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# Louse-borne relapsing fever (*Borrelia recurrentis*) in asylum seekers from Eritrea, the Netherlands, July 2015

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**Two patients from Eritrea, recently arrived in the Netherlands, presented with fever and were investigated for malaria. Bloodfilms showed spirochetes but no blood parasites. Louse-borne relapsing fever caused by *Borrelia recurrentis* was diagnosed. Treatment was complicated by severe Jarisch–Herxheimer reactions in both patients. Physicians should be aware of the possibility of *B. recurrentis* infection in migrant populations who travel under crowded conditions, especially after passing through endemic areas such as Ethiopia and neighbouring countries.**

*Borrelia recurrentis* has for many centuries caused infections of often epidemic proportions known as relapsing fever. Since the infection is exclusively transmitted by body lice and humans are their only host, large scale outbreaks are only expected under circumstances conducive to louse infestation. We here report the first introduction of louse-borne relapsing fever into the Netherlands after World War II.

## Case descriptions

### Patient 1

On 4 July 2015, a young adult from Eritrea was referred from the National Reception Centre for Asylum Seekers (Asielzoekercentrum, AZC) to a regional hospital in the northern Netherlands with a five-day history of headache, dizziness, right upper quadrant pain, myalgia, and fever (39.3 °C). Malaria was suspected. The patient had been in the Netherlands for only two days after arriving in Europe 14 days earlier. En route to Europe, they travelled through Ethiopia, Sudan and Libya. Previously, they had noticed chills while sheltering in an unofficial street camp in Rome where they stayed with a small group of fellow Eritreans before travelling to the Netherlands.

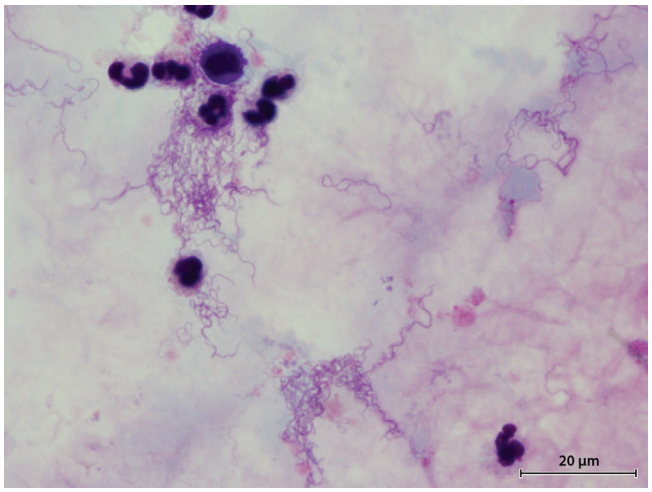
Thick and thin bloodfilms did not show malaria parasites and commercial malaria antigen tests were negative. However, filamentous unidentified structures were reported in the thick film by the laboratory of the peripheral hospital. The patient received empirical treatment with a single dose of ceftriaxone (2,000 mg intravenously) for suspected bacterial septicaemia. After administration, their condition deteriorated and the patient was transferred within the next two hours to the University Medical Center Groningen (UMCG) where they arrived at the emergency department with headache, peripheral hypothermia (35.3 °C), hypotension (systolic/diastolic blood pressure 78/52 mmHg, heart rate of 106 beats per minute), abdominal pain but no hepatosplenomegaly, and shortness of breath (respiratory rate 23 breaths/min).

Laboratory analysis showed leucocytopenia (leucocytes: $1.6 \times 10^9$/L, norm: $4.5–10 \times 10^9$/L), anaemia (haemoglobin: 6.5 mmol/L, norm: 8.6-11.2 mmol/L) and thrombocytopenia (thrombocytes: $16 \times 10^9$/L, norm: $150–450 \times 10^9$/L). C-reactive protein (CRP) was 254 mg/L (norm: < 1 mg/L). Kidney function was normal. The patient's liver function tests showed mildly elevated transaminases (alanine transaminase: 58 U/L, norm: 7–56 U/L; aspartate transaminase: 108 U/L, norm: 10–40 U/L; alkaline phospatase: 124 U/L, norm 20-140 U/L; gamma-glutamyl transferase: 93 U/L, norm 0–51 U/L) and total bilirubin levels of 38 µmol/L (norm: < 26µmol/L) and direct bilirubin 35 µmol/L (norm: < 7 µmol/L). Oxygen saturation was 91% (norm: 95–100%). Giemsa-stained thick and thin films revealed spirochetes in large numbers (Figure 1) and no malaria parasites.

Given the patient's travel history, louse-borne relapsing fever was suspected. Their clinical deterioration was provoked by the ceftriaxone administration leading to a severe Jarisch–Herxheimer reaction [1]. Treatment was switched to doxycyline 200 mg per day intravenously to reduce the risk of relapse [2]. The patient was transferred to the intensive care unit (ICU) for fluid

**Figure 1**

Giemsa-stained thick film from a patient with louse-borne relapsing fever, the Netherlands, 4 July 2015




Photograph courtesy of B. Huizinga, Department of Medical Microbiology, University Medical Center Groningen.

**Figure 2**

Body louse (Pediculus humanus humanus) recovered from the clothing of a patient with louse-borne relapsing fever, the Netherlands, 7 July 2015

Photograph courtesy of M. Fonville, National Institute for Public Health and the Environment (RIVM).

resuscitation, cardiac support with noradrenalin and supportive oxygen delivery via high flow nose mask. *Borrelia recurrentis* was confirmed by 16S rDNA PCR and sequencing directly from blood two days later. The patient stayed at the ICU for two days, made a full recovery and was discharged after six days. The body louse *Pediculus humanus humanus* was recovered from their clothing (Figure 2).

## Patient 2

On 9 July 2015, a second young adult from Eritrea was directly referred by the responsible physician at the AZC to the UMCG. On arrival, the patient presented with general malaise, headache, fever (38.5 °C) and cough. Blood tests showed elevated inflammatory parameters (leucocytes: 12.7 × $10^9$/L, CRP: 320 mg/L), normal kidney function and slightly elevated transaminases, but the blood sample was haemolytic. Thick and thin films showed spirochetes and treatment was started with doxycyline 200 mg orally. Two hours later the patient developed a severe Jarisch–Herxheimer reaction which required admission to intensive care where they received fluid resuscitation, inotropic treatment with noradrenalin and oxygen via a face mask. *B. recurrentis* was confirmed by 16S rDNA sequencing.

