and/or duration of cooking of the sprouts were obviously not adequate to kill *S.* Newport bacteria. Our findings demonstrate once again that consumption of raw or briefly cooked sprouts is associated with a considerable risk of foodborne illness. Since sprouts are known to be frequently contaminated with microorganisms [31,32], consumer advice clearly stating the health risks associated with sprout consumption and the safe preparation of sprouts before consumption is essential for prevention of illness in the general population. In addition, more frequent routine microbiological examination of sprouts and seeds may help to increase consumer safety and avoid distribution of contaminated lots.

General consumer advice on the consumption of sprouts was already published in June 2010 and updated in May 2011 by the BfR. Thorough washing of sprouts before consumption is recommended to reduce the risk of infection [31]. Persons who may be vulnerable because their immune system is not fully developed or weakened (children, pregnant women, elderly and immunocompromised) should refrain from consuming raw or lightly cooked sprouts [31-33], and insufficiently heated sprouts should not be served to them in institutional settings [34].

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Conflict of interest

None declared.

*Erratum:

References 27 to 34 were left out in the originally published reference list. They were added on 13 January 2014. We apologise for this mistake.

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The French human *Salmonella* surveillance system: evaluation of timeliness of laboratory reporting and factors associated with delays, 2007 to 2011

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Given the regular occurrence of salmonellosis outbreaks in France, evaluating the timeliness of laboratory reporting is critical for maintaining an effective surveillance system. Laboratory-confirmed human cases of *Salmonella* infection from whom strains were isolated from 2007 to 2011 in France (n=38,413) were extracted from the surveillance database. Three delay intervals were defined: transport delay (strain isolation, transport from primary laboratory to national reference laboratory), analysis delay (serotyping, reporting) and total reporting delay. We calculated the median delay in days and generated the cumulative delay distribution for each interval. Variables were tested for an association with reporting delay using a multivariable generalised linear model. The median transport and analysis delays were 7 and 6 days respectively (interquartile range (IQR: 6–10 and 4–9 respectively), with a median total reporting delay of 14 days (IQR: 11–19). Timeliness was influenced by various external factors: decreasing serotype frequency, geographical zone of primary laboratory and strain isolation on Sundays were the variables most strongly associated with increased length of delay. The effect of season and day of the week of isolation was highly variable over the study period. Several areas for interventions to shorten delays are identified and discussed for both transport and analysis delays.

Introduction

A primary aim of infectious disease surveillance is to detect changes in disease incidence in order to mount an appropriate public health response [1]. There are inherent delays in surveillance between symptom onset and reporting to health agencies, and the reporting chain consists of multiple steps at which delays can accumulate [2].

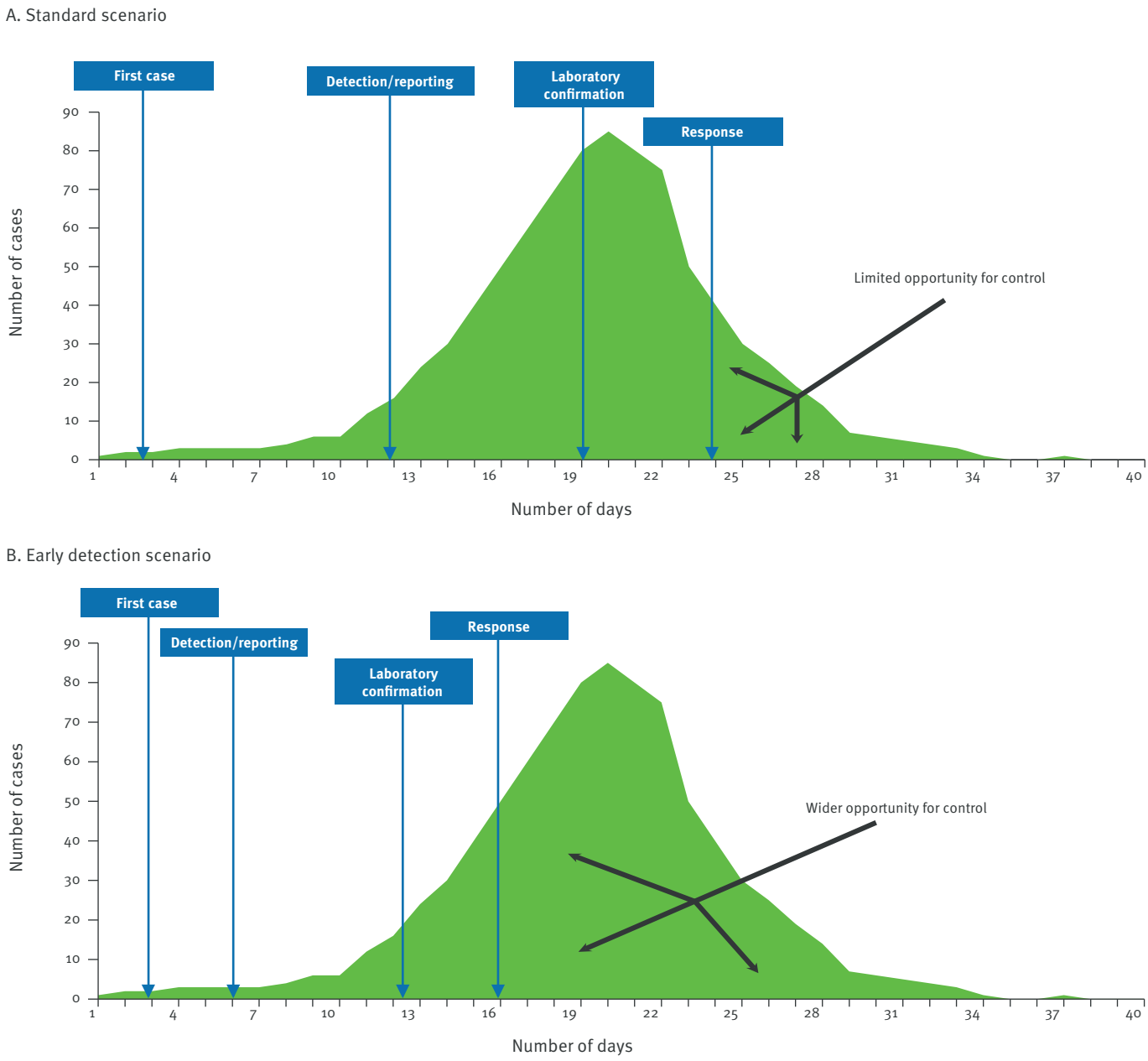
In France, the human *Salmonella* surveillance system is a voluntary laboratory-based network headed by the National Reference Centre for *Salmonella* (NRC) based at the Pasteur Institute in Paris. Participating laboratories (1,392/2,253 (62%) in 2011) send around 8,000 *Salmonella* isolates to the NRC per year. The NRC performs serotyping analysis and runs weekly outbreak detection algorithms, notifying exceeded thresholds to the French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS) [3]. The NRC also signals in real time to the InVS any suspected clusters based on observations of serotyping results in the course of their analysis. During outbreaks, serotyping results are notified to the InVS in real time. In 2008, it was estimated that the *Salmonella* surveillance system detected 66% of laboratory-confirmed human *Salmonella* infections in France [4].

Reporting timeliness reflects the speed with which a case of a reportable disease passes through each stage in the reporting chain from symptom onset to reporting to health authorities and is a key component of any disease surveillance system [5]. For *Salmonella*, which is a consistent source of outbreaks in France (representing 92 of 210 laboratory confirmed food-borne outbreaks (44%) in 2010) [6], rapid detection of clusters is a critical element of an outbreak investigation. Every day gained from timely reporting of cases can aid health authorities in leading an investigation that can identify the source of contamination and control the spread of illness (Figure 1). Therefore, regularly evaluating the timeliness of surveillance systems is crucial for maintaining an effective system [7,8].

Previous timeliness studies have been conducted for a number of countries and reportable diseases, including salmonellosis, but no standardised method has been established [1,7,9-11]. The impact of individual factors, such as reporting method (paper or electronic), has been studied [12,13]; however, the effect of multiple factors, including those related to the structure of the surveillance system (primary laboratory type and location) or to the disease in question (seasonality, serotype), has not been explored. Identifying areas for improvement would allow for targeted efforts to

Figure 1

Effect of early detection and response to an outbreak on the number of cases observed: standard scenario (A) and early detection scenario (B)



Cases preceding the 'first case' represent a baseline of cases that may occur sporadically.

reduce delays. Furthermore, the information obtained on reporting delays could be integrated into methods of prediction or estimation of cases that are based on historic data [14].

