Impact factor 5·9

Eurosurveillance

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

• Featuring a selection of articles published between 1996 and 2016

ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Editorial team

Based at the European Centre for
Disease Prevention and Control (ECDC),
171 65 Stockholm, Sweden

Telephone number

+46 (0)8 58 60 11 38

E-mail

eurosurveillance@ecdc.europa.eu

Online submission system

http://www.editorialmanager.com/eurosurveillance/

www.eurosurveillance.org

Editorial advisors

Contents

Editorial

20 years of communicating facts and figures

Ines-Steffens[1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Correspondence: Ines-Steffens (ines.steffens@ecdc.europa.eu)

Since 1995, when a first pilot issue was published, *Eurosurveillance* has provided the European public health community with a platform to exchange relevant findings on communicable disease surveillance, prevention and control. From the outset, the journal has been open access and has not charged article processing costs.

In 2016, we celebrate 20 years of regular publication. A glimpse at the *Eurosurveillance* archives demonstrates how the journal has matured over the years in terms of format and content. It shows, for example, the merging of the formerly weekly and monthly issues, acceptance of the 'weekly' for indexing in PubMed/MEDLINE and the evolution from a print and online journal to a full online journal and a gradual geographical expansion of the origin of published articles.

However, already from the start, topics covered were remarkably similar to those that are high on the public health agenda today. One of the articles in the pilot issue in 1995 gave an overview of immunisation schedules in Europe [1], a topic still of interest nowadays. Our aim to provide insightful and balanced information on vaccination was shown after the later retracted publication by Wakefield et al. that included subsequently falsified claims of an association of measles mumps and rubella vaccines with autism [2]. Just one week afterwards, *Eurosurveillance* ran a commentary in its weekly edition, followed, two months later, by one entitled 'Further evidence that MMR vaccine, inflammatory bowel disease, and autism are not linked' [3,4]. The public health challenges that Europe faces in reaching the measles elimination goal in Europe were marked in a 'Spotlight on measles' series on ongoing outbreaks and their implications [5].

Since the early days of the journal, surveillance network outputs and outbreak reports have been regular content [6], with topics such as HIV/AIDS and other sexually transmitted infections [7-9], emerging (vector-borne) diseases [10], influenza [11], antimicrobial resistance [12], tuberculosis [13,14] and food- and waterborne diseases [15]. As illustrated by the following subjective selection of articles from the past two decades, public health events and other topics with general public health relevance have also been covered, such as the preparedness for bioterrorism after the 11 September attacks in 2001 in the United States [16], the outbreak of severe acute respiratory syndrome (SARS) [17], the 2009 influenza pandemic [18], the emergence of Middle East respiratory syndrome (MERS) [19], as well as the setup of the European Programme for Intervention Epidemiology Training (EPIET) programme [20] and discussions about establishing a European Centre for Disease Control [21].

Rapid communications were an early feature for the journal at a time when rapid processing of articles was not a common element of scientific journals. The evolution, growth and opportunities offered by the Internet facilitated timely communication and fast turnaround times tremendously. The initially short news-like items are the element of the journal that has most evolved. Today, rapid communications are well-recognised short scientific dispatches. Several of them are among our most highly cited articles, but more importantly, their value has been in their impact on public health practice.

While we have been able to present 'firsts' on several occasions [22,23] and track epidemics and emerging diseases in a timely manner [24], we are publishing an increasing number of (systematic) reviews to provide sound evidence and support for decisionmaking [25].
Working with *Eurosurveillance* is rewarding. The journal has many supporters and collaborators in Europe and beyond whom we are not able to name individually. We would like to express our gratitude to them and also thank our board members, colleagues and publisher wholeheartedly for their continued support. Our 20th anniversary is a reason to celebrate. We marked the occasion on Wednesday 30 November with a lunchtime seminar '20 years of communicating facts and figures in a changing environment', held on the margins of the European Scientific Conference on Applied Infectious Diseases Epidemiology (ESCAIDE). Two eminent speakers, David Heymann and Lawrence Madoff, highlighted changes in sharing information about communicable diseases from a public health perspective over the past 20 years. In addition, we present this selection of articles as a snapshot of the journal's publications and evolution. The topics covered match those that have remained relevant over two decades and we hope our readers will enjoy browsing through this compilation.

References

1. Guérin N, Roure C. Immunisation schedules in the countries of the European Union. Euro Surveill. 1995;0(0):5-7. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=201 PMID:12631752
2. Retracted: Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Lancet. 2010 Feb 6;375(9713):445. doi: 10.1016/S0140-6736(10)60175-4.
3. Miller E. Reported association between measles, mumps, and rubella (MMR) vaccine, autism, and bowel syndrome. Euro Surveill. 1998;2(10):1247. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1247
4. Pebody RG. Further evidence that MMR vaccine, inflammatory bowel disease, and autism are not linked. Euro Surveill. 1998;2(19):1219. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1219
5. Slaymaker E, Joseph C, Bartlett CL. Travel associated legionnaires disease in Europe: 1997 and 1998. Euro Surveill. 1999;4(11):120-4. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=62 PMID:12631885
6. Rogalska J, Santibanez S, Mankertz A, Makowka A, Szenborn L, Stefanoff P. Spotlight on measles 2010: An epidemiological overview of measles outbreaks in Poland in relation to the measles elimination goal. Euro Surveill. 2010;15(17):19549. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19549 PMID:20460084
7. Nicoll A. Global HIV epidemic. Euro Surveill. 1998;2(26):1196. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1196
8. Infuso A, Hamers FF, Downs AM, Alix J. HIV reporting in western Europe: national systems and first European data. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=29
9. Velicko I, Kühlmann-Berenzon S, Blaxhult A. Reasons for the sharp increase of genital chlamydia infections reported in the first months of 2007 in Sweden. Euro Surveill. 2007;12(10):E5-6. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=737 PMID:17997928
10. Zeller H, Zientara S, Hars J. West Nile outbreak in horses in Southern France: September 2004. Euro Surveill. 2004;8(41):2564. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2564
11. Watson J. Four cases of H5N1 influenza in Hong Kong. Euro Surveill. 1997;1(33):1010. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1010</jrn</jrn
12. van den Hof S, van der Kooi T, van den Berg R, Kuijper EJ, Notermans DW. Clostridium difficile PCR ribotype 027 outbreaks in the Netherlands: recent surveillance data indicate that outbreaks are not easily controlled but interhospital transmission is limited. Euro Surveill. 2006;11(1):E060126.2. PMID:16801713
13. Handysides S. Surveillance of tuberculosis in Europe: first data emerge from “EuroTB”. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1031
14. Ködmön C, Zucs P, van der Werf MJ. Migration-related tuberculosis: epidemiology and characteristics of tuberculosis cases originating outside the European Union and European Economic Area, 2007 to 2013. Euro Surveill. 2016;21(12):30164. PMID:27039665 http://dx.doi.org/10.2807/1560-7917.ES.2016.21.12.30164
15. de Valk H, Jacquet C, Goulet V, Vaillant V, Perra A, Simon F, et al.; Listeria Surveillance Feasibility Study Participants. Surveillance of listeria infections in Europe. Euro Surveill. 2005;10(10):251-5. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=572 PMID:16282642
16. Coignard B; Members of the Eurosurveillance editorial board. Bioterrorism preparedness and response in European public health institutes. Euro Surveill. 2001;6(11):159-66. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=383 PMID:11891386
17. Radun D, Niedrig M, Ammon A, Stark K. SARS: retrospective cohort study among German guests of the Hotel ‘M’, Hong Kong. Euro Surveill. 2003;8(12):228-30. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=436 PMID:14724331
18. Surveillance Group for New Influenza A(H1N1) Virus Investigation and Control in Spain. New influenza A(H1N1) virus infections in Spain, April-May 2009. Euro Surveill. 2009;14(19):19209. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19209 PMID:19442404
19. Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, et al. Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. Euro Surveill. 2013;18(36):20574. PMID:24079378 http://dx.doi.org/10.2807/1560-7917.ES2013.18.36.20574
20. Giesecke J, Moren A, Rowland M, Van Loock F. The European Programme for Intervention Epidemiology Training. Euro Surveill. 1996;1(4):30-1. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=171 PMID:12631845
21. Pritchard L. European Commissioner again pledges European centre for disease control by 2005. Euro Surveill. 2002;6(40):1888. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1888
22. Frank C, Faber MS, Askar M, Bernard H, Fruth A, Gilsdorf A, et al. Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19878
23. López MA, Amela C, Ordobas M, Domínguez-Berjón MF, Álvarez C, Martínez M, et al.; Working group of Ebola outbreak investigation team of Madrid. First secondary case of Ebola outside Africa: epidemiological characteristics and contact monitoring, Spain, September to November 2014. Euro Surveill. 2015;20(1):21003. PMID:25613651 http://dx.doi.org/10.2807/1560-7917.ES2015.20.1.21003
24. Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E, et al. Concurrent outbreaks of dengue, chikungunya and Zika virus infections - an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012-2014. Euro Surveill. 2014;19(41):20929. PMID:25345518 http://dx.doi.org/10.2807/1560-7917.ES2014.19.41.20929
25. Coque TM, Baquero F, Cantón R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill. 2008;13(47):19044. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19044 PMID:19021958