The patient reported symptoms of chills and fever two weeks before presentation at our hospital. Their journey through North Africa followed the same route as that of Patient 1, but Patient 2 had arrived in Europe a week earlier. Patient 2 had camped out in the streets for five days in Rome (as had Patient 1). Patient 2 arrived in the Netherlands two weeks before presenting at our hospitals after travelling through Austria and Germany. The patient made a full recovery and was discharged after five days. Lice could not be recovered from the clothing.

## Discussion

*B. recurrentis* should be suspected in patients presenting with fever and a recent history of migration from or through endemic countries (Ethiopia, Sudan, Eritrea, and Somalia). The infection is transmitted through body lice (*P. humanus humanus*, formally known as *P. humanus corporis*) which typically lives and breeds in the seams of clothes but can occasionally also be found in bedlinen. Immigrants may share their clothing and that can pose an additional risk of transmission. The incubation period for relapsing fever is usually four to eight days with a range of two to 15 days [3]. It should be noted that head lice (*P. humanus capitis*) which are not uncommon in Northern Europe are incompetent vectors and cannot transmit *B. recurrentis*. The spirochetes are easily visible under a microscope in a Giemsa-stained thick or thin blood film as used for the diagnosis of *Plasmodium* spp [4]. In our patients, the diagnosis was confirmed in both cases by 16S rDNA PCR and sequencing from blood.

Published evidence supports a single dose of tetracycline 500 mg intravenously as the conventional treatment, but considering the limited availability of this drug, doxycycline 200 mg can be used as an effective alternative [2,5]. In young children, pregnant women, or patients with a tetracycline allergy, erythromycin 500 mg can be used instead [6].

Both patients had travelled independently along a similar route before arriving in the Netherlands. Given the incubation period, it cannot be ruled out that the infection was acquired within Europe. Crucial information about risk factors such as exact travel history, recollection of louse infestation or bites and onset of symptoms was, however, impossible to obtain from our

patients. Apart from being very sick and the fact that communication required an interpreter versed in Tigre our patients appeared to be traumatised and intimidated and not eager to volunteer information for fear of legal consequences.

Both patients developed a severe Jarisch–Herxheimer reaction after starting antibiotic treatment. *B. recurrentis* evades host immune defences, resulting in very high bacterial loads ($10^6$–$10^8$/µl), and effective antibiotic therapy is followed by severe reactions characterised by sudden rigors, fever and hypotension in virtually all treated patients [3]. Clinical symptoms are associated with increased plasma concentrations of tumour necrosis factor alpha (TNF-alpha), interleukin-6 and interleukin-8 [7]. Treating physicians should be aware of this complication and the chances that ICU admission may be warranted. It is advised that patients receive two well-placed intravenous lines for rapid fluid resuscitation. Treatment of Jarisch–Herxheimer reaction consists mainly of supportive care. Corticosteroids seem to have limited beneficial effect but studies suggests that TNF-alpha blockers may be useful [8].

An ad hoc survey at the AZC on 16 July found body lice on two newly arrived Eritreans. Since then, all asylum seekers arriving from endemic countries to the AZC have been segregated into a different compound, where they turn over all of their personal clothes in exchange for disposable overalls. Personal clothes are then washed and returned on the next day. Used overalls and bed linen are subsequently destroyed. In addition to delousing, all arrivals receive a single dose of ivermectin as pre-emptive treatment against scabies and Eritreans who arrive with clinically manifest scabies (about 80% of all new arrivals) receive a second dose a week later. No new cases of *B. recurrentis* infection have been identified since mandatory delousing was implemented.

## Conclusion

Because infections with *B. recurrentis* pose a significant health risk to other migrants, aid workers, healthcare personnel and arguably to the general population, screening and delousing should be considered for arriving migrants already at ports of entry into the European Union. Our patients may have acquired body lice before arriving in Europe but transmission of infected lice between migrants after arrival in Europe cannot be ruled out and could pose an additional public health challenge.


### Acknowledgements

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### Conflict of interest

None declared.

### Authors' contributions

KRW had responsibility in the clinical management of the patients and wrote the initial manuscript. YS had responsibility in the clinical management of the patients contributed to the collection of epidemiological information. BS carried out diagnostic confirmation. MB carried out the entomological investigation. DC had responsibility in the clinical management at the primary health care level and contributed to the entomological investigation. HG made the tentative diagnosis and wrote the initial manuscript. All authors contributed to the editing of the final manuscript.

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# Outbreak of *Salmonella* Enteritidis phage type 13a infection in Sweden linked to imported dried-vegetable spice mixes, December 2014 to July 2015

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**From 24 December to 24 July 2015, 174 cases were reported in a nationwide salmonellosis outbreak in Sweden: 108 cases were connected to a single restaurant. A spice mix, containing dried vegetables from the restaurant tested positive for the outbreak strain. Additional spice mixes with similar content from different suppliers also tested positive. The outbreak investigation suggests there could be a risk of contaminated products being also on the market in other countries.**

At the end of March 2015, 17 cases of *Salmonella* Enteritidis phage type (PT) 13a had been reported to SmiNet, the national surveillance system, from four counties in Sweden. The first case included in the outbreak was reported with onset of symptoms on 24 December 2014. By 24 July 2015, a total of 174 cases had been reported connected to the outbreak, making it one of the largest salmonellosis outbreaks in Sweden. The median age of the cases was 44 years (range: 1–93), 83 were female and 91 male, suggesting a food vehicle that could be consumed by a broad range of the population. The outbreak is still ongoing.

## Epidemiological and environmental investigation

An outbreak team was formed at the Public Health Agency of Sweden at the end of March 2015. The characteristics of the first 17 cases in terms of age and sex did not differ from the distribution in the general population. The local county medical offices conducted standardised interviews with the cases using a questionnaire. The interviews were conducted either by telephone, face-to-face or the questionnaire was filled in by the case. No specific food items stood out in the responses. However, in one of the counties, four of nine cases had eaten at the same sausage food stand and in another county, three of five cases had eaten at the same lunch restaurant. In mid-April, the Public Health Agency of Sweden initiated regular telephone conferences with all the relevant local authorities. The teleconferences were held on a weekly basis till the end of June to liaise with all relevant actors at national and local level and to keep everyone updated. In July, the teleconferences were held more often, due to the development in Kalmar county (see below).