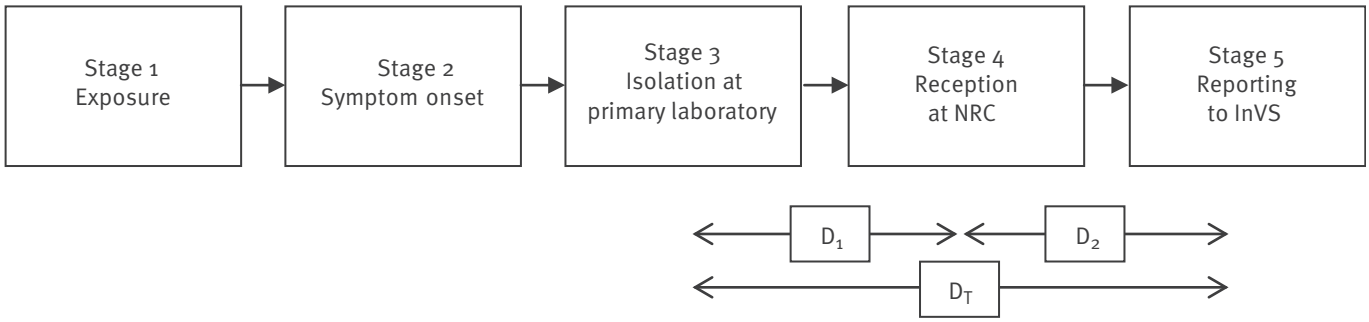
As *Salmonella* surveillance in France is based on a voluntary laboratory network coordinated by the NRC, delays can occur at two levels: (i) at the primary laboratory (strain isolation and transport to the NRC) and (ii) at the NRC (serotyping and transmission of the results to InVS). The aim of this study is to assess the timeliness of the French human *Salmonella* surveillance system, to identify factors associated with reporting delays and to identify opportunities to shorten these delays.

Methods

The NRC extracted from the *Salmonella* surveillance database the case number, serotype, specimen type, isolation date at primary laboratory, date of reception at NRC, date of validation (serotyping report available to InVS), reporting year, primary laboratory type and

Figure 2

Stages in the reporting chain and delay intervals for human *Salmonella* surveillance in France



D_1: transport delay
D_2: analysis delay
D_T: total reporting delay

InVS: French Institute for Public Health Surveillance (Institut de Veille Sanitaire); NRC: National Reference Centre for *Salmonella*.

laboratory and patient postal codes. We generated additional variables from the data including serotype frequency, whether the serotype was a monophasic variant of Typhimurium, geographical zone of the primary laboratory, season of reception of isolates at the NRC, isolation year and day of the week of isolation at primary laboratory. The study period included strains isolated from 1 January 2007 to 31 December 2011.

Reporting delays

We defined five stages in the reporting chain (Figure 2). Exposure date (stage 1) is only available from outbreak investigations for which a specific source or exposure is known. Date of symptom onset (stage 2) is not routinely collected by the surveillance system. Surveillance data included dates associated with stages 3, 4 and 5, permitting the calculation of three delay intervals: transport delay (D_1) from strain isolation at the primary laboratory to reception at the NRC (partial serotyping for certain primary laboratories and transport of the sample), analysis delay (D_2) from reception at the NRC to serotyping report available to InVS (serotyping and transmission of results) and total reporting delay (D_T) from strain isolation to the serotyping report being available to InVS. If one of these dates was missing, only the delay interval for which both dates were available was calculated.

Timeliness was evaluated based on the observed delays for the three delay intervals for each isolate in the database. Incoherent dates and aberrant delays were identified and verified. The median delay of each interval was calculated along with the interquartile range (IQR). The empirical cumulative delay distribution, $F(t)$, was generated, as this represents delays better than other indicators such as the mean, which is influenced by extreme values, or the median, which may mask differences occurring before or after the 50% point.

To analyse factors associated with reporting delays, eight variables were selected from the available data: (i) type of primary laboratory of strain isolation (private or hospital); (ii) season of isolate reception at the NRC; (iii) specimen type (blood, stool or other); (iv) serotype frequency (number of isolates received at the NRC in 2011: very frequent (›200), frequent (53–200), fairly frequent (12–52), rare (‹12)); (v) monophasic variant of Typhimurium 1,4,[5],12:i:- (yes or no); (vi) isolation year; (vii) geographical zone of primary laboratory (Paris, North, West, East, South, South-west, South-east and overseas); and (viii) day of the week of strain isolation at primary laboratory (day of week). Monophasic variants of Typhimurium were selected due to recent emergence, their initially time-consuming typing protocol and placement as the second most-frequent serotype in France [4]. The cumulative delay distributions were plotted for selected variables and statistical analyses carried out to determine the variables significantly associated with reporting delays.

Statistical analyses

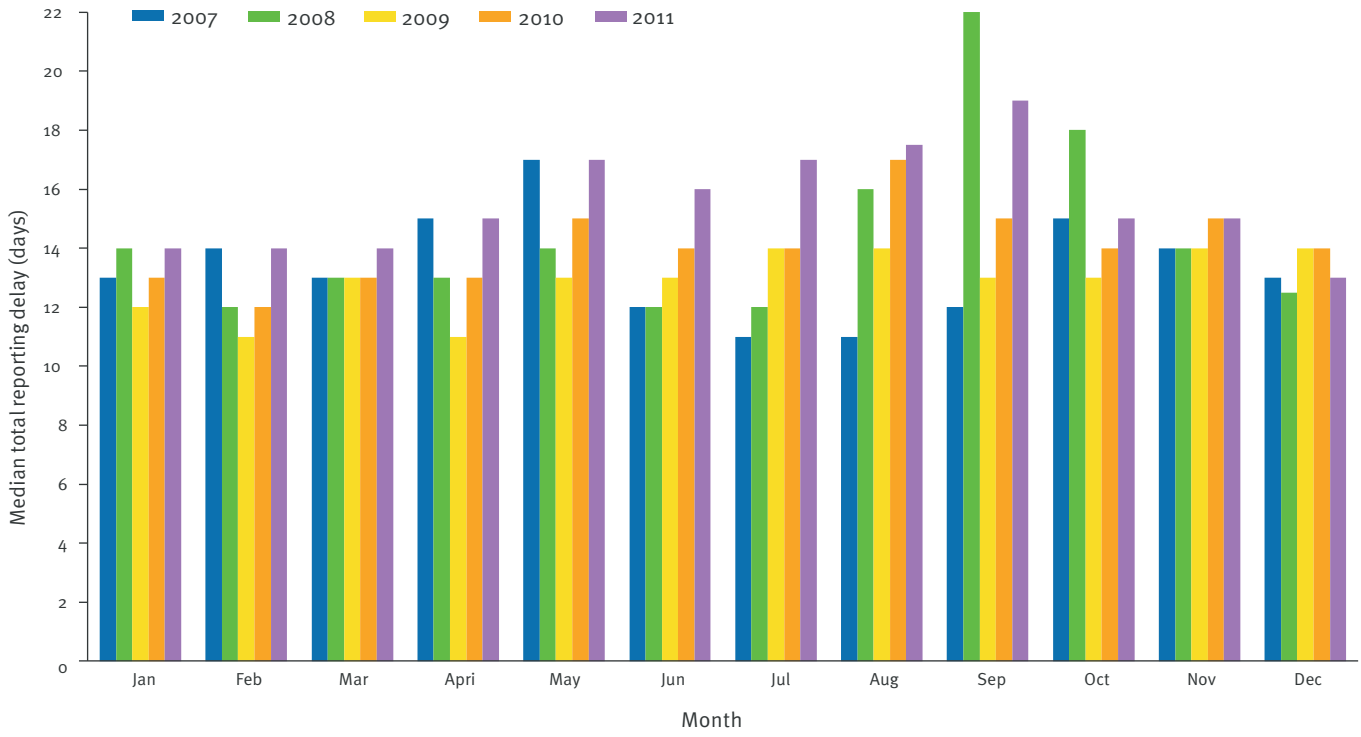
We used a regression model based on the method proposed by Brookmeyer and Liao [15] for comparison of the cumulative delay distributions. We conducted univariable analysis ($p<0.05$) followed by multivariable analysis using a generalised linear model (GLM) with the complementary log-log link function. A threshold of $p<0.05$ was selected due to the large number of isolates (good statistical power). Analyses were conducted using Stata version 11 (StataCorp LP, College Station, TX, United States). A detailed description of the statistical analyses used is included at the end of the article.

Results

A total of 38,413 *Salmonella* strains were isolated from January 2007 to December 2011. Of these, 1,619 (4.2%) were excluded before analysis (multiple dates missing,

Figure 3

Median total reporting delay in the French human *Salmonella* surveillance system in days by sampling month, 2007–2011



sample originating from laboratories overseas that do not regularly participate in surveillance). For the remaining isolates (n=36,794), it was possible to calculate the transport delay (D_1) for 35,450 isolates (96%), the analysis delay (D_2) for 36,630 isolates (99%) and the total reporting delay (D_T) for 35,287 isolates (96%).

The median transport delay was 7 days (IQR: 6–10), the median analysis delay 6 days (IQR: 4–9) and the median total reporting delay 14 days (IQR: 11–19) with intra- and inter-year variability observed for monthly median delays. The median total reporting delay by month ranged from a minimum of 11 days to a maximum of 22 days over the study period (Figure 3). Variability was observed between years, with intra-year differences of as little as 3 days to as many as 10 days between months.