Immunisation schedules in the countries of the European Union

N. Guérin[1], C. Roure[2]
1. Communicable diseases and immunization - Centre International del'Enfance, Paris
2. Programme Elargi de Vaccination - Bureau Régional de l'OMS pourl'Europe, Copenhague

Citation style for this article:
Guérin N, Roure C. Immunisation schedules in the countries of the European Union. Euro Surveill. 1995;0(0):pii=201. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=201

Article published on 01 September 1995

The WHO Regional Office for Europe organises meetings on immunisationprogrammes for national experts from all countries of the European Union (EU) and data on the incidence of diseases and immunisation coverage are regularlysubmitted to WHO. We have analysed immunisation schedules from informationincluded in national plans developed by each country. It is difficult to keep this information up to date since immunisation policies are often adapted inresponse to epidemiological changes and the development of new vaccines. Furthermore, policies may vary between regions within the same country.Belgium, Denmark, Italy, and Spain have decided not to use or to discontinueBCG immunisation. Other countries immunise children at high risk only: neonatesin Austria, Germany, and Luxemburg, or at 6 months of age in the Netherlandsand Sweden. Some countries immunise children at a particular age: at birth inFinland, Ireland, and Portugal, at 6 years in France and Greece, and at 12 years in the United Kingdom. France and the United Kingdom immunise high riskchildren at birth.

In addition to this range of policies on primary immunisation, tuberculin testsand reimmunisation of children with negative skin reactions are carried out at the age of 10 and 15 in France, between 13 and 14 years and 20 and 25 years in Greece, 12 years in Ireland, 5 and 11 years in Portugal. In France, a maximumof two intradermal immunisations is recommended.

Diphtheria, Tetanus, Pertussis (DPT), and Poliomyelitis

Although diphtheria, tetanus, pertussis and polio immunisations are generally combined in young children, vaccination schedules vary so much that it isclearer to present them individually.

Diphtheria

All the countries of the European Union give at least three doses of diphtheriavaccine during the first two years of life. France, Greece, Ireland, Luxemburg,Portugal, and the United Kingdom start at 2 months; Austria, Belgium, Finland,Germany, Italy, the Netherlands, Spain, and Sweden at 3 months; and Denmark at 5 months. Consecutive injections are usually separated by one or two months,but there are nine months between the second and third doses in Denmark. Booster doses are given in most countries one year after the third injection,then approximately every 5 years. Childhood immunisation stops at the age of 6years in Belgium, Ireland, Italy, and Portugal, 10 years in the Netherlands and Sweden, 15 years in Austria, Greece, and Luxemburg, 15 to 19 years in the United Kingdom, and 18 to 20 years in France. Only Austria, Finland and Germany systematically maintain adult immunity with tetanus toxoid and a low dose ofdiphtheria vaccine (th) every 10 years. the recent epidemic of diphtheria in the former Soviet Union led WHO to recommend systematic immunisation of travellers to these states.

Tetanus

Tetanus and diphtheria vaccinations are always given in combination to youngchildren. Primary immunisation of children consists of four doses of tetanusantitoxin in their first 2 years in Austria, Belgium, Finland, France, Germany,Greece, Italy, Luxemburg, the Netherlands, Portugal, and Spain, but only threedoses in Denmark, Ireland, Sweden, and the United Kingdom. Children in the United Kingdom receive a fourth dose at school entry. A booster dose is givenat the age of 15 to 16 years. Boosters of tetanus vaccine in adults are givenmore systemically than for diphtheria: in addition to Austria, Finland andGermany, they are recommended every 10 years in the French, Greek and Portuguese programmes.

Pertussis

Denmark, Ireland, Spain, and the United Kingdom give three doses of pertussisvaccine in the first year of life. Austria, Belgium, Finland, France, Germany,Greece,

TABLE 1

Calendriers vaccinaux dans l'Union Européenne - Août 1995 (w = week / m = month / y = year)

Countries	BCG	DPT	DT	TT	OVP	IPV
Austria	At birth [1]	3,4,5,16-18 m	7,14-15 y	Every 10 y adults/pref.th [4]	4-5,6-7,16-18 m 7,14-15 y	
Belgium		3,4,5,13 m	6 y	16 y	3,5,13 m; 6 y	
Denmark			5,6,15 m [2]		2,3,4 y	5,6,15 m
Finland	At birth	3,4,5,20-24 m	11-13 y th [4]			6,12,20-24 m; Every 10 y
France	At birth [1] 6,10,14,18 y	2,3,4,18 m	6,11,15,18 y	Every 10 y		2,3,4,18 m; 6,11,15 y
Germany	At birth [1]	3,4,5 m; 2 y	6,11-15 y	Every 10 y adults/pref.th [4]	3,5 m; 2,10 y	
Greece	5-6y;13-14y; 20-25y	2,4,6,18 m; 4 y	14-16 y	Every 10 y	2,4,6,18 m; 4 y	
Ireland	At birth; 12 y	2,3,4 m	5 y		2,3,4 m; 5 y	
Italy		3,4,7,18 m; 5 y	ou 3,4,7,18 m; 5 y		3,4,10 m; 3 y	
Luxemburg	At birth [1]	2,3,4,18 m	5,15 y		3,4,10,18 m; 3 y	
Netherlands	6 m [1]	3,4,5,11 m	4,9 y			3,4,5,11 m; 4,9 y
Portugal	At birth - 5,11 y	2,4,6,18; 5 y		Every 10 y	2,4,6 m; 5 y	
Spain		3,5,7 m	18 m [3]	6,14 y	3,5,7,18 m; 6,14 y	
Sweden	After 6 m [1]		3,5,12 m; 10 y			3,5,12 m; 5-6 y
United Kingdom	At birth [1]; 12 y	2,3,4 m	4 y, 16 y , th (4)		2,3,4 m; 4,15 y	

[1] for at risk only
[2] pertussis vaccine given alone at 5, 9w and 10m
[3] DPT in a few autonomous communities
[4] th Tetanus and low title Diphteria associated vaccin

TABLE 2

Calendriers vaccinaux dans l'Union Européenne - Août 1995 (w = week / m = month / y = year)