The initial investigations, summed up at the first teleconference on 17 April, suggested that the vehicle of infection was most likely a food item with a long shelf life, e.g. something frozen, nuts or spices, given that the first case had been reported in December. By the end of May, the number of cases had risen to 48 (Figure) and were distributed over 14 counties. When interviewing the cases up to this time point, use of specific spice mixes containing dried vegetables, buying food from specialised food stores, consumption of chicken stood out as over-represented among the cases, but without statistical significance. Various food items were analysed for *Salmonella* spp., including two different brands of spice mixes containing dried vegetables. Up to the end of June, all tested negative for *Salmonella* spp.

At the beginning of June (week 23), Kalmar county reported a case who had fallen ill after eating at a restaurant that served many of their dishes on wooden boards. Cases continued to be reported and at the end of the following week (week 24), a total of six cases of salmonellosis had been reported from that same county. Interviews showed that all the six cases had eaten at the same restaurant in the county within the incubation period for symptom onset. As they all shared the outbreak strain, they could be connected to the *S.* Enteritidis PT 13a outbreak. The restaurant had served many guests, around 200, during the Swedish

midsummer holiday (19–20 June), some of whom were from Denmark, Norway and Germany. Local authorities reported that the majority of the cases at the restaurant had eaten pork tenderloin, served on a wooden board together with mashed potatoes. The pork tenderloin was one of the first main suspected contaminated food sources at the restaurant outbreak; however, analyses for *Salmonella* spp. were negative.

A municipal environment and health team collected additional food items, serving utensils and other kitchen utensils for testing from the restaurant. On 9 July, the laboratory at the National Veterinary Institute (SVA) in Uppsala, detected a *Salmonella* isolate in an unopened package of a spice mix collected from the restaurant (Brand A) originating from Serbia, containing dried vegetables, e.g. onions, carrots and parsnips. This spice mix had been used in many of the dishes. In addition, a pastry bag used for piping mashed potatoes servings was found positive for the outbreak strain. The mashed potatoes had been seasoned with the spice mix (Brand A). In total, 108 cases were connected to the restaurant, including staff members. Local authorities reported that many of the cases had severe symptoms, including bacteraemia, and were hospitalised. The exact numbers hospitalised is currently not yet verified.

## Microbiological investigation

In Sweden, *S.* Enteritidis is the second most common serotype comprising 19 % (97/501) of the domestic cases reported in 2014 [1]. The majority of isolates from domestic cases (›90%) are sent to the Public Health Agency of Sweden in Solna for subtyping for outbreak detection and surveillance purposes. Each year, two to five cases with PT 13a are reported. In 2014, four of the domestic *S.* Enteritidis cases were PT 13a [1].

Phage typing has been the gold standard for subtyping of *S.* Enteritidis [2]; however, multiple-locus variable-number of tandem-repeats analysis (MLVA) [3] was recently set up at the Agency, enabling further subtyping of isolates. Whole genome sequencing as a first-line tool for typing is under development at the Agency. All three methods were used in the current outbreak.

As at 24 July, 174 cases in the outbreak had been laboratory confirmed with salmonellosis. Of these, 123 had been further subtyped as *S.* Enteritidis PT 13a; the additional 51 salmonellosis cases were epidemiologically linked to the outbreak. In addition, 102 human isolates and all six environmental isolates had been further subtyped by MLVA. Before the onset of the outbreak in Kalmar county, three profiles had been identified in the national outbreak. According to the nomenclature SENT7-SENT5-SENT6-SENT4-SE3 [3], their profiles were 2-10-7-5-2, 2-12-8-5-2 and 2-11-6-5-2. Cases with the same MLVA profile were at that time point more or less evenly distributed both in time and place, i.e. there was no clustering of a specific MLVA profile in one county or on one specific occasion limited in time. In the Kalmar county restaurant outbreak the profile 2-11-6-5-2 was the only one identified.

A subset of human isolates (n = 12), four from each MLVA profile, was analysed by whole genome sequencing using the Ion Torrent platform at the Agency. Within each MLVA profile, the isolates were identical or differed by one single nucleotide polymorphism (SNP). Between the three MLVA profiles, the isolates differed by about 40–50 SNPs.

All three MLVA profiles were regarded as being part of the same outbreak because the particular PT (13a) is normally only seen up to five times a year in Sweden. Moreover, the scenario that three parallel outbreaks (parallel in terms of time and place) of PT 13a infection were taking place seemed unlikely. A plausible scenario was that the source of the bacteria was contaminated with multiple *S.* Enteritidis subtypes. All isolates from the cases connected to the outbreak in Kalmar county that had been typed as at 24 July (n = 43) had the same MLVA profile (2-11-6-5-2), as did isolates from the Brand A spice mix (n = 3) and the pastry bag from the restaurant (n=1).