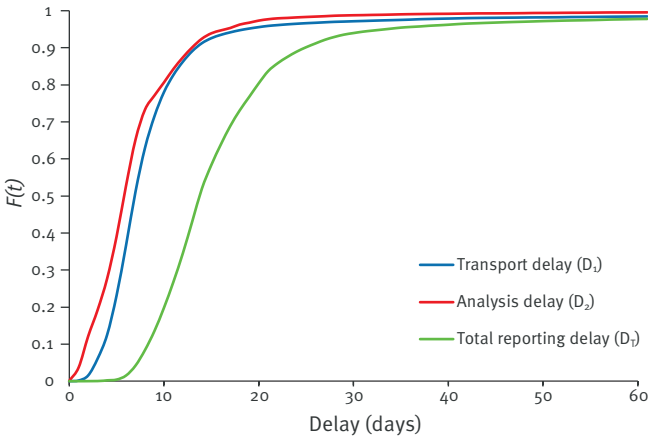
Comparison of cumulative delay distributions

The cumulative delay distribution represents at time *t* the probability that the reporting delay is less than or equal to *t*. Figure 4 compares the distribution of the three delay intervals for delays from 0 to 60 days. A higher curve indicates shorter delays relative to a lower curve.

Visual comparison of the cumulative delay distributions for the transport delay (D_1) and the analysis delay (D_2) reveals a disproportionate effect of certain variables on these delay intervals. The variables for which the transport delay distributions were most

Figure 4

Cumulative delay distribution of transport delay (D_1), analysis delay (D_2) and total reporting delay (D_T)[a], French human *Salmonella* surveillance system, 2007–2011



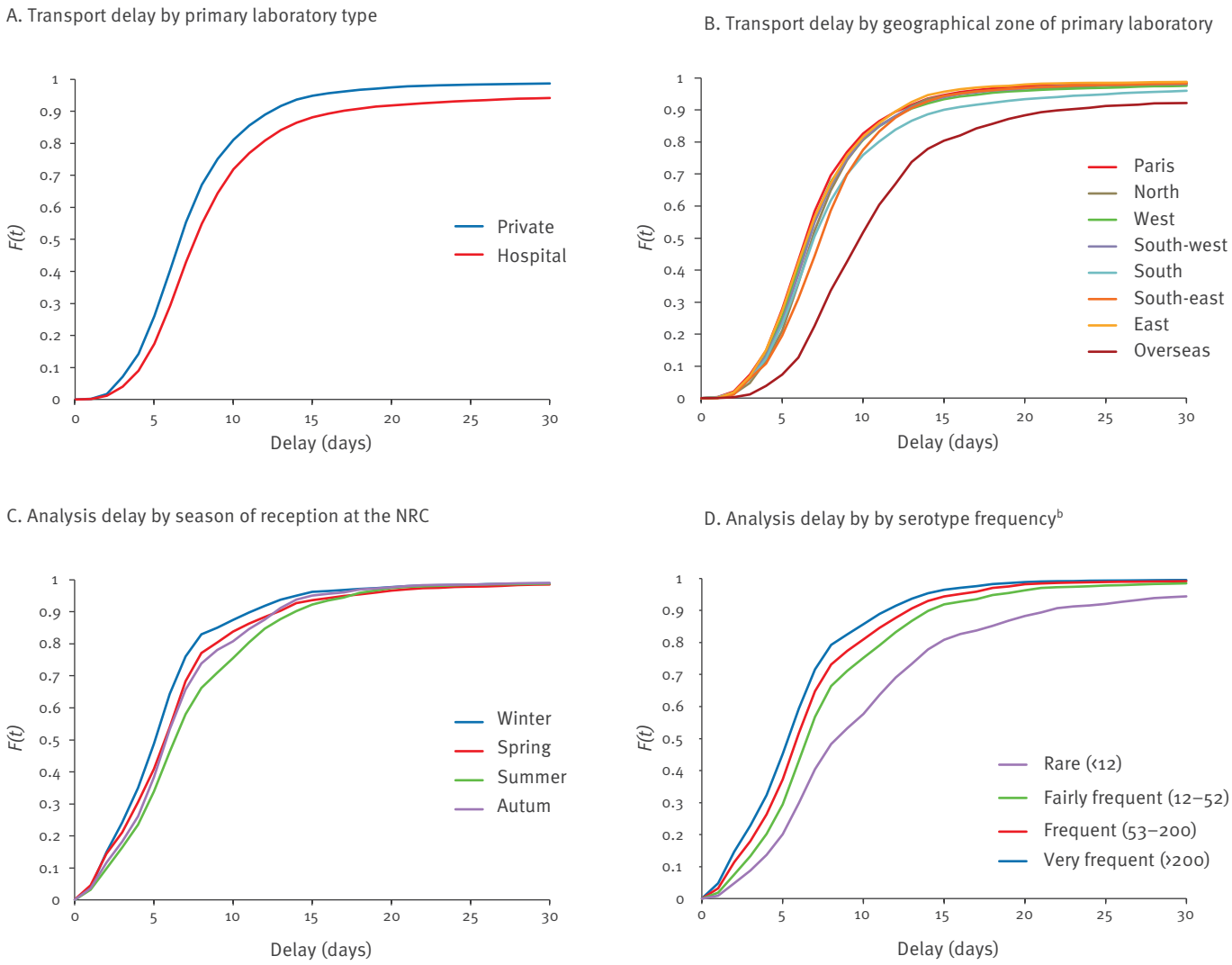
F(t): empirical cumulative delay distribution.

A cut-off of 60 days was used for clarity of graphical representation as more than 95% of isolates were received at the National Reference Centre for *Salmonella* and/or reported to the French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS) within this time period.

[a] For delays from 0 to 60 days.

Figure 5

Cumulative delay distributions[a] for transport and analysis delays, French human *Salmonella* surveillance system, 2007–2011



F(t): empirical cumulative delay distribution; NRC: National Reference Centre for *Salmonella*.

A cut-off of 30 days was used for clarity of graphical representation as more than 95% of isolates were received at the National Reference Centre for *Salmonella* and/or reported to the French Institute for Public Health Surveillance (Institut de Veille Sanitaire, InVS) within this time period.

[a] For delays from 0 to 30 days.

[b] Number of isolates received at the NRC in 2011.

greatly influenced were laboratory type (Figure 5a) and geographical zone of primary laboratory (Figure 5b). Hospital laboratories had a greater transport delay than private laboratories, as did laboratories overseas relative to those in mainland France. Day of the week of strain isolation also influenced D_1, but the grouping of the distribution curves prevented further interpretation (data not shown).

Conversely, season (Figure 5c) and serotype frequency (Figure 5d) showed the greatest degree of divergence for the analysis delay distributions. The analysis delay increased with decreasing serotype frequency and seasonal trends showed the shortest delays in winter and the longest delays in summer.

Delays for both D_1 and D_2 differed by isolation year, but no trends were observed, indicating that these delays did not increase or decrease consistently over the five-year study period (data not shown). Specimen type and monophasic variants of Typhimurium showed little difference in their effect on D_1 and D_2 (data not shown).

Table

Association between variables and total reporting delay using a multivariable generalised linear model and stratified by isolation year, French human *Salmonella* surveillance system, 2007–2011

	2007		2008		2009		2010		2011	
	$\hat{\beta}$	SE	$\hat{\beta}$	SE	$\hat{\beta}$	SE	$\hat{\beta}$	SE	$\hat{\beta}$	SE
Laboratory type										
Private	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Hospital	0.352***	0.035	0.411***	0.030	0.421***	0.031	0.349***	0.030	0.295***	0.027
Monophasic variant of Typhimurium										
No	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Yes	1.965***	0.111	1.477***	0.060	0.797***	0.040	0.423***	0.039	0.104***	0.029
Serotype frequency										
Very frequent	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Frequent	0.726***	0.044	0.822***	0.040	0.440***	0.038	0.421***	0.036	0.440***	0.035
Fairly frequent	0.869***	0.046	0.754***	0.038	0.668***	0.040	0.556***	0.037	0.666***	0.037
Rare	1.256***	0.056	1.273***	0.048	1.166***	0.051	1.088***	0.049	1.320***	0.050
Geographical zone										
Paris	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
North	0.712***	0.071	0.577***	0.060	0.786***	0.065	0.613***	0.060	0.702***	0.062
West	0.334***	0.051	0.144**	0.043	0.185***	0.046	0.153***	0.043	0.153***	0.041
South-west	0.288***	0.050	0.178***	0.044	0.239***	0.046	0.177***	0.044	0.115**	0.040
South	0.450***	0.055	0.416***	0.049	0.314***	0.049	0.384***	0.051	0.282***	0.043
South-east	0.136**	0.052	0.149***	0.042	0.209***	0.041	0.248***	0.041	0.223***	0.039
East	0.336***	0.057	0.337***	0.050	0.258***	0.051	0.330***	0.050	0.288***	0.046
Overseas	0.783***	0.072	0.971***	0.076	0.829***	0.066	0.569***	0.060	0.631***	0.058
Season of isolate reception at NRC										
Summer	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Winter	0.551***	0.044	−0.120**	0.038	−0.117**	0.037	−0.007	0.040	−0.123**	0.036
Spring	0.547***	0.043	−0.123**	0.038	−0.020	0.035	0.048	0.034	0.001	0.031
Autumn	0.566***	0.043	−0.026	0.034	0.155***	0.034	-0.036	0.032	−0.381***	0.030
Day of the week of strain isolation										
Monday	ref	ref	ref	ref	ref	ref	ref	ref	ref	ref
Tuesday	0.052	0.053	−0.077	0.045	0.053	0.045	−0.040	0.044	0.138***	0.040
Wednesday	0.102	0.054	0.070	0.046	0.186***	0.046	0.000	0.045	0.130**	0.040
Thursday	0.088	0.053	0.044	0.047	0.128**	0.046	0.051	0.046	0.232***	0.040
Friday	0.167**	0.055	0.066	0.046	0.159**	0.048	0.039	0.045	0.157***	0.040
Saturday	0.163**	0.057	0.061	0.049	0.188***	0.049	0.071	0.048	0.149***	0.043
Sunday	0.639***	0.082	0.324***	0.061	0.415***	0.062	0.452***	0.068	0.463***	0.058

NRC: National Reference Centre for *Salmonella*; ref: reference; SE: standard error.