Countries	MMR	Measles	Rubella	Mumps	Hib/Hib	VHB/HBV
Austria	14 m;6 y		Girls : 13 y		3,4,5,14-18 m	[1] et [2]
Belgium	15 m				3,4,5, 13 m	[1]
Denmark	15 m;12 y				5,6,16 m	[1]
Finland	14-18 m; 6 y; 11-13 y [5]				4,6,14-18 m	[1] et [2]
France	12 m	9 m [4]	Girls : 11 y	11 y	2,3,4,15 m	Infants 12y; [1] and [2]
Germany	15 m; 6 y		Girls : 11,15 y		3,5,15 m	[1]
Greece	15 m; 10 y					[1] et [2]
Ireland	15 m; 12 y				2,4,6 m	
Italy	15 m		Girls : 11 y			3,4,10 m; 12 y
Luxemburg	15 m				3,5,15 m	[1]
Netherlands	14 m; 9 y				3,4,5,11 m	[1] et [2]
Portugal	15 m; 11 y					[1]
Spain	15 m; 11 y					12y [3]
Sweden	18 m; 12 y				3,5,12 m	
United Kingdom	12 m		Girls : 10 y [5]		2,3,4 m	[1] et [2]

[1] for at risk only
[2] infants born of HbsAg positive mother
[3] in a few autonomous communities
[4] for children living in collectivities
[5] if MMR not already given

Luxemburg and the Netherlands recommend four doses; three in the firstand one in the second year. Italy and Portugal recommend 5 doses: 3 in the first year, one in the second year, and a booster in the sixth year. Swedendoes not immunise against pertussis, but immunisation policies may change inthe light of recent results of clinical trials of acellular vaccines in Swedenand Italy which showed acellular pertussis vaccines to be more protective andelicit fewer adverse reactions than a whole cell vaccine.

Poliomyelitis

All countries vaccinate against poliomyelitis but some recommend the inactivated injectable vaccine (IPV) (Finland, France, the Netherlands, andSweden) and others the live oral polio vaccine (OPV) (Austria, Belgium,Germany, Greece, Ireland, Italy, Luxemburg, Portugal, Spain and the UnitedKingdom). In Denmark IPV is recommended at 5, 6, and 15 months and OPV at 2, 3,and 4 years of age. In Europe the first vaccination is given between 2 and 6months. Intervals between the doses of the primary course vary from one countryto another, between four and six weeks. Booster doses are given up to the age of 6 years in Belgium, Denmark, Greece, Ireland, Italy, Luxemburg, Portugal,and Sweden; 10 years in Germany, and the Netherlands; 14 to 15 years inAustria, Spain, and the United Kingdom; in Finland every 10 years or every 5 years when traveling to polio endemic areas, and adulthood in France.

Measles, Mumps, and Rubella (MMR)

All countries in the European Union have introduced MMR immunisation in the second year in their child immunisation schedules. Belgium, France, Italy,Luxemburg, and the United Kingdom currently recommend only one dose. In 1994 analysis of surveillance data, including mathematical modelling, in the UnitedKingdom led the Department of Health to conduct a national campaign ofvaccination against measles and rubella for children aged 5 to 16 years of age to prevent a measles epidemic predicted for 1995 and 1996. Most of the othercountries in Europe recommend two doses of combined MMR vaccine. the second dose is given at the age of 6 in Austria, Finland and Germany and between 9 and 10 in Denmark, Greece, Ireland, the Netherlands, Portugal, Spain, and Sweden. Among the countries that have not yet included a second dose of MMR vaccine,three recommend immunisation against rubella for girls aged 12 to 13 and, in France, immunisation against mumps is recommended for all children at 11 yearsof age.

Haemophilus influenzae type b (Hib)

Immunisation against Hib infections was first introduced in Finland, but other European countries followed as soon as the conjugate PRP-Tbecame available. In Austria, Denmark, Finland, Germany, Ireland, Luxemburg,Sweden, and the United Kingdom three doses are given, the first between 2 and 5 months and the third between 4 and 18 months. In Belgium, France, and theNetherlands four doses are given starting at 2 or 3 months. the first 3 dose sare each separated by a month, and, the fourth is given at 11, 13, or 15months. Greece, Italy, Portugal, and Spain have not introduced routine immunisation against Hib.

Hepatitis B virus (HBV)

Most countries in the European Union immunise health care workers and “highrisk” groups. Austria, Finland, France, Italy, Greece, Netherlands and the United Kingdom also immunise children born of HBsAg positive mothers. Italy and France now immunise all infants and cohorts of children aged 10 to 12 years for 12 years in order to quickly increase the protection in young people. In Spain, some autonomous communities have chosen to immunise infants and others have chosen to immunise children aged 10 to 12 years. Ireland and Sweden have no systematic immunisation policy against hepatitis B.

Conclusion

All countries in the EU share the same aims for the control, elimination, oreradication of vaccine preventable diseases, as defined by WHO. Important variations exist in strategies for child immunisation and programmes set up to achieve these aims. All countries aim to immunise all children against hiphtheria, tetanus, poliomyelitis, measles, rubella, and mumps by the age of 2 years. On the other hand, immunisation against pertussis, Hib, and hepatitis Bare not systematically applied, and adult immunisation policies are stilldeveloping.

Immunisation schedules and policies for each country depend more on health caresystems, established immunisation practices, and the results of national surveys than on real differences in the epidemiology of infectious diseases. Harmonisation of immunisation policies within the EU could be considered while maintaining some flexibility in schedules. It is difficult to compare theeffectiveness of immunisation programmes, particularly their impact on themorbidity and mortality of the target diseases, due to variations in the epidemiological surveillance of infectious diseases between countries in the Europe Union. One of the goals of collaborative projects currently underdevelopment is to streng then and harmonise surveillance activities.

The European Programme for Intervention Epidemiology Training

A. Moren, M. Rowland, F. Van Loock, J. Giesecke

Citation style for this article:
Moren A, Rowland M, van Loock F, Giesecke J. The European Programme for Intervention Epidemiology Training . Euro Surveill. 1996;1(4):pii=171. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=171

Article published on 01 April 1996

The European Programme for Intervention Epidemiology Training (EPIET) provides practical experience in infectious disease epidemiology. EPIET aims to create a network of professionals throughout Europe trained to use a standard approach in intervention epidemiology including field work, surveillance, applied research, communication, and the use of epidemiological information as a basis for public health action. EPIET is financed through a grant from the Directorate General V of the Commission of the European Communities.

So far 17 fellows have been recruited, in two cohorts, representing 13 European nations. The fellows are now based in various European public health institutes offering action orientated training. The first cohort of EPIET fellows was enrolled in November 1995 and the second cohort of nine EPIET fellows entered the programme in June 1996. It is hoped to enrol the third cohort in 1997.

Each cohort of EPIET fellows starts its two year training period with a residential introductory course held in Veyrier du Lac, France. The course aims to provide participants with basic knowledge in epidemiological methods, outbreak investigation and surveillance techniques, and communication skills. During each course a field survey is developed and completed by the fellows. Surveys conducted at the World Health Organization (WHO) and United Nations headquarters in Genève in 1995 and 1996 concerned staff compliance with WHO recommendations for overseas travel and to smoking in the work place. The team of facilitators for these courses was composed of experienced epidemiologists and senior trainers who were to host fellows during their subsequent placement.

In addition to the introductory course, up to seven additional weeks of course work are included in the two years training. A module on immunisation, organised by the National Public Health Institute (KTL) in Helsinki, in March 1996, provided up to date information on vaccines and discussed epidemiological methods used in vaccine trials, programme evaluation, and in developing vaccination strategies. A second module, at the PHLS Communicable Disease Surveillance Centre in London, focused on communication techniques including radio and television interviews. A module on multivariate analysis will be held in the Istituto Superiore di Sanità, Roma, in January 1997.

Since the first cohort began their training in November 1995 they have carried out various tasks including outbreak investigations, evaluations of surveillance systems, and applied research studies. Some of the studies crossed national borders - for example, the Franco-Belgian collaborative study on hantavirus. A scientific seminar held at the end of the second introductory course in June 1996 enabled fellows from the first EPIET cohort, and their colleagues from the Spanish, German, and Hungarian Field Epidemiology Training Programmes (FETP) to present work in progress at host institutes to the directors and supervisors from participating countries and to fellows from the second training cohort.