## International aspects

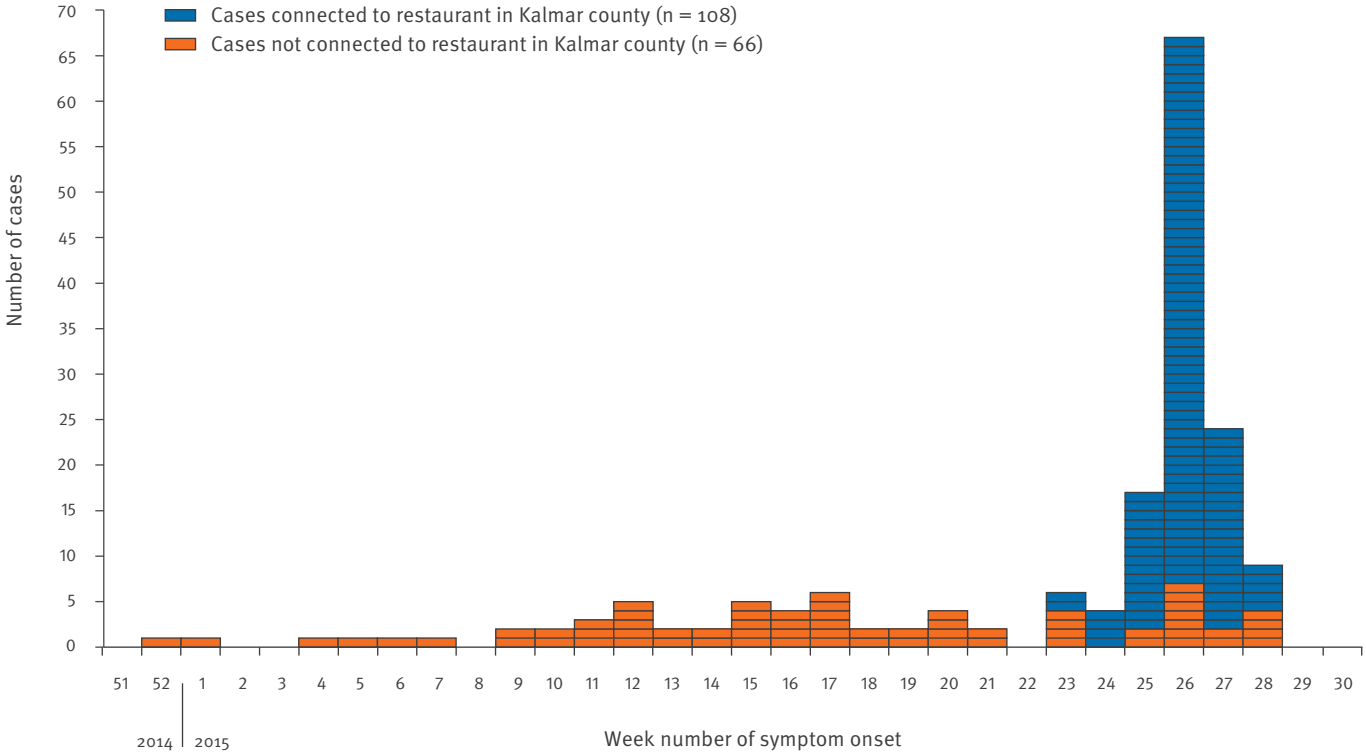
Sweden launched an 'urgent inquiry' in the Epidemic Intelligence Information System (EPIS) [4], a web-based communication platform run by the European Centre for Disease Prevention and Control (ECDC), on 2 April 2015. No other country has to date reported an increase of this specific subtype of *S.* Enteritidis. However, many European Union Member States only subtype *S.* Enteritidis in outbreak situations or cluster investigations and in addition, phage typing or MLVA is not performed in all EU countries. Thus, a relatively minor increase in number of cases with this *S.* Enteritidis PT could go unnoticed.

A Rapid Alert System for Food and Feed (RASFF) [5] alert (reference 2015.0825) was issued by Austria on 26 June, in which it was reported that a *Salmonella* spp. had been detected in a spice mix/seasoning Brand B from a manufacturer in Croatia, with a similar content of dried vegetables as Brand A. Batches of this product, which had been distributed to Austria, Germany and Slovakia, were voluntarily recalled from the market by the manufacturer (announced in a press release by the company on 1 July). The specific batch of Brand B from which *Salmonella* spp. had been detected had not been distributed to Sweden. The serotype, however, was *S.* Oranienburg (not *S.* Enteritidis PT 13a), according to the Austrian Salmonella Reference Centre (Christian Kornschober, personal communication, 27 July 2015).

The Swedish National Food Agency issued a RASFF notification for information on 9 July (reference 2015.0873), after the Brand A spice mix had been found positive for *Salmonella* Enteritidis in the outbreak in Kalmar county.

**Figure**

Number of salmonellosis cases by week number of symptom onset connected to the *Salmonella* Enteritidis phage type 13a outbreak, Sweden, 24 December 2014–24 July 2015[a] (n = 174)




[a] Week 52 2014 to week 30 2015.

Whole genome sequencing data were shared with Public Health England, United Kingdom, which had during 2009 to 2014 reported the largest number (48%) of *S.* Enteritidis PT 13a to the European Surveillance System (TESSy) [6], the ECDC system for reporting data. In addition, comparison with an *S.* Enteritidis PT 13a isolate from Luxemburg from 2014, sharing one of the MLVA profiles, was compared using whole genome sequencing data. No clustering of the isolates indicating relationship with the Swedish outbreak could be seen, as assessed by whole genome sequencing.

## Testing of food items

In addition to the *S.* Enteritidis PT 13a-positive Brand A spice mix from an unopened package from the restaurant, two more samples (opened packages) of Brand A, from two other counties in Sweden tested positive for PT 13a in July. These two additional positive samples of Brand A had the same MLVA profile as the isolate from the spice mix from the restaurant in Kalmar county (2-11-6-5-2). Two Brand C spice mix samples, manufactured in Bosnia and Herzegovina and Serbia, which came from opened packages from two different households in Sweden, had two different profiles: 2-10-7-5-2, which is one of the profiles seen in cases in the ongoing outbreak, and 2-10-8-8-2, which is a profile that has not been identified in cases in this outbreak. When an opened package is found positive for *Salmonella* spp., this could mean that the spice mix had been contaminated after opening. The manufacturer of Brand C voluntarily recalled the product on 6 July as a precaution, after they were informed about a possible link of the product to the outbreak.

During the analysis of the food items, and specifically the spice mixes, it was observed that a more sensitive method than the commonly used Nordisk Metodikkommitté för Livsmedel (NMKL) number 71 method, corresponding to International Organization for Standardization (ISO) 6579:2002/Corr 1:4 [7], could be needed. Additional dilution steps and number of replicates were often necessary in order to detect and isolate *Salmonella* spp., indicating very low concentration of the pathogen in the spices.

## Background

*Salmonella* spp. can survive for an extended period of time (exceeding one year) in dried products such as spices, due to high tolerance to desiccation stress [8]. Contamination of spices with *Salmonella* spp. can be from a wide variety of sources, such as soil, water, rodents, birds and insects [8]. The United States (US) Food and Drug Administration (FDA) have shown a wide diversity of *Salmonella* spp. serotypes in spices imported to the US, which may well be a reflection of the wide range of the sources of contamination [8]. Several outbreaks of *Salmonella* spp. infection

associated with spices in Europe identified many different serotypes within the same outbreak [9,10].

Spices and herbs contaminated with *Salmonella* spp. have been associated with a variety of food-borne outbreaks in Europe and North America [11]. The largest spice-associated salmonellosis outbreak occurred in 1993 in Germany, in which it was estimated that there were more than 1,000 cases of salmonellosis [9]. A variety of *Salmonella* serotypes were identified during the outbreak and *Salmonella* Saintpaul, Javiana and Rubislaw were isolated from paprika and paprika-powdered potato chips and from patients. Trace-back investigations and product testing identified paprika, used in seasoning for potato chips, as the contaminated food vehicle.