* $p<0.05$

** $p<0.01$

*** $p<0.001$

$\hat{\beta}$ is a regression coefficient adjusted on other coefficients (not shown) representing reporting delays grouped into time intervals.

For the total reporting delay, D_T, the variables having the greatest influence on delays were primary laboratory type, serotype frequency, season, geographical zone of primary laboratory and isolation year. The influence of these variables on the individual delay intervals D_1 and D_2 were reflected in the cumulative delay distributions of the total reporting delay.

Analyses of variables associated with reporting delays

Results of the multivariable model for the association between six variables (significant in univariable analysis) and the total reporting delay are shown in the Table. The analysis was stratified by isolation year due to its statistically significant interactions with season of isolate reception at the NRC and day of week the strain was isolated ($p<0.05$).

The association between reporting delays and most variables remained significant for the entire study period. The coefficient $\hat{\beta}$ represents the effect on a delay by a given variable and adjusted for all other variables in the model. A positive $\hat{\beta}$ value represents a longer delay and a negative $\hat{\beta}$ value a shorter delay. The force of the association is shown by the relative $\hat{\beta}$ value. All geographical zones had significantly longer delays than Paris, but the overseas and North zones exhibited consistently longer delays and the force of the association was similar. Hospital laboratories had longer delays than private laboratories and the association was relatively stable over time. Similarly, the association between serotype frequency and reporting delays was maintained, with delays increasing as serotype frequency decreased. A trend was observed for monophasic variants of Typhimurium, with reporting delays for such variants consistently longer than for non-monophasic variants, but this decreased sharply from $\hat{\beta}=1.965$ in 2007 to $\hat{\beta}=0.104$ in 2011.

Two variables, season and day of week, stood out. The effect of these variables on the total reporting delay fluctuated over the study period. Only samples isolated on Sunday had consistently longer delays than those isolated other days (largest $\hat{\beta}$ value). Otherwise, the associations between these variables and shorter or longer delays ranged from statistically significant one year to non-significant the next, with no trends over time.

Discussion and conclusions

In this first study of laboratory reporting timeliness of human *Salmonella* surveillance in France, we identified key intervals in the reporting chain and calculated the associated delays. Direct international comparison of timeliness for *Salmonella* reporting is difficult due to differences in the structure of public health and surveillance systems (e.g. mandatory vs voluntary reporting, steps in the reporting chain). The delay intervals described in our study are solely laboratory related, while previous studies in the United States and Europe included a greater number of intervals from symptom onset to reporting to health authorities [8,10,13,16]. One study in Ireland allows direct comparison: the transport and analysis delays we identified in France of 7 and 6 days respectively are longer than those reported in 2008 in Ireland for the equivalent intervals (4 and 5 days respectively) [7].

In this study, the emergence of the monophasic variant of Typhimurium 1,4,[5],12:i:- (not expressing the 1,2 phase) followed by a better knowledge of the clone and improved analysis techniques, is evident. Longer delays were observed at the beginning of the study period (due to, for example, multiple confirmatory analyses carried out by testing several colonies, the use of polymerase chain reaction and sequencing to identify the *fljB* gene encoding the 1,2 antigen), followed by a sharp decrease as the lack of this second phase was more readily recognised and such techniques were no longer used [17]. Thus, by 2011 these variants no longer represented one of the strongest factors in increased reporting delays. This trend was most pronounced for the analysis delay, but was also observed for the transport delay.

Trends related to the transport delay can be explained in part by two factors: the structure of the laboratory network in different geographical zones (laboratories may refer their isolates to hospital or specialised laboratories for transport) and the capacity of laboratories to perform partial serotyping before the isolate is sent to the NRC. The analysis performed was able to identify a systematic difference in the delays for samples originating from the overseas and North zones, information that can be used to target efforts to decrease delays related to the primary laboratories in these zones.

Trends related to the analysis delay, notably the increase in delay with decreasing serotype frequency, can be associated in part to factors associated with the analysis protocol at the NRC (verification of serotyping results for rare serotypes) [18].

The variables season and day of week demonstrate the inconsistent effect of certain factors on reporting delays over time. The NRC in France adapts its staff availability to *Salmonella* seasonality by assigning all technicians to serotyping in the summer in an effort to minimise backlogs; however, personnel turnover or large outbreaks during peak vacation periods (when there are fewer personnel) may still result in increased delays in some years. An example was observed clearly in September 2008 (Figure 3), where a large peak in delays coincided with personnel turnover in the preceding months and a backlog in the NRC analysis. Limited staffing situations may be a risk for increased delays, with an immediate observable effect. Such information should be taken into account in the current public health context in which budget restrictions render such conditions more and more common.

This study has several limitations. First, the surveillance system does not collect the date of symptom onset and therefore the delays in the reporting chain from symptom onset to strain isolation at the primary laboratory cannot be systematically evaluated. The date of symptom onset can be obtained from case interviews in outbreak investigations and an analysis of 16 investigations at the InVS from 2007 to 2011 containing the date of symptom onset showed a median delay from date of symptom onset to strain isolation at the primary laboratory of 4 days (unpublished data). This delay was shorter than the 7 days observed in studies on *Salmonella* reporting timeliness in both the United States and Ireland [7,10]. In addition, the median delay between symptom onset and strain isolation differed between hospital laboratories (3 days) and private laboratories (5 days). The information gained from the cases for whom date of symptom onset was available highlights the value of including such information in surveillance data, which would allow for more complete timeliness evaluation.

Second, information regarding protocols at the primary laboratories is limited. Delays incurred by factors – such as transportation service (large services with daily collection vs small services with less frequent collection) or partial serotyping (use of a limited number of antisera to identify the most prevalent serotypes) before sending the isolate to the NRC – cannot be fully evaluated. A 2008 study of 3,217 medical laboratories in France found that 35% of laboratories performed partial serotyping of *Salmonella* isolates (65% hospital laboratories vs 31% private laboratories) [19]. Partial serotyping allows more rapid clinical results and orientation of patient treatment (non-typhoidal vs typhoidal salmonellosis), but may also increase the transport delay for isolates difficult to type at the primary laboratory. These additional delays may affect surveillance and delay detection of clusters by the NRC.

For *Salmonella* surveillance, it is critical that delays are as short as possible in order to rapidly detect outbreaks, to monitor their progression and to assess the impact of control measures. In outbreak detection and investigation, every day gained in early detection counts since the implementation of control measures may avoid additional cases. The delay until detection varies widely between outbreaks and depends on characteristics of the outbreak, the algorithm used and reporting delays. The characteristics of the outbreak (e.g. sudden increase or prolonged onset, serotype frequency, proportion of sporadic cases) cannot be influenced and there is little opportunity to decrease detection delay. It is possible to reduce the detection delay by setting lower thresholds in the algorithm. However, this will decrease the specificity of cluster detection and lead to a potentially large number of false alerts. Currently, the delay between the identification of the outbreak and notification to the InVS is less than one day. Therefore, efforts to reduce the detection delay should focus on the reporting delays.

In this study, we were able to analyse timeliness of laboratory-based surveillance of human *Salmonella* over a five-year period as the system remained unchanged. Timeliness of *Salmonella* reporting in France depends on a number of variables that intervene along the reporting chain. These factors can be distinguished by their effect on the transport delay or conversely on the analysis delay at the NRC. Both the transport and the analysis delay demonstrate potential for improvement through targeted measures. For example, time can be gained by encouraging immediate transport of samples to the NRC, by efficient serotyping or by more frequent running of algorithms. Additionally, the implementation of a web-based system for real-time reporting of partial serotyping results (scheduled for implementation in 2014) would permit the NRC to rapidly obtain serotype information from laboratories before submission of the actual isolate. Such a system would provide pertinent real-time information about cases not yet received by the NRC that may serve to alert the NRC to potential clusters or to supplement data during alerts.

For human *Salmonella* surveillance in France, obtaining information regarding primary laboratory protocols should be encouraged as part of the surveillance network and efforts to increase awareness for timely submission of isolates should be emphasised in order to decrease the transport delay, particularly in certain geographical zones. On the basis of the results of this study, we recommend that participating laboratories be requested to transport a sample of any *Salmonella* isolate immediately to the NRC before partial serotying. While laboratories could continue to perform partial serotyping to orient clinical treatment, simultaneously sending a sample to the NRC could serve to reduce the delays incurred by waiting for the results of partial serotying before sample submission.