During its first year the EPIET programme has already contributed to the promotion and the development of intervention epidemiology in Europe[1]. “Learning by doing” and the collaboration process involved is fostering strong professional, institutional, and interpersonal links between fellows, course facilitators, and training supervisors. In collaboration with other European public health projects, the EPIET programme is contributing to coordinated surveillance and intervention within the European Union (EU). It is already becoming clear that requests for collaborative links and help will be sought from the EU by countries beyond its borders.

Reference

1. Ziese T., Anderson Y., de Jong B., Löfdahl S., Ramberg M. Outbreak of Escherichia coli O157 in Sweden. Eurosurveillance 1996; (1)1:2-3

Four cases of H5N1 influenza in Hong Kong

Citation style for this article:
Watson J. Four cases of H5N1 influenza in Hong Kong. Euro Surveill. 1997;1(33):pii=1010. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1010

Article published on 11 December 1997

A total of four cases of influenza A virus subtype H_5N_1 infection have been reported, among people living in Hong Kong. The first case was a 3 year old boy who became ill in May 1997 and died (1,2). The boy was believed to have acquired the infection through contact with chickens. Epidemiological investigations of the local population during the following months revealed no transmission to other cases. Three further cases have occurred in November; the first one a 2 year old child who recovered, and two other cases in late November, one a 13 year old girl who is recovering and the other a 54 year old man who has died.

The United States' Centers for Disease Control and Prevention (CDC) are assisting the Hong Kong health authorities in their investigations into the source of infection of the three new cases. So far there is no evidence of human to human transmission, but contacts of the known cases are being investigated intensively. The season when influenza activity in Europe is likely to occur has just begun. Although the probability of any cases of the new influenza A H_5N_1 strain being imported into Europe is currently very low, laboratories are nevertheless advised to remain vigilant about the travel history of any suspected cases of influenza.

References :

1. Handysides S. New influenza virus strain confirmed in human case, but no person to person transmission identified so far. Eurosurveillance Weekly 970828
2. de Jong JC, Claas ECJ, Osterhaus ADME, Webster RG, Lim WL. A pandemic warning? Nature 1997; 389: 554

Reported by John Watson (jwatson@phls.co.uk), PHLS Communicable Disease Surveillance Centre, England

Answers to questions likely to be asked by members of the public and journalists about influenza A H_5N_1 in Hong Kong

Adapted from: the Public Health Laboratory Service in England and Wales (http://www.open.gov.uk/cdsc/flufact.htm)

What is influenza A H_5N_1?

Influenza A H5N1 is a subtype of the influenza A virus that has previously been found only in birds. This 'avian' influenza virus was obtained from specimens from a 3 year old child in Hong Kong who became ill in May 1997. The child subsequently died with a respiratory illness and Reye's syndrome and the identity of the virus was confirmed in August. This is the first known case of illness in a human due to infection with this virus. Influenza A H5N1 is known to be circulating among chickens in Hong Kong. The child was reported to have had contact with chickens before the onset of illness.

How many people have been infected with influenza A H_5N_1?

Four cases of infection in humans had been identified by 8 December 1997: one case occurred in May 1997 and the other three in November 1997. All were residents of Hong Kong. Influenza surveillance was intensified in Hong Kong and southern China following the identification of influenza A H5N1 infection in the first case in August.

How did the people become infected with influenza A H_5N_1?

Following the first case of influenza H5N1 infection, intensive local surveillance efforts were put in place to determine if there was any evidence of transmission to other people who had come into contact with the child or in the local population. No evidence of transmission was found. No evidence has been found to indicate transmission between the four cases so far identified in residents of Hong Kong.

It is thought most likely that the first case contracted influenza infection from contact with infected chickens. Information is not yet available on where the further three cases are likely to have contracted their infections, including whether or not they had significant exposure to chickens or other birds. These cases are being investigated actively.

How likely is a pandemic (worldwide epidemic) due to influenza A H_5N_1?

To date, only four cases have been identified in six months in one small geographic area. A pandemic occurs when a new influenza virus not only causes illness in humans but also spreads from person to person. No links between the cases have yet been identified and there is no evidence, as yet, of person to person transmission.

How severe is the illness caused by influenza A H_5N_1?

Influenza virus infection can by asymptomatic or cause illness of varying severity, from a cold to a rapidly fatal pneumonia. Most influenza illnesses, though unpleasant, are self limiting and do not require medical attention. Although two of the four patients with illness found to be due to influenza A H5N1 infection have died, there is still insufficient information to be able to say how severe the illness might be in the population if the infection spread. The only cases identified so far have been in hospital, where patients with more severe illness would be likely to be identified.

Is there a vaccine to protect against influenza A H_5N_1?

Not at present. Work is currently underway, as a precautionary measure, to see how best to develop a vaccine. Widescale production would not take place unless there was good evidence of extensive spread of the virus in the human population.

What is the role of this year's influenza vaccine?

The current vaccine contains components of three strains of influenza virus (two subtypes of influenza A - H1N1 and H3N2 - and one influenza B strain) that are most likely to circulate in the northern hemisphere this winter. Influenza A viruses similar to each of the vaccine strains have been isolated in the United Kingdom in November and December although current levels of influenza activity are at baseline levels. Thus the current vaccine remains the best protection against the most likely strains of influenza in this country this winter.

Is the antiviral drug amantadine effective against influenza A H_5N_1?

Influenza A viruses are generally susceptible to amantadine. The first two isolates of influenza A H5N1 have been susceptible to amantadine and future strains are also likely to be so.

What should individuals do who become unwell with an influenza-like illness?

Rest, take analgesics (paracetamol for all ages, but aspirin may also be taken by adults), and drink plenty of fluids. Symptomatic treatment at home reduces the amount of further spread in the community. Medical advice should be sought if symptoms become severe or recovery does not start after about a week. People with chronic illnesses may need to seek advice earlier.

What advice should be given to people travelling to Hong Kong?

There is currently no evidence of human to human transmission of influenza A H5N1 infection in Hong Kong and only four cases have been confirmed to date. People at high risk of complications of influenza infection as a result of pre-existing disease should be immunised, as usual, with the current vaccine. From information received so far (1100 on Thursday 11 December) - from Belgium, Denmark, England and Wales, Finland, Scotland, Spain, and Sweden - no additional measures or restrictions have been recommended for travellers to and from Hong Kong.

The Portuguese Influenza Surveillance System has registered only mild activity in recent weeks (maximum: 11 cases/100 000 inhabitants). Regarding influenza A H5N1 in Portugal are existing surveillance systems giving special attention to identifying influenza cases occurring in people coming from Asian countries (mainly Hong Kong and Macao); any local increase in numbers of cases in comparison with the same period last year; and the occurrence of severe cases. Health authorities and the media are being kept informed.

Finnish authorities are reinforcing their laboratories' diagnostic capacities and consulting vaccine manufacturers. The public and media are being kept informed. Virologists in Scotland are being contacted to encourage the taking of specimens for virus isolation and typing.

Prepared by PHLS Surveillance of Influenza, Respiratory Diseases Section (respcdsc@phls.co.uk), PHLS Communicable Disease Surveillance Centre and Influenza Section (mzambon@phls.co.uk), Enteric and Respiratory Viruses Laboratory, PHLS Central Public Health Laboratory, England. Thanks also to members of the Eurosurveillance editorial board for further material.

Further information can be found at:

- Government of Hong Kong Special Administrative Region Department of Health (http://www.info.gov.hk/dh/new/index.htm)
- World Health Organization, Emerging and other Communicable Diseases (EMC) (http:/www.who.ch/programmes/emc/news.htm)

Surveillance of tuberculosis in Europe: first data emerge from "EuroTB"

Citation style for this article:
Handysides S. Surveillance of tuberculosis in Europe: first data emerge from "EuroTB". Euro Surveill. 1997;1(27):pii=1031. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1031

Article published on 30 October 1997

Recommendations for standardising the reporting of tuberculosis cases in Europe made by a working group set up by the World Health Organization (WHO) and the European Region of the International Union Against Tuberculosis and Lung Disease (1) were approved by representatives of 37 countries in September 1995. The results of a one year pilot study have just been published (2).