A more recent outbreak associated with spices occurred in the United Kingdom in 2013. A total of 413 cases of gastroenteritis were associated with a street market spice festival [10]. Uncooked curry leaves that were contaminated with *Salmonella* Agona PT40 were the source of infection. Additional serotypes, as well as other species of *Enterobacteriaceae*, were isolated from patients; however, *S.* Agona PT40 was predominant.

## Ongoing assessments and conclusion

As at 24 July, the outbreak in Sweden currently includes 174 cases, reported from 17 of the 21 counties in Sweden. No other EU countries have confirmed outbreaks or an increase in the number of cases with the outbreak strain. In countries with a high number of reported cases of *S.* Enteritidis infection, however, a small increase in number of cases with the outbreak strain could go undetected. The detection of *Salmonella* spp. in two different brands of spice mixes sold in Sweden and the RASFF alert from Austria during the outbreak period, however, indicates that there could be a common ingredient in these mixes that could be contaminated with *Salmonella* spp. Thus, there is a risk that contaminated products are also on the market in other countries.

Information and updates about the outbreak have been posted on the websites of the Public Health Agency of Sweden and the National Food Agency. Press releases from local investigators as well as from the national authorities have also been issued. The outbreak received a lot of media attention in connection with the increasing number of cases connected to the restaurant in Kalmar county, which has been closed due to the outbreak. The national outbreak is still ongoing and more spice mixes are being analysed. This outbreak highlights the complexity of a stealth food vehicle ('stealth foods are those that people may eat but are unlikely to remember' [12]) with a long shelf life.


### Acknowledgements

We would like to acknowledge all the members of the Outbreak Control Team: county medical offices, clinical microbiological laboratories, municipality environmental and health authorities, the Swedish Veterinary Institute and the National Food Agency for their valuable work in the investigation. We would especially like to thank the local authorities in the county of Kalmar.

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### Conflict of interest

None declared.

### Authors' contributions

Cecilia Jernberg drafted the manuscript, was part of the outbreak investigation team and responsible for the international communications and data sharing. Emma Löf was responsible for the epidemiological data collection during the study period and coordination of the local authorities, and critically reviewed the manuscript. Marika Hjertqvist was responsible for epidemiological coordination in the Kalmar outbreak. Anna Pääjärvi was responsible for the microbiological analysis during the study period and Camilla Sundborger for the microbiological analysis during the Kalmar outbreak. Elsie Castro, Margareta Löfdahl, Lena Sundqvist were part of the outbreak investigation team. All authors read and approved the manuscript.

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# Future directions for the European influenza reference laboratory network in influenza surveillance

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**By defining strategic objectives for the network of influenza laboratories that have national influenza centre status or national function within European Union Member States, Iceland and Norway, it is possible to align their priorities in undertaking virological surveillance of influenza. This will help maintain and develop the network to meet and adapt to new challenges over the next 3–5 years and underpin a longer-term strategy over 5–10 years. We analysed the key activities undertaken by influenza reference laboratories in Europe and categorised them into a framework of four key strategic objectives areas: enhancing laboratory capability, ensuring laboratory capacity, providing emergency response and translating laboratory data into information for public health action. We make recommendations on the priority areas for future development.**

## Introduction

Functional reference laboratory networks capable of undertaking detailed strain characterisation are an important element of communicable disease control. Such networks are major contributors to public health intervention policies through provision of timely and detailed scientific data.

Continuing emergence of influenza viruses emphasises the need for accurate and rapid detection capability and detailed strain characterisation. The influenza A(H1N1)pdm09 pandemic [1], the emergence of influenza A(H3N2)v in the United States (US) in 2012 [2] and the more recent emergence of influenza A(H7N9) [3], influenza A(H9N2) [4], influenza A(H10N8) [5] and the novel Middle East respiratory syndrome coronavirus (MERS-CoV) infections in humans in 2013 [6] have highlighted the continual threat to human health from new and emerging respiratory viruses with pandemic potential.

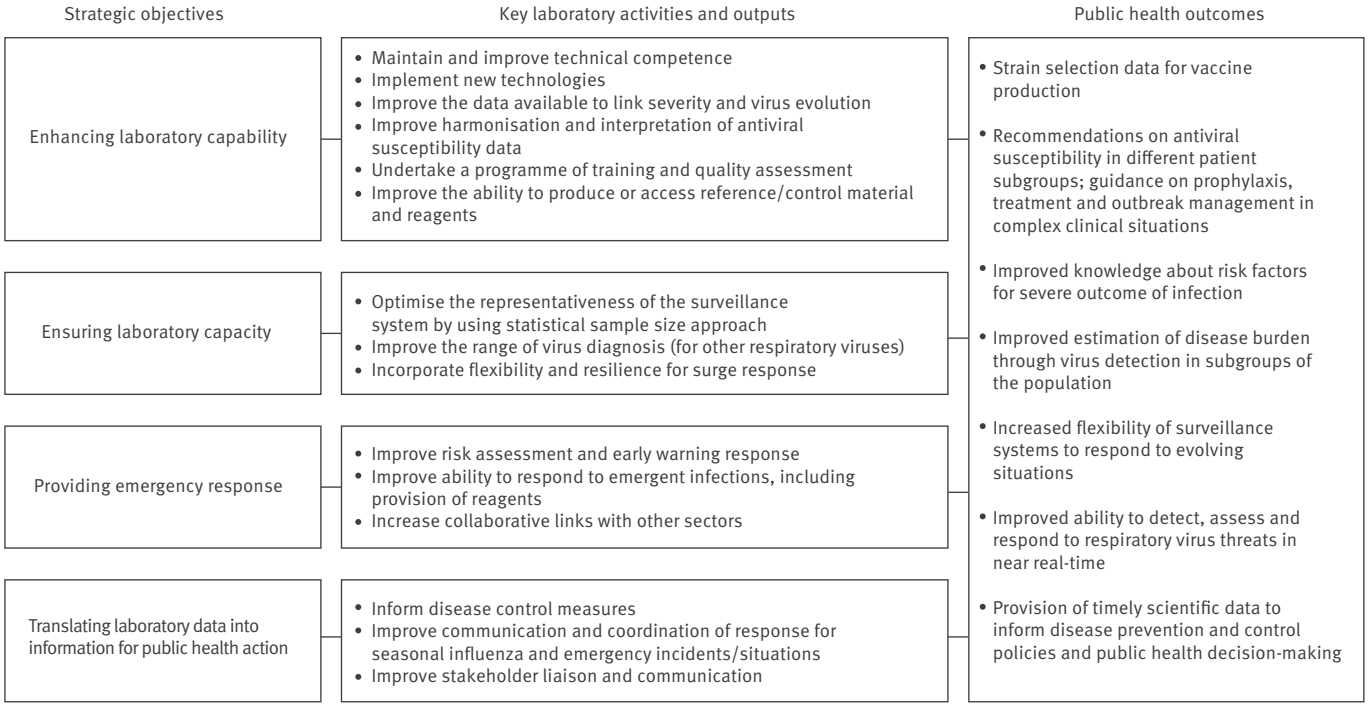
Virological data are collected through continuous global surveillance to monitor the characteristics of circulating influenza viruses, for example, in virus antigenicity, genetics and antiviral susceptibility to guide appropriate response and intervention activities. Data are used to inform the selection of the best candidate vaccine strains for the annual influenza vaccine. The surveillance activities inform a proportionate response that can be escalated in a rapid and coordinated manner as required.