Current efforts to minimise delays at the NCR focus on assigning all technicians to serotyping during the summer to coincide with seasonality. However, from a technical standpoint, changes in serotyping methods could also be considered, to reduce the analysis delay. Notably, molecular methods could replace the classic serotyping by agglutination currently used at the NRC (White-Kauffmann-Le Minor scheme [18]). Achtman et al. recently evaluated multilocus sequence typing (MLST) for *Salmonella*, finding it a suitable alternative to classic serotyping [20]. It is likely that as costs of molecular methods continue to decrease, such methods can feasibly be adopted by the NRC to decrease the analysis delay.

The fluctuating association between delays and the variables season, monophasic variants of Typhimurium and day of the week demonstrate the potential instability of delays over time. Therefore, timeliness of the surveillance system should be regularly evaluated to target areas for improvement and to determine if implemented changes have been effective. Furthermore,

methods of prediction or estimation based on reporting delays from historical data should be mindful of hypotheses regarding the stability of delays over time.

This study proposes a new method for the evaluation of timeliness of laboratory reporting for *Salmonella* surveillance and for identifying factors impacting reporting delays. It can be adapted for any infectious disease surveillance system. A better understanding of reporting delays and associated variables is essential for optimising disease surveillance systems and maximising their capacity to mount an appropriate and effective public health response.

Additional information: statistical analyses

We used a regression model for comparison of the cumulative delay distributions. According to the method proposed by Brookmeyer and Liao [15], isolates were grouped by sampling month and then categorised by reporting delay into m index periods of finite values in days designated $t_1,\ldots,t_m$. For each $j=1,\ldots m$, the number of isolates per sampling month reported at time t_j was designated Y_j and the total number of isolates with a reporting delay less than or equal to t_j was designated n_j. In order to represent the reporting delays for *Salmonella* isolates, a nonparametric estimate of the cumulative delay distribution, *F(t)*, was used. *F(t)* can be expressed as the product of conditional probabilities, p_j, which represents the probability that the reporting delay is equal to t_j given that the delay is less than or equal to t_j. The estimate of the conditional probability can be expressed as $pj = Y_j/n_j$ and the estimate of the cumulative delay distribution:

$$\hat{F}(t_s) = \prod_{j=s+1}^{m} \left(1 - \frac{Y_j}{n_j}\right) \quad s = 1, \ldots, m-1$$

The above method is particularly useful as it is adaptable for analysis of multiple variables. The database of isolates can be stratified into K strata, which are generated by crossing variables of interest present in the database. The notation in the previous paragraph is extended using the subscript k to index the strata (p_{jk} is the conditional probability at time t_j in the stratum k).

A GLM with the complementary log-log link function was chosen as it provides the following relationship between the dependent variable (here the conditional probability of reporting within time t) and the explicative variables Xk:

$$\log\left(-\log\left(1 - p_{jk}\right)\right) = \alpha_j + \beta X_k$$

where a_j and $\beta=(\beta_1,\ldots,\beta_q)$ are the regression parameters. The association between variables and the total reporting delay, adjusted for the covariables in the model, is represented by the estimate of the parameter β. Interpretation of the coefficient β_q associated with the covariable x_q is as follows: a positive βq represents a longer delay for increasing values of x_q, while a negative β_q indicates a shorter delay with increasing values of x_q.

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The Danish Microbiology Database (MiBa) 2010 to 2013

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The Danish Microbiology Database (MiBa) is a national database that receives copies of reports from all Danish departments of clinical microbiology. The database was launched in order to provide healthcare personnel with nationwide access to microbiology reports and to enable real-time surveillance of communicable diseases and microorganisms. The establishment and management of MiBa has been a collaborative process among stakeholders, and the present paper summarises lessons learned from this nationwide endeavour which may be relevant to similar projects in the rapidly changing landscape of health informatics.

Background

In Denmark, surveillance of infectious diseases is based on notifications from physicians in hospitals and general practices as well as statutory reporting from departments of clinical microbiology (DCM) to Statens Serum Institut (SSI), the national public health institute for infectious diseases (www.ssi.dk). Until 2013, all reported data were handled manually before being processed. This system was not flexible and lacked timeliness as well as complete reporting.

In the late 1990s, electronic submission of laboratory reports to general practitioners (GPs) was initiated in Denmark and currently, all communication regarding test results and test ordering between GPs and DCMs (Figure 1) are electronic, using national standard protocols [1]. Electronic reporting from the local DCM to hospitals was initiated on a small scale in 2005, but has now become widespread. However, the hospital or the GP information systems offer no common facility for sharing pertinent microbiological information between healthcare providers, and this lack of data sharing represents an increasing challenge due to frequent transfer of patients between hospitals.

Based on a pilot project involving two DCMs with different data structure and coding, the SSI took in 2008 the initiative to establish a nationwide database for microbiology in order to enable real-time surveillance of communicable diseases and microorganisms as well as provide nationwide access for healthcare personnel to microbiology reports. By addressing these two aims in one project, the benefit of this new activity was maximised.

Organisation of the MiBa project

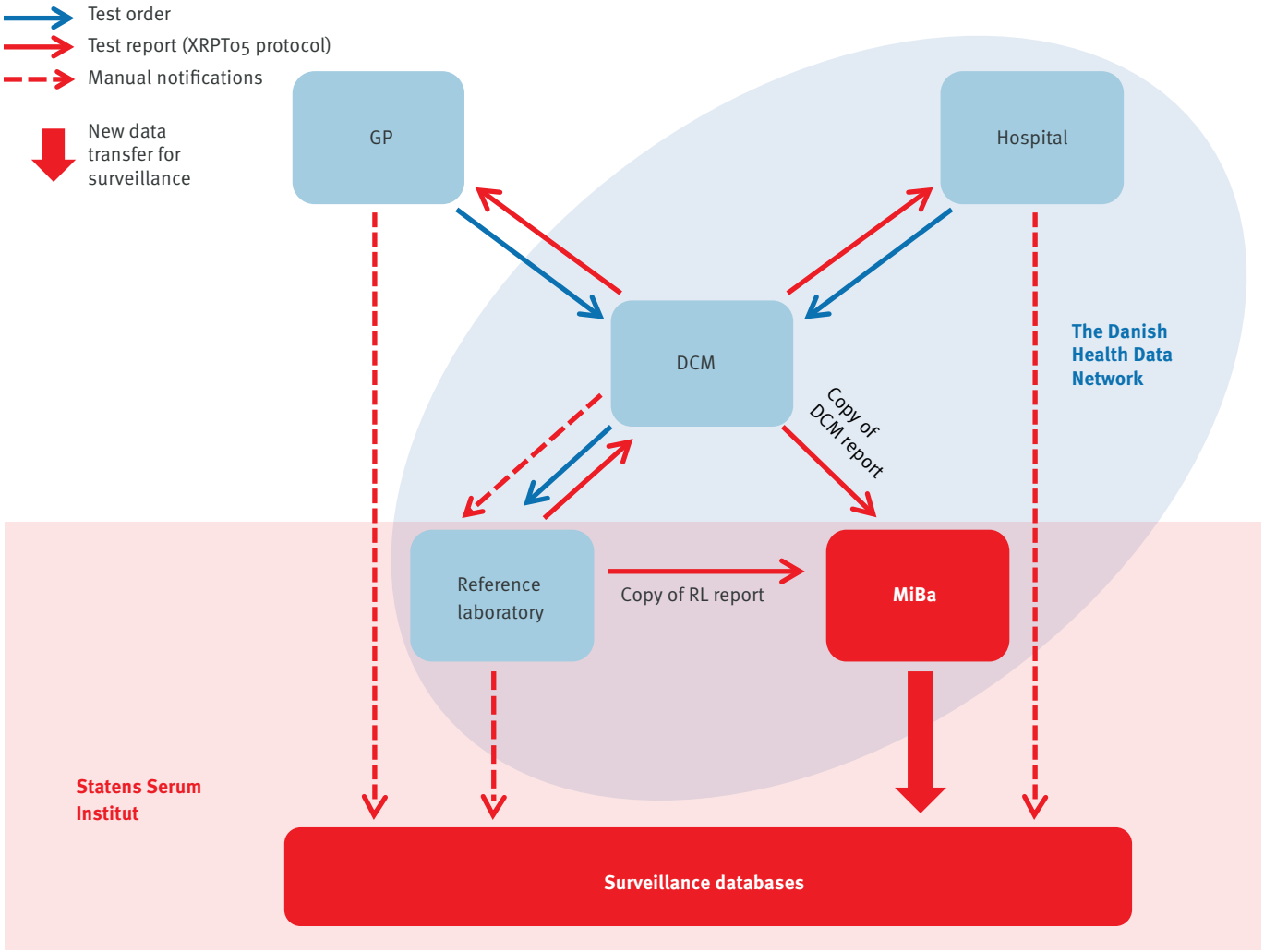
The Danish Microbiology Database (MiBa) project was based on a practical philosophy of data sharing and on a number of core principles:

- MiBa was established by a collaborative effort of all Danish DCMs;
- The DCMs transmit to MiBa a copy of all final electronic reports sent to clinical departments or GPs submitting patient samples for clinical microbiology;
- All data transferred to MiBa from a local DCM remain the property of the local DCM and thus respect local authority and data control;
- Decisions on standardisation of coding and data structure should be made jointly and implemented stepwise respecting local challenges;
- Access to patient reports is only permitted for healthcare providers engaged in diagnosis, treatment or consultation concerning the patient; the statutory national surveillance of infectious diseases is excluded from this clause;
- Access to relevant patient information must be easy, without compromising a high level of data protection;
- Access to data for purposes other than surveillance must comply with Danish law and be approved by the each DCM involved.