A national correspondent was identified in 49 of the 50 countries of WHO's European region (not Ukraine). Forty-six of the 48 countries sent the total numbers of cases of tuberculosis notified in 1995 and 41 provided further details: 40 gave the sex of cases, 39 the site of disease, 34 the new or recurrent status of the cases, 34 supplied bacterial confirmation, 33 the age of cases, 27 the result of sputum smear examination, and 21 the geographic origin of patients. Nineteen countries provided computerised information about individual cases.

Forty-six countries of WHO's European region notified 276 811 cases of tuberculosis in 1995, representing an overall incidence of 34.6 cases per 100 000 population (range 2.7 in Malta to 101.9 in Romania). The incidence was lower than 20/100 000 in 22 countries, all of which were in western Europe apart from Albania, the Czech Republic, and Israel. These countries accounted for 44% of the population but contributed 16% of the cases of the 46 countries. Countries where the incidence was 20/100 000 or more were mostly in eastern Europe, with the exception of Portugal and Spain. Countries of the European Union (EU) notified 54 133 cases in 1995, an incidence of 14.5/100 000 (range 6.4 in Sweden to 56.8 in Portugal). Thirteen of the 15 EU countries had incidences lower than 20/100 000.

Countries where the incidence of tuberculosis was lower reported greater numbers of adult and elderly patients proportionately. More male than female cases were notified. Patients originating from parts of the world where the incidence of tuberculosis is high accounted for substantial numbers of cases in several countries of western Europe. Most cases were new episodes in people never diagnosed previously, and 80% of cases were of pulmonary tuberculosis. Less than a half of all notified cases were bacteriologically confirmed, and 40% were sputum smear positive.

References :

1. Schwoebel V, Rieder HL, Watson JM, Raviglione MC for the Working Group for Uniform Reporting on Tuberculosis Cases in Europe.
2. EuroTB (CESES/KNCV) and the National Coordinators for Tuberculosis Surveillance in the WHO European Region. Surveillance of tuberculosis in Europe: report on the feasibility study (1996-1997), tuberculosis cases notified in 1995. St Maurice: CESES, 1997

Copies of the report can be found at: http://www.b3e.jussieu.fr/ceses/eurotb or obtained from: EuroTB, CESES - Hôpital National de Saint-Maurice, 14 rue du Val d'Osne, 94410 Saint-Maurice, France

Reported by Stuart Handysides (shandysi@phls.co.uk) PHLS Communicable Disease Surveillance Centre, England

Global HIV epidemic

Citation style for this article:
Nicoll A. Global HIV epidemic. Euro Surveill. 1998;2(26):pii=1196. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1196

Article published on 25 June 1998

A detailed report on the global HIV epidemic issued this week by the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO), and based on data to the end of 1997, forms a backdrop for the 12th World AIDS Conference that starts next week (1). The global estimates are similar to those issued last year(2): 30.6 million people living with HIV infection, 5.8 million new infections (16 000 per day), and 2.3 million deaths attributable to HIV in 1997 (1).

The report describes the continuing spread of HIV, its emergence in some countries previously little affected, and its increased transmission in countries where infection was already established. The very low prevalence in China seems to have doubled in two years and infection reports in many parts of the former Soviet Union have risen dramatically; prevalence in the Ukraine has risen 70-fold in four years. Rapid increases in Southern Africa are illustrated by the most recent reports of HIV prevalence in pregnant women: 43% in Francistown (Botswana), 32% in Harare (Zimbabwe), and 28% in Kwa Zulu~Natal (South Africa) (1). Nigeria with 2.3 million estimated infections, equivalent to a prevalence of 4.1% in those aged 15 to 49 years) and India (4.1 million, 0.82%) cause particular concern because, although prevalences are lower than in some nearby counties, the potential exists for large rises in numbers of infections. India is already estimated to have more prevalent infections than any other country.

HIV has become one of the top ten causes of death worldwide (1). Independent surveys show that the emergence of HIV infection has more than doubled death rates among young adults in some African countries. Life expectancy in hard hit areas of Uganda has fallen by 16 years. The report is by no means overwhelmingly gloomy, however, and presents national successes in HIV prevention in Thailand, Uganda, Senegal, and the United Kingdom, for instance.

The report estimates that 30 000 new HIV infections arose in western Europe in 1997. The HIV infection rate is decreasing in general, and the new infections are concentrated among drug injectors in southern Europe. A 38% decrease in the number of new AIDS cases - 23 954 in 1995 to 14 874 in 1997 - reflects prevention measures taken in the late 1980s in the gay communities, and increased condom use among young people, but most significant has been the use of antiretroviral treatment which has postponed the onset of AIDS.

References :

1. Joint United Nations Programme on HIV/AIDS and the World Health Organization (WHO). Report on the global HIV/AIDS epidemic June 1998. Geneva: UNAIDS/WHO 1998. (here)

Reported by Angus Nicoll (anicoll@phls.co.uk), PHLS Communicable Disease Surveillance Centre, London, England

Reported association between measles, mumps, and rubella (MMR) vaccine, autism, and bowel syndrome

Citation style for this article:
Miller E. Reported association between measles, mumps, and rubella (MMR) vaccine, autism, and bowel syndrome. Euro Surveill. 1998;2(10):pii=1247. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1247

Article published on 05 March 1998

Last week's Lancet contained a paper reporting 12 children with bowel symptoms and "regressive developmental disorder", 10 of whom had autism or autistic like features (1). In 8 of the 12 children a temporal association between onset of symptoms and measles, mumps, and rubella (MMR) immunisation was reported. An abstract of the paper published last year was reviewed by the United Kingdom's Joint Committee on Vaccination and Immunisation, which did not recommend any changes to the MMR vaccination policy.

A statement in the Lancet paper that both measles virus and measles vaccination have been implicated as risk factors for Crohn's disease (1) contradicts a review of evidence recently published in the World Health Organization's (WHO) Weekly Epidemiological Record (2). WHO's review concluded that the hypothesis remains unproven and that immunisation programmes throughout the world should continue. Further negative virological evidence from workers at the UK's National Institute for Biological Standards and Control was published in last week's Lancet (3). The BMJ said recently that the links between measles and Crohn's disease "were dead" (4).

The Lancet published an independent commentary from Dr Robert Chen, of the United States Centers for Disease Control, which made the point that although hundreds of millions of individuals worldwide have received measles containing vaccine since the mid-1960s, this syndrome of regressive developmental disorder with bowel symptoms has not previously been reported (5). Chen also drew attention to the lack of any virological or epidemiological evidence in the accompanying paper to support a causal association with MMR vaccine and warned of the dangers of confusing a chance temporal association with causality.

Several studies on autism have reported on the occurrence of associated medical conditions (6) but none has mentioned an association with Crohn's disease. About one third of children with autistic disorder exhibit regression after apparently normal development in the first year of life (7). The mean age at which parents of children with autism first report concern about their child's development is 18 to 19 months, and 14 months for experienced parents (8,9). Since over 90% of children receive MMR vaccine before their 2nd birthday, the probability that parents of autistic children will first notice abnormal behaviour shortly after MMR vaccination in a child who was previously developing normally is therefore high.