The influenza laboratory network in Europe is well established and has performed virological surveillance since 1952 through World Health Organization (WHO)-recognised national influenza centres (NICs) [7]. The European Influenza laboratory network was further strengthened in 1995 by the formation of the European Influenza Surveillance System (EISS) [8], which in 2003 became the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL) [9]. Since then, the European Influenza laboratory network has been coordinated by the European Centre for Disease Prevention and Control (ECDC) and the WHO Regional Office for Europe (WHO/Europe) with the assistance of a group of experts from within the network, and is now termed the European Reference Laboratory Network for Human Influenza (ERLI-Net). The key capabilities of this network within Europe currently include:

- ability to respond to new and emerging influenza viruses through the development of laboratory diagnostic capabilities and data capture systems that link to clinical surveillance;
- provision of an appropriately trained cohort of virologists/microbiologists with the necessary laboratory surveillance techniques, in particular skills in virus detection and virus isolation;

**Figure 1**

Key laboratory activities and outputs and the resulting public health outcomes for each strategic objective




- generation of genetic and antigenic data on circulating influenza viruses;
- generation of phenotypic and genotypic data on influenza antiviral susceptibility;
- capability and capacity to respond to a rapid upsurge in laboratory activity as a result of an epidemic or pandemic of influenza viruses, with resilience to sustain a long-term response to an emerging threat;
- participation in regular external quality assurance (EQA) activities, underpinned by targeted training where necessary, to ensure reliability of results generated for diagnostic and surveillance purposes.

Changing health service priorities and political structures within the WHO European Region mean it is timely to consider the alignment of key laboratory networks and their future roles, to ensure maximum benefit from specialist activities.

## Objectives of this analysis

To help align the diverse priorities of ECDC and WHO/Europe, the development of common strategic objectives was undertaken to provide a framework for the maintenance and further development of a high-quality, cohesive laboratory network for the virological surveillance of influenza within the European Region. Strategic priorities were developed through consultation and a Delphi structured communication process over a period of six months in 2013 involving ERLI-Net members. This was used to further refine the strategic priorities before submission to ECDC and WHO/Europe.

## Strategic priorities for the European influenza laboratory network

While national influenza laboratory surveillance systems across Europe differ in their foundation, organisation, funding basis and regional interaction with national authorities, the influenza virological surveillance activities undertaken by NICs are largely consistent.

Laboratory activities were categorised into four key strategic objectives in order to provide a framework to guide laboratories in prioritising their influenza surveillance activities, and to aid the identification of operational issues and challenges for the maintenance and development of the laboratory network in order to meet and adapt to new challenges, including the emergence of other respiratory virus threats, over the coming years: (i) enhancing laboratory capability; (ii) ensuring laboratory capacity; (iii) providing emergency response; and (iv) translating laboratory data into information for public health action.

The key laboratory activities and outputs, and the resulting public health outcomes in each of the strategic objectives are illustrated in Figure 1. The priority areas for development in each strategic objective are shown in the Table.

## Enhancing laboratory capability

The key priorities in ensuring that the laboratory network has the capability to characterise unusual influenza A viruses, send isolates for vaccine strain selection to the WHO Collaborating Centre for Reference and Research on Influenza, London, and operate under

**Table**

Recommendations for the development of the influenza laboratory network in Europe in key strategic objectives

| Strategic objectives | Suggested actions in priority areas |
|---|---|
| Enhancing laboratory capability | • Undertake gap analysis to identify network requirements and develop workplan of remedial actions.<br>• Improve coordination of training activities between WHO/Europe and ECDC.<br>• Ensure expertise and technical support for developments so that network is responding to the latest technological advances (e.g. generic PCRs, multiplex-PCRs, next generation sequencing, point-of-care testing).<br>• Develop structured training activities to (i) address performance issues identified through EQA and (ii) provide refresher/advanced training to laboratories with more established capabilities.<br>• Align laboratory core competences based on expert consultation of technology feasibility and network priorities for public health benefit.<br>• Improve the completeness and quality of antiviral susceptibility data reporting to inform use of antiviral therapies and clinical guidance.<br>• Development of web-based in silico (sequence questionnaire tools) to enhance diagnostic capability within the network in the event of an emerging virus. |
| Ensuring laboratory capacity | • Optimise the representativeness of country-level surveillance systems, e.g. using statistical sample size.<br>• Ensure robust virological data from a range of healthcare settings e.g. primary care, secondary care (both routine hospital admissions and critical care), and from a spectrum of clinical illness from subclinical (through population-based surveillance studies and serological assessment) to severe illness (SARI) and death.<br>• Virologists from network laboratories should be encouraged to liaise with their epidemiology colleagues to develop plans for statistical and systematic approaches to support evidence-based decisions. |
| Providing emergency response | • Improve risk assessment and early warning response, e.g. in the event of the emergence of a new virus subtype, it may be necessary to quickly modify the sampling strategy to target population subgroups preferentially affected (e.g. young children or pregnant women) to obtain data to inform the response.<br>• Effective use of virological data to enable early recognition of infection threats or prediction of epidemiological trends, and facilitate early public health intervention.<br>• Incorporate flexibility and resilience for surge response with respect to diagnostics and workforce.<br>• Assess current status of BSL-3 facilities within the laboratory network, and develop plan to ensure all countries have access to BSL-3 facilities to respond to highly pathogenic influenza virus strains.<br>• Develop a European laboratory network outbreak/emergency response plan so that network members are aware of coordination arrangements and what outputs can be expected.<br>• Assessment and further development of laboratory testing algorithms. |
| Translating laboratory data into information for public health action | • Use laboratory surveillance data more effectively in order to inform disease control measures.<br>• Reshape the outputs of the laboratory network to improve the public health focus and visibility to network members, politicians, academia, etc.<br>• Develop more high-quality automated data processing to ensure that surveillance outputs are timelier, more accessible and user-customisable to meet the requirements of relevant stakeholders. |