The director of the Department of Infectious Disease Epidemiology at the SSI is legally the owner of the MiBa project and is financially responsible for management and development. The overall steering of MiBa is undertaken by a board of representatives from each of the 14 DCMs in Denmark and the director who serves as head. The board works in close collaboration with an executive MiBa manager, who from January 2010 has been a medical doctor, specialised in clinical microbiology. Additional staff are a part time software developer and a secretary. Most of the development and continuous upgrading of the MiBa database software has been outsourced to a private supplier of laboratory information systems and funded by the Ministry of Health.

Figure 1

Electronic communication of microbiological laboratory test orders and reports, Denmark



DCM: department of clinical microbiology; GP: general practitioner; MiBa: the Danish Microbiology Database; RL: Reference laboratory; XRTP05: MedCom XRPT05 xml data transfer protocol.

Principles of dataflow from the departments of clinical microbiology to the MiBa database

A national standard xml transfer protocol for microbiology reports (XRPT05) was issued in 2007 in collaboration with MedCom [1,2]. MedCom facilitates the cooperation between authorities, organisations and private companies linked to the Danish healthcare sector, with special focus on solutions for electronic communication. Several different laboratory information systems are in use in Denmark. The protocol enables standardised data output from DCMs with different laboratory information systems and DCM-specific data structure. Data are transferred to MiBa via The Danish Health Data Network which allows the health sector in Denmark to communicate and transfer person-based information to connected organisations through one secure digital solution [1].

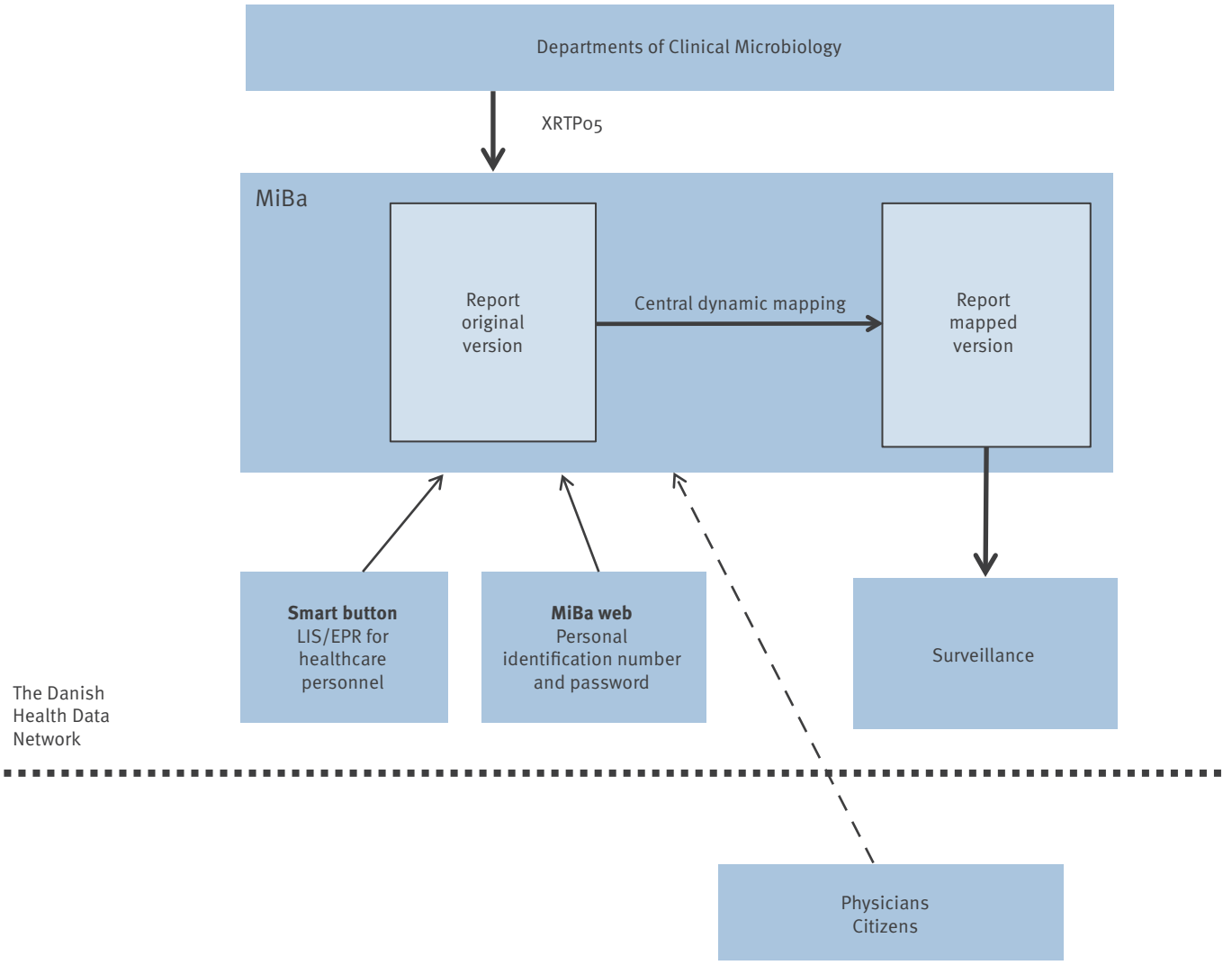
The key principle is the simultaneous submission to MiBa of an electronic copy every time a new or updated report is sent from a DCM to the GP or hospital department that requested the test. From the XRPT05 xml-protocol, the report is imported as a 'local version', i.e. the version available to the healthcare provider (Figures 2 and 3).

Each report is identified by a MiBa identification (ID) number, the patient's unique civil registry number (the CPR number) [3] and the report ID number used by the local DCM. By use of these numbers, reports can be updated if later changes are made by the local DCM. Only the latest version of a report is available in MiBa.

Since January 2010, copies of all reports submitted by Danish DCMs have been transferred to MiBa. By

Figure 2

Electronic communication of microbiological laboratory test orders and reports, Denmark



EPR: electronic patient record system; LIS: laboratory information system; MiBa: the Danish Microbiology Database; XRTP05: MedCom XRPT05 xml data transfer protocol.

December 2013, approximately 11 million reports had been uploaded.

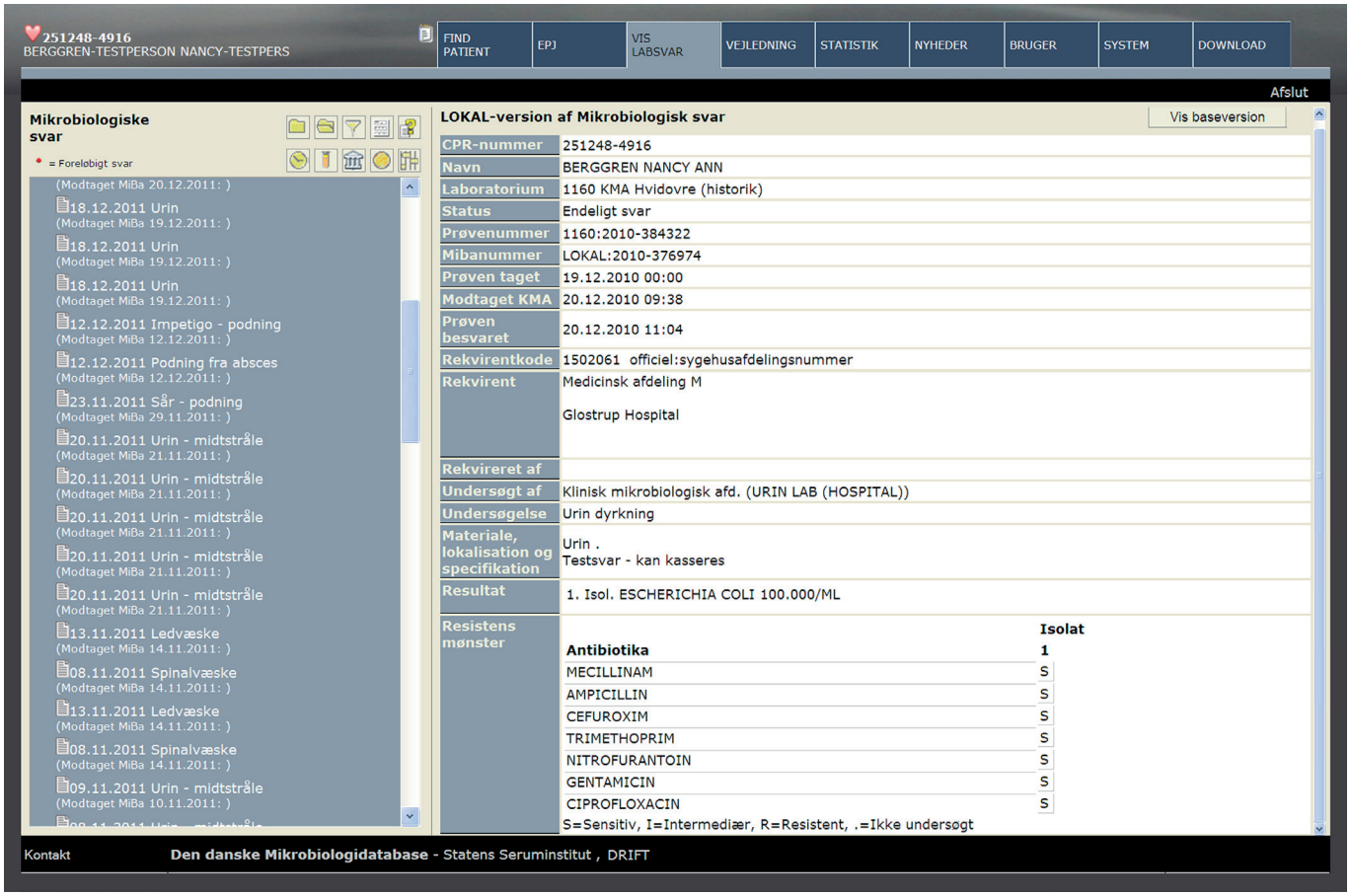
Access to patient test reports

Access to the reports in MiBa can be obtained in three different ways (see Figure 2):