References :

1. Wakefield AJ, Murch SH, Anthony A, Linnell J, Casson DM, Malik M, et al. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. Lancet 1998; 351: 637-41
2. WHO. Expanded programme on immunization (EPI) - association between measles infection and the occurrence of chronic inflammatory bowel disease. Wkly Epidemiol Rec 1998; 73: 33-40
3. Afzal MA, Minor PD, Begley J, Bentley ML, Armitage E, Ghosh S, et al. Absence of measles-virus genome in inflammatory bowel disease. Lancet 1998; 351: 646
4. Editor's choice: beware individual issues of journals. BMJ 1998; 316: (Jan 17)
5. Chen RT, DeStefano F. Vaccine adverse events: causal or coincidental? Lancet 1998; 351: 611-2
6. Fombonne E, Du Mazaubrun C, Cans C, Grandjean H. Autism and associated medical disorders in a French epidemiological survey. J Am Acad Child Adolesc Psychiatry 1997; 36: 1561-9
7. Mauk JE. Autism and pervasive developmental disorders. Pediatr Clin North Am 1993; 40: 567-78
8. Siegal B, Pliner C, Eschler J, Elliot G. How children with autism are diagnosed: difficulties in identification of children with multiple developmental delays. J Dev Behav Pediatr 1988; 9: 199-204
9. de Giacomo A, Fombonne E. Parental recognition of developmental abnormalities in autism. European Child and Adolescent Psychiatry, in press

Reported by Elizabeth Miller emiller@phls.co.uk PHLS Communicable Disease Surveillance Centre, London, England.

Travel associated legionnaires' disease in Europe: 1997 and 1998

E Slaymaker[1], CA Joseph[2], CLR Bartlett[3] on behalf of the European Working Group for Legionella Infections (EWGLI)
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
2. Anti-infectives and Vaccines Office, European Medicines Agency (EMA), London, United Kingdom

Correspondence: Marco Cavaleri (Marco.Cavaleri@ema.europa.eu)

Citation style for this article:
PHLS Communicable Disease Surveillance Centre (CDSC), London, England

Article published on 01 November 1999

Introduction

The European Surveillance Scheme for Travel Associated Legionnaires' Disease was set up by the European Working Group on Legionella Infections (EWGLI) in 1987 to identify cases of legionella infection in returning travellers and to detect outbreaks and clusters of legionnaires' disease. The scheme was initially run from the Swedish Institute for Infectious Disease Control (SIIDC) in Stockholm where it was funded by the World Health Organization (WHO). In 1993 it moved to its present location in the Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre (CDSC) in London, a move which coincided with the beginning of funding by Directorate General V of the European Commission.

Methods

The surveillance scheme methods have been described previously (1). Twenty-four countries took part in the scheme in 1997 and seven joined in 1998. There are now 36 collaborating centres in 31 countries (figure 1). A case of travel associated legionnaires' disease is defined as follows:

- Clinical or radiographic evidence of pneumonia accompanied by appropriate laboratory diagnosis.

- A history of travel in the ten days before the onset of illness. Travel is defined as staying away from home for one night or more. Overnight stays in private accommodation are not included.

Cases diagnosed by the detection of specific legionella antigen in urine using validated reagents have been regarded as confirmed since 1 January 1998. Details of cases ascertained by national or regional surveillance systems of participating countries are sent by fax or email to the coordinating centre at CDSC. The information collected includes the full travel itinerary and clinical and microbiological data. When a new case is added to the database at CDSC, the database is searched to see if previous cases have been reported at the same accommodation site. The collaborator, or ministry of health, in the presumed country of infection is immediately informed by fax of all cases associated with their country. All collaborators and WHO are informed immediately if the case is part of a cluster. Clusters are defined as two or more cases associated with the same accommodation site who became ill within six months of each other. If the case stayed at accommodation associated with previous cases, but became ill over six months later, then the cases are said to be linked. Collaborators and WHO are informed of linked groups at the end of each month. Some countries choose to inform representatives of their national organisations of tour operators about cases that arise in tourist accommodation.

All collaborating countries maintain a copy of the EWGLI data set. Most countries have a copy of the EWGLI database which is updated by email at the end of each month.

Results

The number of cases reported has increased from three cases in 1987 to 242 cases in 1997 and 232 in 1998 (table 1). Clustered cases accounted 32% (78) of the total in 1997 and 26% (62) in 1998, within the range of previous years. The average size of a cluster in both 1997 and 1998 was three cases (figure 2).

The most commonly used methods of diagnosis have changed during 1997/8. Forty-one per cent (99 in 1997, and 96 in 1998) of cases are now being diagnosed by detection of urinary antigen. The increase has been at the expense of serological methods; the proportion of cases confirmed by culture and by other methods remain similar (figure 3).

Figure 1
Pays participant au programme de surveillance en 1998.
Countries participating in the surveillance scheme in 1998.

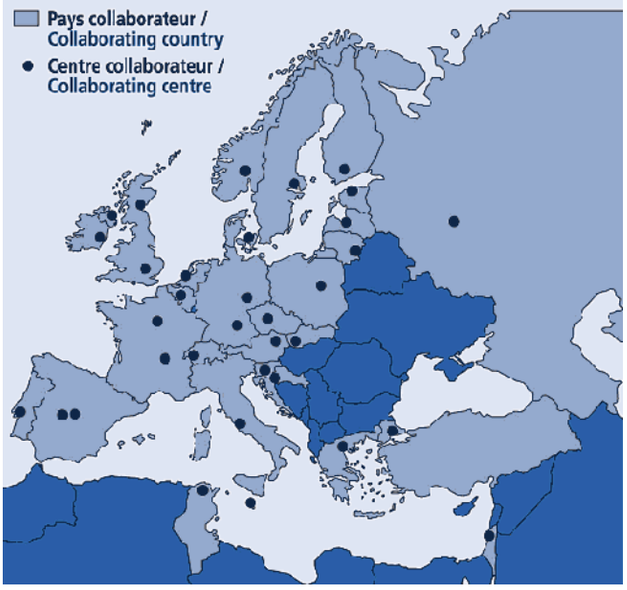


Figure 2
Catégorie de cas par année d'apparition des symptômes
Category of cases by year of onset

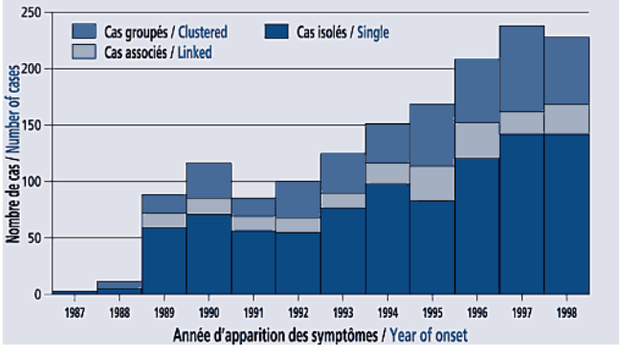


Figure 3
Méthode de diagnostic par année de survenue de la maladie
Method of diagnosis by year of onset

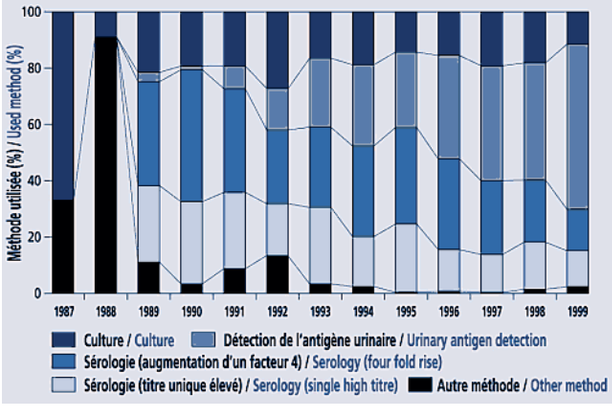
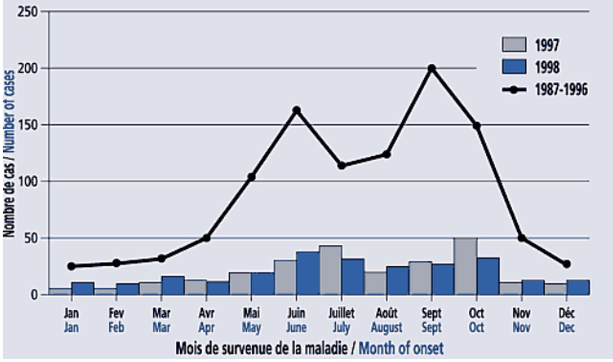


Figure 4
Distribution saisonnière des cas de 1987 à 1998
Seasonal pattern of cases from 1987 to 1998



The age and sex profile of cases is similar to previous years (3). In 1997 and 1998 there were more than twice as many men as women (table 1). The age of cases was normally distributed around a mean of 57 in 1997 and 56 in 1998.