BSL-3: biosafety level 3; ECDC: European Centre for Disease Prevention and Control; EQA: external quality assessment; PCR: polymerase chain reaction; SARI: severe acute respiratory illness; WHO/Europe: World Health Organization Regional Office for Europe.

biosafety level 3 (BSL-3) conditions to detect highly pathogenic influenza viruses, as well as other emerging respiratory virus threats such as MERS-CoV, are as follows. First, maintain technical skills such as virus isolation in embryonated chicken eggs and antigenic characterisation of virus isolates that are beginning to decline due to a shift to molecular technologies. Second, increase laboratory preparedness and resilience through the introduction of new capabilities such as molecular detection, sequencing and next generation sequencing.

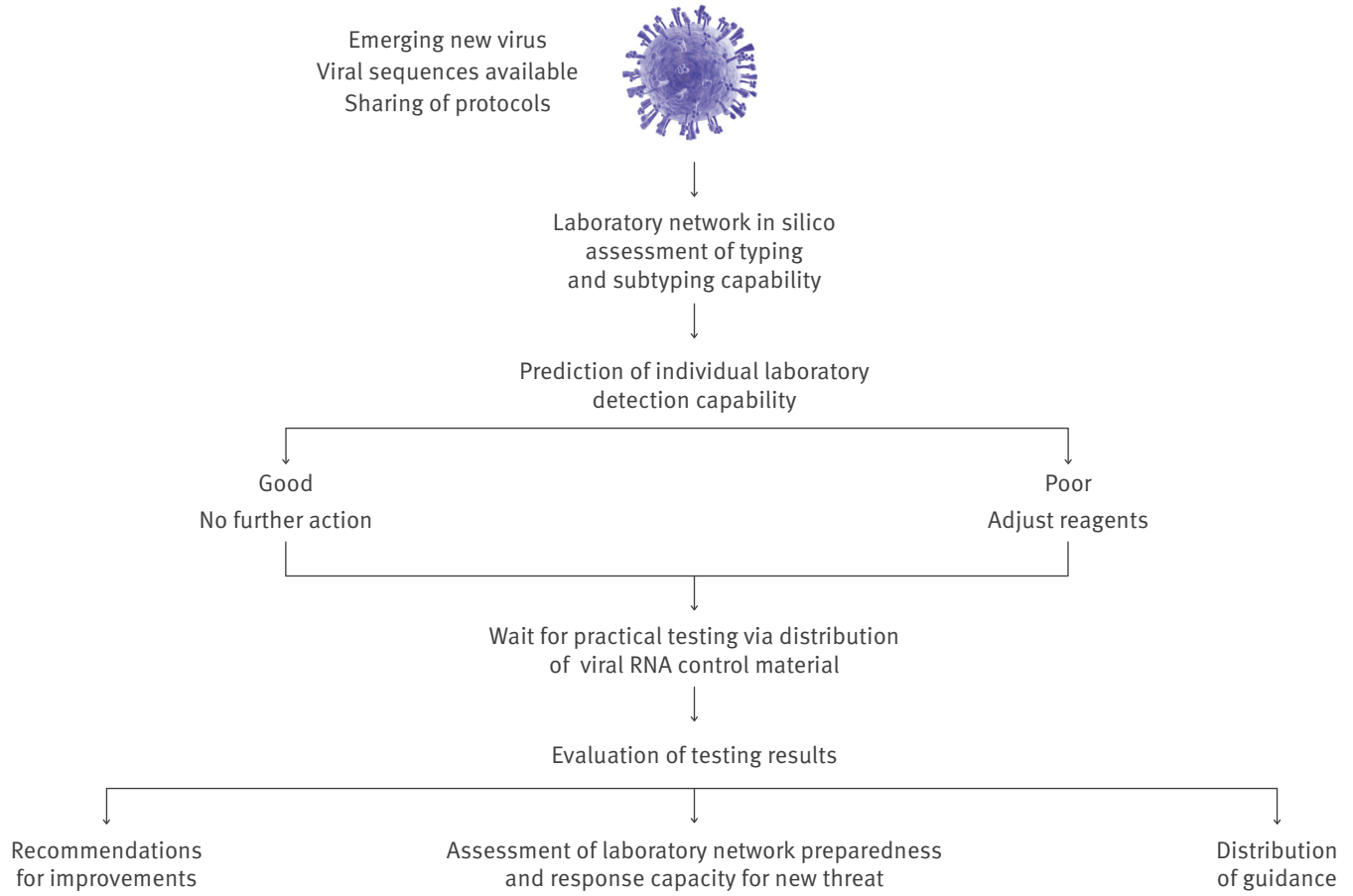
To meet the diverse requirements of the European Region, where laboratories are geographically separated and developments are often undertaken at different speeds, a coordinated programme of training is required including the following: 'wet laboratory' practical training; a shift towards e-learning and distance-learning technologies; annual meetings; twinning and use of EQA to identify gaps and weaknesses in capability.

The rate of introduction of new skills into the laboratory network is dependent on underlying funding mechanisms and decisions about which capabilities to introduce versus which to omit must be undertaken at a strategic level.

Initiatives have been under way within ERLI-Net over recent years to offer network members a comprehensive programme of laboratory training, including practical-based training underpinned by theoretical instruction (for example, influenza virus isolation and characterisation and antiviral susceptibility testing), lecture-based theoretical instruction (for example, influenza sequencing) and more recently, e-learning webcast (for training sessions on primer design). A twinning initiative has been established to foster interaction and support between laboratory network members, supported by a small financial grant to cover

**Figure 2**

Improving the capability for virus diagnosis within the European influenza laboratory network




Source of image: ©Istockphoto

travel and subsistence costs to visit a partner laboratory. It is hoped that long-standing collaborations will be established between network laboratories to help improve resilience and communication.