- 'MiBa buttons' integrated in local laboratory information systems and electronic patient record systems:
- The healthcare provider has already identified themselves during the procedure for access to the local system. When the MiBa button is pressed, information on the local user ID, the user´s organisation and the patient identifier in question are automatically forwarded to MiBa. A new login session is not necessary.
- Login by access from a website using a personal user ID and password:
- A selected group of persons (such as physicians in DCMs, public health personnel) can access MiBa this way, providing more functionality than the MiBa buttons described above. For both solutions, it is a prerequisite that the computer used is covered by a contract with the Danish Health Data Network; this is the case for all hospital computers [1].
- GPs and other healthcare professionals not covered by contracts with the Danish Health Data Network can obtain access to MiBa via a national web portal (www.sundhed.dk). This access requires a digital signature which is a national solution to enable online electronic ID verification on public websites [1].

Figure 3

Local version of a laboratory report, Denmark



The reports shown are from a fictitious person.

When admitted to hospital patients provide consent for sharing their data within the healthcare system. In rare cases, consent is refused, and these reports are not accessible in MiBa. All activities in MiBa, including user login, are logged and are traceable. Patients do not yet have access to their own microbiology reports. However, we expect that patients in the near future will be able to access their own personal information in MiBa through the national web portal [1]. When access is obtained, an overview of all the patient´s reports is shown (Figure 3). Different views and search functions are available. During the first year, access was only obtained by clinical microbiologists, hereafter other healthcare staff were increasingly using the database (Figure 4).

The principle of central dynamic mapping in MiBa

Although all DCMs provide information like 'microorganism identified' and 'test performed' in coded form, a national standard for coding is not applied for all variables (Box 1). Some elements in the reports follow national coding standards whereas other elements are coded according to local standards. Uniform coding and terminology are required for data extraction and statistics. By use of the function Central Dynamic Mapping, all local codes are mapped to shared codes within MiBa. All reports in MiBa are copied and saved as a 'mapped version', in which all codes have been mapped automatically into shared codes (Figure 1). If the key for mapping between a given local code and the shared code is changed, the code in question is remapped in all uploaded reports (mapped version) from the DCM in question. The local version of the report remains unchanged.

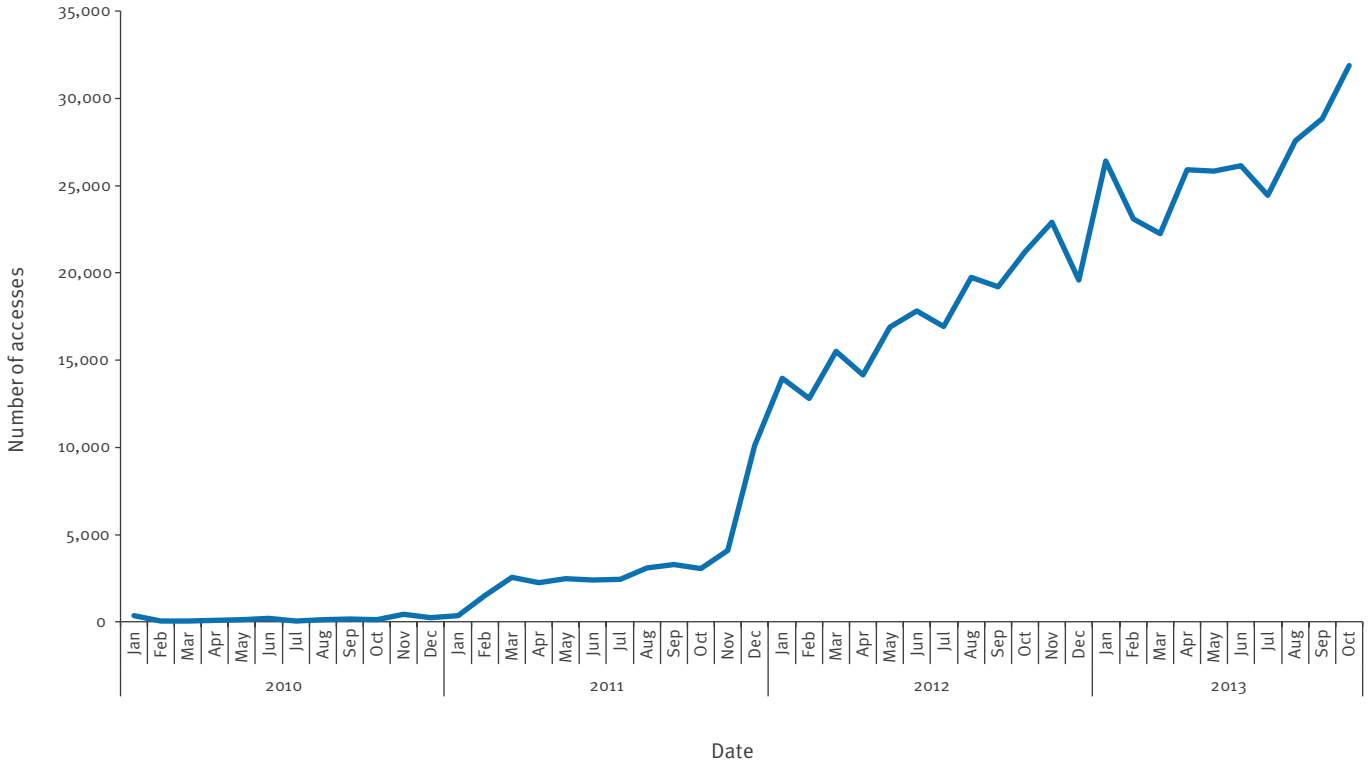
In the MiBa collaboration, we are developing and maintaining shared codes (national terminologies) for reporting laboratory findings. Existing and new working groups have contributed, including a standing committee working on national request codes for detailing sample materials and examinations [4].

Data extraction from MiBa and national surveillance

Data extraction from MiBa is based on the shared codes in the mapped version of the report (Figure 1).

Figure 4

Accesses to the MiBa database per month, Denmark, 2010-12



MiBa: the Danish Microbiology Database.

The use of MiBa in nationwide sharing of reports is illustrated in the figure as accesses per month defined as the number of times a healthcare employee looks up reports on a civil registry number (patient identification) per month.

Functionalities are created to specify case definitions and manage automatic transfer of data to specific databases used for laboratory-based surveillance. MiBa can be applied in a real-time workflow to enhance the detection of outbreaks and timely analysis of trends. MiBa contains negative test results as well, facilitating an understanding of diagnostic practices, which is important in the interpretation of surveillance trends. Examples are given in Box 2. Aggregated and graphical surveillance data will be accessible on an interactive website (http://www.ssi.dk/Smitteberedskab/Sygdomsovervaagning.aspx). Reports from patients who have refused the sharing of their data among healthcare personnel will still be accessible for data extraction and surveillance.

Individual cases of notifiable diseases are still reported on paper forms by the attending physicians. These forms are often delayed or forgotten. In the future, digital public health surveillance, MiBa-based laboratory surveillance and notifications by clinicians will be integrated in the same workflow in order to provide complete and timely notification to the public health officers at SSI.

MiBa will also serve as a resource for researchers and stimulate standardisation and quality assurance in public health microbiology and public health surveillance [5]. The microbiologist, clinician or researcher cannot extract data themselves. They can only look up records on specified patients, one patient at a time. For data extraction, an application is needed. As data are owned by the local DCMs, approval for projects on MiBa data must be obtained from each individual DCM. In Denmark it is possible to link data across national registries by the CPR number. Data from MiBa and the Danish vaccination register [6] have been linked to estimate vaccine effectiveness [7]. In that study, patients with negative test results were used as controls.

Limitations for the use of MiBa for national surveillance

A limitation to the current version of MiBa is the lack of standardisation of some types of information (e.g. data on subtyping). The consequence is that key information may be reported as plain text which is not well suited for data extraction.