The outcome of illness was reported for most cases, but if the case was still ill when reported the information was often not updated and the outcome remained unknown. Fifty-six per cent of cases in 1997 are known to have recovered and 57% in 1998. Twenty-six deaths were reported in 1997 and 25 in 1998, a provisional case fatality rate of 11% in both years. Cases whose outcome was unknown at report accounted for 5% of reports in 1997 and 10% in 1998.

The seasonal pattern, based on dates of onset, has not changed significantly from previous years. Two peaks were seen in both 1997 and 1998, the first in June/July and the second in September/October. The timing of these peaks varies slightly from year to year (figure 4). A small peak seen around Easter time in 1998 had not been observed in previous years.

Most cases are reported from countries in northern Europe - in particular from England and Wales, Scotland, the Netherlands, Sweden, France, and Denmark - but 12 cases (almost 3%) were reported from Italy in 1997/8. Infections are usually diagnosed after return to the country of residence. Twelve countries reported cases in 1997 and 16 in 1998.

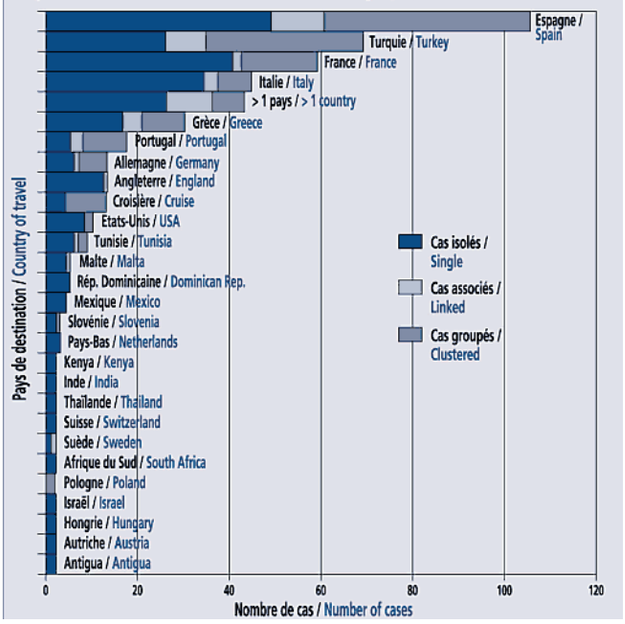
Travel

The 474 cases with onset in 1997/8 had made 697 visits to 51 countries. The Mediterranean region was the most popular destination. In both 1997 and 1998 more than 20% of cases had visited Spain. Italy, Greece, and Turkey combined accounted for 30% of cases. France and Germany had 15% of the visitors in 1997 and 14% in 1998 (figure 5). Cases who took their holidays in northern Europe stayed in a wider variety of places than those who went to Mediterranean coastal resorts.

Figure 5

Type de cas par pays visités - 1997-1998 (17 pays, chacun visité par un cas, n'ont pas été pris en compte dans ce graphique)

Type of case by country visited - 1997-1998 (17 countries, each visited by one case, have been omitted from this graph)



The number of cases associated with a country is usually proportionate to the total number of people who visit the country. Therefore, although Spain has a high number of cases, the rate per million travellers from the United Kingdom (the only country for which information on the number of travellers is available) is no higher than in countries that receive fewer visitors (Office for National Statistics, unpublished data) (table 2).

Twenty-five clusters were detected in 1997 and 19 in 1998. Six of the clusters in 1997 and 10 in 1998 would not have been detected without the surveillance scheme since each included only one national from several countries. Most of the clusters detected occurred in the most visited countries, but there were some exceptions. For instance, the number of clusters on cruise ships was higher than would be expected given the numbers of people who take cruise holidays.

Outbreaks and clusters

Cruise ship 1: An outbreak of six cases (one fatal) in English and Scottish residents occurred on a Rhine cruise ship in 1997. The cases arose between July and October 1997 and had travelled on four separate cruises. The Dutch owned ship was taken out of operation when the outbreak was detected. The temperature of the hot water system was found to be inadequate and the whirlpool spa had been improperly maintained. *Legionella pneumophila* serogroup (sg) 4 was isolated from this pool but although there was strong epidemiological evidence that this was the source (4,8,9) evidence of *L. pneumophila* sg 4 infection was found in none of the patients.

Table 1

Summary of results from 1997 and 1998

1997	1998
242 cases	232 cases
168 male - 74 female (2,27:1)	169 male - 62 female (2,73:1)
	1 unknown
26 deaths	25 deaths
Reported from 12 countries	Reported from 16 countries
Travelled to 37 countries	Travelled to 39 countries
25 clusters detected: Spain (6), Turkey (4), Germany (3), Greece (3),	19 clusters detected: Spain (8),
Italy (3), cruise (1), Hungary (1), Poland (1),	Turkey (4), France (3), cruise (1),
Portugal (1), Tunisia (1), USA (1)	Greece (1), Italy (1), Portugal (1)

Turkey: Sixteen cases and one suspected case of legionnaires' disease were identified in an outbreak at a hotel in Istanbul in September and October 1997. Four people died. Sixteen of the cases were French and one was Belgian. Isolates were obtained from six patients; typing showed that all were infected with the same strain of *L. pneumophila* sg 1 of a distinct, and previously unknown, type. There was no opportunity for environmental investigation of the hotel and the source of infection was never found. The epidemiology strongly suggested an extended point source. Two tour companies used the hotel and reported that the hotel was closed for renovation after the outbreak was detected (B Decludt, personal communication).

Cruise ship 2: Three cases of legionnaires' disease and one case of non-pneumonic legionellosis arose on a British ship in May and June 1998. The ship had previously been registered in Italy and the new owners were unaware that it had been associated with two previous cases of legionnaires' disease, one of which had been fatal. The ship was inspected and serious flaws were found in the temperature regulation of the hot and cold water system and in the electrical system. The ship's itinerary was disrupted while these faults were rectified. *L. pneumophila* sg 1 was isolated from the ship's water supply but no clinical isolates were available for comparison (5).

Table 2

Rates of UK cases of legionnaires disease per million travellers from UK to some of the most popular countries

Country of travel	UK cases/million travellers from UK			
	(percentage of cases that came from the UK)			
	1997		1998	
Spain	4.1	(72)	4.7	(75)
Turkey	14	(42)	22.7	(64)
France	0.8	(32)	1.1	(41)
Italy	3.3	(25)	1.4	(16)
Greece	4.7	(44)	2.7	(39)
Germany	-		0.5	(14)
Portugal	7.7	(71)	0.8	(25)
USA	1	(43)	0.8	(60)
Tunisia	-		2.9	(20)

France: An increase in the number of cases reporting travel to Paris was observed in June 1998. None of the cases was associated with the same buildings but several were visiting France for the football world cup. Investigation by the French authorities and case searching through EWGLI resulted in the detection of nine travel associated cases: four English, three Scottish, one Swedish, and one Danish. Eleven cases were French residents. A case control study by the Institut de Veille Sanitaire (formerly known as Réseau de Santé Publique (RNSP)) demonstrated an association with an area of Paris and cooling towers in this area were sampled. Several towers yielded legionella and isolates from one tower were indistinguishable by subtyping and subgrouping analysis from clinical isolates (6).

Spain: A outbreak of 11 cases occurred at a hotel in Benidorm between August and December 1998. Two cases had previously been associated with the hotel, one in 1990 and one in 1996. The first case in the cluster was reported to EWGLI in September 1998. The second and third cases were reported on 21 and 24 December, and a cluster alert was issued. The tour operators using the hotel withdrew their clients on 24 December. Inspections of the hotel and sampling of the water systems were carried out by the local health authorities and a private company. The water system had been chlorinated before samples were taken and no legionella were isolated. Over the next few weeks, as more cases were reported to CDSC, it transpired that eight cases had occurred by the time the cluster alert was issued but that they had not been reported (7).