Both WHO/Europe and ERLI-Net coordinate regular EQA programmes to evaluate the technical capability of the influenza network of laboratories. ERLI-Net undertakes biennial EQA of laboratory capability in European Union (EU)/European Economic Area (EEA) countries, results of which inform a programme of training activities. Through the regular collection of EQA data for the ERLI-Net, it is possible to undertake comparative analyses and identify changes in performance. The most recent EQA in 2013 included virus isolation and antigenic and genetic characterisation, and phenotypic and genetic characterisation of antiviral susceptibility techniques, which are not currently covered by the WHO EQA Project (EQAP) [10]. The 2011 antiviral EQA assessment by ERLI-Net was the first of its kind globally [11].

Laboratory preparedness and technical performance has also been assessed, through an 'in silico' exercise in 2011 to detect novel reassortant and circulating triple reassortant (TRA) influenza A(H3N2) swine viruses in humans [12], and more recently, in 2013, in collaboration with ECDC and WHO/Europe, for the rapid assessment of laboratory preparedness for detection of the novel avian influenza A(H7N9) virus in EU/EEA countries [13].

In the future, rapid assessment activities, as illustrated in Figure 2, should be extended to all laboratories in the European Region to provide assurance on the range of virus diagnosis.

## Ensuring laboratory capacity

The key priorities in ensuring capacity of laboratories in the network include ensuring robust staffing mechanisms and succession planning are in place, and ensuring the capacity to scale up the surveillance response and link to clinical surveillance programmes.

Network activities need to focus on staff succession planning to ensure that the European influenza laboratory network has the capacity to meet the shifting technological priorities. Surveillance development initiatives need to link virological data to clinical surveillance programmes, and be underpinned by robust statistical data to ensure the network will target its

resources in the most efficient way, help inform effective intervention strategies, and provide a scaleable response in the event of a future epidemic or pandemic.

### Providing emergency response

To provide timely and effective emergency response, the European influenza laboratory network needs to have coordinated arrangements for risk assessment and early warning.

Following the emergence of the influenza A(H7N9) virus in early 2013, ECDC, WHO/Europe and the ERLI-Net Coordination Team convened regularly by teleconference to plan activities to assess network capability, distribute positive-control viruses and virus RNA, provide relevant information on primers/probes for diagnosing influenza A viruses with pandemic potential, and to draft a technical briefing note on diagnostic preparedness [14]. Since then, regular communication has taken place between all partner organisations as part of early warning and response activities.

### Translating laboratory data into improved public health outputs

The influenza laboratory network needs to maximise the public health outputs from routinely generated virological surveillance data. The priorities include: (i) targeted surveillance of deaths and serious illness due to influenza; (ii) estimating influenza vaccine effectiveness and identifying influenza vaccine failures; (iii) timely analysis and interpretation of data to inform public health action; and (iv) improved technical data provision through improved visual display and dissemination.

A number of initiatives are already under way to use the wealth of virological data generated by the influenza laboratory network to strengthen evidence-based decision-making: for example, some laboratories currently undertake surveillance for severe disease and deaths due to influenza (SARI surveillance). These data are incorporated into the routine weekly influenza surveillance overview (WISO) produced by ECDC [15]. Further work is underway to improve the quality and comparability of SARI surveillance data across the European region [16], and increase the number of countries who currently participate.

ERLI-Net produces regular influenza virus characterisation surveillance reports for ECDC, which present the results of the antigenic and genetic analysis of influenza viruses isolated in Europe undertaken by WHO Collaborating Centre for Reference and Research on Influenza in London [17]. The reports provide network virologists with detailed technical data that allow them to compare viruses they have isolated in a wider background context. This may help assist evaluation of circulating strains within different countries.

A single software platform was implemented in the 2014/15 influenza season for data collection across the whole European region, rather than the previous separate ECDC- and WHO/Europe-run systems. Data collected via this platform have been used to produce a single joint surveillance bulletin [18], thereby improving consistency in communication across the region.

## Conclusions

Successful implementation of these strategic objectives will produce a European laboratory network that can deliver the following:

- the capability and capacity to monitor seasonal influenza and to respond to the emergence of novel influenza virus subtypes and reassortants, as well as other newly emerging respiratory virus infections in a timely and coordinated manner;
- a scalable response in the event of a newly emerging influenza virus or other novel respiratory virus threat;
- expertise and technical developments to ensure that it is responding to the latest technological advances (e.g. generic PCRs, multiplex PCRs, next generation sequencing, point-of-care testing);
- outputs required by stakeholders to inform public health action;
- the provision of timely and accurate surveillance data to health professionals and commissioners of healthcare services to inform effective intervention (e.g. vaccination programmes or antiviral treatment) and improve patient outcomes;
- a programme of EQA to ensure the technical capability of laboratories within the network;
- a programme of training and development for virologists within the network to maintain technical competence and ensure continuing professional development;
- identification of areas for further research and development in diagnostics, surveillance and control of infection (through antiviral drugs and vaccination programmes).

Influenza laboratories across Europe operate in different contexts, for example, public health institutes, universities or research agencies, with little or no consistency in funding mechanisms. As a result, the laboratories use different technical approaches and diagnostic platforms according to local need, which is challenging for the standardisation of molecular activities in particular. As the financial pressures of the current economic climate are expected to continue over the coming years, a more systematic and strategic approach to virological surveillance is needed to respond to shifting priorities.

Optimisation of surveillance systems using, for example, statistical sample size calculations, will provide a desired level of confidence in the data to inform situational assessments, improve system approaches, focus resources and justify funding needs. By enhancing the public health benefit of the data through timely reporting and analysis, it will be possible to strengthen the evidence-based decision-making to inform policymakers and disease prevention and control strategies within a country and across the wider European Region.

Strategic planning for the European influenza laboratory network coincides with ongoing surveillance development activities under way within Europe [19] and in the US [20]. A number of the activities outlined in the Table are already under way within ERLI-Net. In order to achieve efficient and effective implementation of the recommendations across the European Region, ECDC and WHO/Europe will need to develop a systematic approach to assessing current laboratory capability and capacity, and provide the tools necessary to aid planning and resource allocation within defined timescales. This will ensure that the network is able to respond in a strategic and efficient manner to new challenges over the next 3–5 years and develop a longer-term strategy over the next 5–10 years, thereby contributing effectively to the global surveillance of influenza and other emerging respiratory virus infections.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## Conflict of interest

None declared.

## Authors' contributions

MZ, NG and HRA initiated the discussions on strategic development; all co-authors were involved in subsequent discussion and development of key strategic development areas. NG and HRA presented the strategy at European influenza network workshops and meetings and facilitated discussion with network members. NG drafted the initial manuscript with HRA; all co-authors reviewed and commented including approval of the final version.

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