Another limitation is that the uploaded reports contain information relevant for the treatment of the patient and are targeted for the attending physician and for

Box 1

Variables in MiBa, Denmark

General information
- Patient name and CPR number
- Dates (sampled, received in laboratory, sent from laboratory, received in MiBa)
- Requestor identification (national organisation codes)
- Laboratory sending the record (national organisation codes)
- Clinical information (text)
- The test(s) requested (national order codes)[a]
- Test material (national order codes)
- Anatomical location of sampling (national order codes)

Specific test (i.e. specific PCR or serological tests)
- The analysis performed (local code)
- Test result
- Interpretation of test result (i.e. positive or negative)

Complex test (i.e. culture)
- Microorganism(s) found (local code)
- Antimicrobial resistance pattern (local code)
- Microscopy result (text or local code)

Interpretation
- Overall interpretation of test results (text)
- Comments on test or test results (text)

CPR number: the patient's unique civil registry number; MiBa: the Danish Microbiology Database; PCR: polymerase chain reaction.

[a] Whether a test is a screening test or a test in disease-related diagnostics, is not registered by a separate variable, but this information may be evident from the order codes used or may be included in the report as a text comment.

inclusion in the patient's health record. Additional data may be needed for surveillance purposes. For instance, information on antimicrobial susceptibility patterns can be restricted in accordance with local antibiotic policies, leaving out information important for surveillance of multidrug-resistant microorganisms.

A new collaborative project has been initiated to overcome the above limitations. This project, which is called the 'subtype project', includes i) construction of a new enlarged xml transfer protocol that allows for special resistance data (not to be shown for the clinician) and subtyping data to be transferred to MiBa in a standardised manner, ii) establishment of a nationally accepted terminology for subtyping data and iii) implementation of necessary changes in registration procedures in local DCMs.

Lessons learned

The most important lesson was that a complex healthcare IT-project like MiBa depends on close collaboration between all stakeholders: the DCMs, suppliers of laboratory information systems, clinical users and political decision makers. Goals, concerns and technical possibilities may change over time and development must proceed in manageable iterative steps to accommodate new realities.

It has been a prerequisite that the project received external funding. In addition, the most important barriers to participation were issues of data ownership and authority over their use as well as concerns about extra workload in the local DCMs during phases of development. As soon as it became clear that data transferred to MiBa remained the property of the local DCMs, and that all major decisions on the development of MiBa must be approved by the MiBa board, the collaboration was dynamic and the MiBa project showed steady progress regarding parameters such as number of accesses, completeness of coding, willingness of changing local data structure, development of national codes, number of research projects in progress, etc.

The emphasis on data transmission while accepting local coding, variations in data standards and allowing the DCMs to harmonise data structure and coding in a stepwise manner, is the main reason why MiBa could be established within a short time span of two years. When local DCMs experienced the benefit of sharing microbiology reports, attitudes changed towards sharing even more data. We must emphasise that MiBa would not have been possible without the commitment and support by the local DCMs, which have always been willing to assist in testing and resolving any issues related to internal procedures or codes. However, it needs to be acknowledged that changes in local IT systems and workflows are time-consuming processes and that differences in reporting laboratory diagnostic work may remain.

Achievements and planned activities

Currently, all physicians have access to microbiological test reports nationwide. All microbiologists, most GPs

Box 2

Examples of how MiBa is used in national surveillance, Denmark

The intermittent workflow

Bordetella pertussis data are extracted every third month (both positive and negative test results).

The variables returned from MiBa are: local record ID, patient CPR number, sex, age, date of sampling, test material, type of test, results of test, ID of the requestor. Information on municipality of residence is obtained from the national CPR register. Using the CPR number, vaccine status can be obtained from the Danish vaccine registry (3). A detailed report is sent back to the DCM.

The continuous workflow

An automatic data transfer is established between a prototype of the National Registry of Enteric Pathogens and MiBa.

Once a day, MiBa returns all new records, in which an enteric pathogen has been found. The number of episodes are calculated and updated every day based on CPR number, the type of pathogen found and the time interval between records.

CPR number: the patient's unique civil registry number; DCM: department of clinical microbiology; ID: identification number; MiBa: the Danish Microbiology Database.

and around one third of clinicians in hospitals have smart-button access solutions.

Laboratory surveillance for pertussis and influenza are now fully based on MiBa data. An electronic weekly report on influenza surveillance was launched in the winter 2012/13 with free access (http://www.ssi.dk/Aktuelt/Nyhedsbreve/INFLUENZA-NYT.aspx).

For validation purposes, MiBa-based surveillance of a number of microorganisms is currently running in parallel with the existing reporting system. In the spring of 2014, surveillance of individual microorganisms will one by one be changed to the MiBa system only after successful validation.

Planned activities include:

- development and implementation of the 'subtype project',
- development of electronic workflows integrating laboratory-based surveillance with notifications from the attending physicians for selected diseases under statutory surveillance,
- provision of smart access solutions to all hospital physicians,
- provision of access for patients to their own reports.

Conclusions

One of the visions for the healthcare system in Denmark is to provide access to test results across the entire health sector, which is vital for the development of secure, efficient work processes and high standards of public health surveillance [1]. MiBa meets this vision. The development of MiBa emerged from a unique collaborative environment including all Danish DCMs, the Department of Infectious Disease Epidemiology, the suppliers of laboratory information systems and MedCom. Development of MiBa with a limited budget and within a short time has only been possible because all stakeholders, including laboratory information system suppliers, were personally engaged.

The nationwide sharing of real-time microbiological reports has been a success. The integration of MiBa in the national eHealth infrastructure is still being improved. Automatic workflows for laboratory surveillance are in progress. The large benefits of a timely and complete surveillance system are still to be experienced, but prototype examples show promising results. Finally, MiBa will provide numerous opportunities as a data source for national surveillance and research projects.

MiBa Board of representatives

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Authors' contributions

M Voldstedlund has drafted the first version, made revisions, and approved the final version of the paper; has been managing MiBa science 2010. K Mølbak revised the first draft of the paper critically and approved the final version; has established MiBa. M Haahr has been engaged in the MiBa project, contributed to the illustrations, and approved the final version of the paper. The MiBa board of representatives have revised the first draft of the paper and approved the final version; each member has been part of the collaborative effort establishing MiBa.

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Conclusions of the fourth CONSISE international meeting

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The Consortium for Standardization of Influenza Seroepidemiology (CONSISE) held its fourth international meeting in Cape Town in September 2013. Conclusions of this meeting and influenza seroepidemiology protocol templates of different study designs are now available on the CONSISE website: http://consise.tghn.org/.

After the 2009 influenza pandemic, it was recognised that there was a need to provide more timely and standardised influenza seroepidemiology results to inform decision making [1,2]. This was the stimulus to form CONSISE. This consortium is composed of globally recognised experts and institutes who are interested in the standardisation of seroepidemiology for influenza and other respiratory pathogens. There are currently more than 100 members of CONSISE based in more than 45 countries. CONSISE has two (epidemiology and laboratory) integrated working groups. Background information and the organisation of CONSISE can be found on the CONSISE website.

The main task of the epidemiology working group is to generate detailed study protocol templates to evaluate the seroprevalence of seasonal, pandemic and zoonotic influenza viruses in specific human populations. So far, seven influenza seroepidemiology protocol templates of different study designs have been drafted and three are available on the CONSISE website at http://consise.tghn.org/articles/available-consise-influenza-protocols/. The templates are based on detailed epidemiologic protocols used previously by members of CONSISE in a range of situations across the globe. In addition, several of these protocols have been adapted for respiratory pathogens other than influenza, such as the Middle East respiratory syndrome coronavirus (MERS-CoV). The working group is also developing a question bank, which holds a collection of questions under major categories (e.g. demographic, background medical history, animal exposures, healthcare facility exposures, etc.) to facilitate the rapid development of questionnaires that should be used in conjunction with each of the protocol templates. An online interface will be developed that allows downloading specific questions into questionnaires.

One of the main tasks of the laboratory working group is to coordinate and standardise the international serology laboratory response to a new emerging influenza virus. The group has developed two consensus protocols for the microneutralisation (MN) and the haemagglutination-inhibition (HI) assays. A comparative influenza A(H1N1)pdm09 virus study to compare results and to assess reproducibility between laboratories using the agreed HI and MN consensus protocols will be conducted during 2014 and a small study group will be established to develop the detailed study protocol. It was agreed that either the two-day or three-day MN assays could be used in the study and various sources of potential antibody standards will be evaluated.

CONSISE has responded to emerging respiratory virus threats such as influenza A(H7N9), A(H5N1) and MERS-CoV. HI and MN assay protocols developed for influenza A(H7N9) by the Chinese Center for Disease Control and Prevention (CDC) and the United States CDC have been shared through postings on the Internet. Several of the CONSISE protocol templates have been adapted for MERS-CoV and are available on the World Health Organization (WHO) and CONSISE websites (http://consise.tghn.org/whats-new/). CONSISE plans to generate a consensus statement for the recommended serological assays, timing of sample collection and criteria for seropositivity for highly pathogenic influenza A(H5N1) seroepidemiological studies. The full report of CONSISE's activities and achievements to date is available on the CONSISE website [3].

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