Discussion

The surveillance scheme has continued to expand during 1997 and 1998. The gradual increase in the numbers of cases reported since the start of the scheme in 1987 is thought to be due to improved detection and reporting, rather than increased incidence.

The characteristics of the cases reported to EWGLI have remained consistent over the past few years, although the methods used to diagnose patients with legionnaires' disease changed during 1997/8 with more widespread use of urinary antigen detection techniques. The proportions of single, linked, and clustered cases have changed very little as have the seasonal distribution and the countries visited by cases. The importance of the scheme, in promptly detecting clusters and outbreaks, is illustrated by the fact that 24% of the clusters in 1997 and 53% in 1998 would not have been detected without EWGLI. Collaboration with tour operators is proving valuable in the prevention of further cases, by ensuring prompt action after clusters are reported at tourist accommodation sites.

Acknowledgements

We would like to thank all the collaborators for reporting their cases and all the people involved in public health control and prevention programmes for travel associated legionnaires' disease.

References

1. Hutchinson EJ, Joseph CA, Bartlett CLR on behalf of the European Working Group for Legionella Infections. EWGLI: a European Surveillance Scheme for Travel Associated Legionnaires' Disease. Eurosurveillance 1996; 1: 37-9.
2. Epidemiology, prevention and control of legionellosis. Memorandum from a WHO meeting. Bull World Health Organ 1990; 68: 155-64.
3. Lane CR, Joseph CA, Bartlett CLR on behalf of the European Working Group for Legionella Infection. European surveillance of travel associated legionnaires' disease 1996. Eurosurveillance 1998; 3: 6-8.
4. Joseph CA. Outbreak of legionnaires' disease among British tourists associated with Rhine cruise. Eurosurveillance Weekly 1997; 1: 971030. (http://www.eurosurv.org)
5. Christie P, Joseph CA. Legionella on board a cruise ship. Eurosurveillance Weekly 1998; 2: 980702. (http://www.eurosurv.org)
6. Decludt B, Guillotin L, Van Gastel B, Dubrou S, Jarraud S, Perrocheau A, et al. Epidemic cluster of legionnaire's disease in Paris, June 1998. Eurosurveillance 1999; 4: 115-8.
7. De Mateo S, Crespi S, Joseph CA. Outbreak of legionnaires' disease in Spain. Eurosurveillance Weekly 1999; 3: 990128. (http://www.eurosurv.org)
8. Joseph CA, Harrison TG, Ilijic-Car D, Bartlett CLR. Legionnaires' disease in residents of England and Wales: 1997. Commun Dis Public Health 1998; 1: 252-8.
9. Rowbotham TJ. Legionellosis associated with ships: 1977 to 1997 Commun Dis Public Health 1998; 1: 146-51.

HIV reporting in western Europe : national systems and first European data

Andrea Infuso, Françoise F. Hamers, Angela M. Downs, Jane Alix
1. European Centre for the Epidemiological Monitoring of AIDS (EuroHIV)*, Institut de Veille Sanitaire, Saint-Maurice, France, for the national correspondents of the European HIV reporting system in western European countries
(JP. Klein, Austria; A. Sasse, Belgium; E. Smith, Denmark; P. Holström, Finland; F. Lot, France; O. Hamouda, Germany; L. Tzala, Greece; H. Briem, Iceland; J. Devlin, Ireland; B. Suligoi, Italy; I. Robert, Luxembourg; J.K. Van Wijngaarden, the Netherlands; O. Nilsen, Norway; M.T. Paixão, Portugal; I. Noguer, Spain; M. Arneborn, Sweden; M. Gebhardt, Switzerland; N. Macdonald, United Kingdom). **Correspondence:** Marco Cavaleri (Marco.Cavaleri@ema.europa.eu)

Citation style for this article:
Infuso A, Hamers FF, Downs AM, Alix J. HIV reporting in western Europe : national systems and first European data . Euro Surveill. 2000;5(2):pii=29. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=29

Article published on 01 February 2000

Introduction

AIDS case reporting has been an essential tool for monitoring HIV infection in western Europe. Recent trends in AIDS have been affected by improved antiretroviral treatments that delay HIV disease progression, however, and no longer serve as indicators of HIV transmission trends. Reporting of all diagnosed HIV infections is increasingly advocated as a central component of surveillance (1). A European HIV reporting system including 39 countries of the World Health Organization (WHO) European Region was set up in 1999 to complement AIDS reporting. This paper describes national HIV reporting systems in western European countries and presents the first data collected

Methods

The characteristics of national HIV reporting were explored in a preliminary survey in 1997 (2) and updated in 1999. Individual anonymous data (or, if not possible, aggregate data) on HIV infections diagnosed at any clinical stage and reported by the end of 1998 were collected from national HIV/AIDS surveillance institutes taking part in European AIDS reporting. The number of HIV cases reported in 1998 was compared to the number of AIDS cases reported in the same year. Western Europe was defined as the 15 countries of the European Union plus Iceland, Norway, and Switzerland

Results

Reporting systems

In 1999, HIV reporting was taking place in 15 of the 18 countries of western Europe (table 1). National systems for HIV reporting existed in 13 of these countries and was implemented in six of the 21 regions in Italy and in the area of Arnhem in the Netherlands. Regional systems existed in 13 of the 23 regions of France until the end of 1998. HIV reporting had not been implemented in Austria and Ireland. National reporting systems are planned in France and in Ireland and regional systems in Italy and the Netherlands will be expanded in the near future.

Thirteen countries began reporting before 1991 and two countries (Greece and Luxembourg) in 1999. Spain began a gradual process to implement national HIV reporting in 1999. Reporting is mandatory in eight countries, in most Italian regions, and in the national system planned in France. Cases of HIV infection are reported by laboratories only in four countries, by clinicians only in two countries, and by both in ten countries. Clinician reporting has recently been added to laboratory reporting in Germany (1998) and in the United Kingdom (UK) (2000).

Apart from Iceland, none of the national HIV reporting systems records named cases. Twelve countries eliminate duplicate reports and carry out linkage with other sources of data (e.g. AIDS or death reports) at national level using date of birth (or part thereof), sex, and other personal information, such as parts of the name (nine countries and most regions in Italy) or parts of the social security number (two countries). Three systems (Denmark, Norway, and the laboratory reporting system in Germany) collect no personal information other than date of birth and sex.In these countries, linkage with other data sets is impossible and HIV reports of cases with a history of previous positive tests are excluded from national statistics to reduce repeat counting of the same case. Recent or planned changes in the personal information collected on HIV reports include a shift to named reporting (Iceland, 1999), and the introduction of name initials (Switzerland, 1999), full date of birth (Germany, planned), and the social security number (Denmark, under discussion).

All countries collect data on the route of HIV transmission and clinical stage, using similar categories. Other data often collected include geographic origin (nationality, country of birth, or country of permanent

Table 1

Characteristics of HIV reporting systems in western European countries

	Start of reporting	Legal status	Source of reports	Nr of laboratories reporting (potentially)	Case identifiers *	
Country					Date of birth	Personal information
Austria	-	-	-	(4)		
Belgium	1986	V	L	8	d/m/y	initials
Denmark	1990	M	L, C	6	y	none
Finland	1986	M	L, C	20	d/m/y	part of SSN
France (13 regions) **	1988-1996)	V	L,C	na	m/y	none
(nationwide)	planned	M	L,C	5000	d/m/y	to be defined
Germany	1988	M	L	153	y	none
	1998	V	C	-	y	name based
Greece	1998	M	L, C	9	d/m/y	initials
Iceland	1985	M	L, C	1	m/y	name (from 1999)
Ireland	planned	-	-	1		
Italy (7 regions)	1985-1999	M	L	na	d/m/y	name based ***
Luxembourg	1999	V	L	1	m/y	initials
Netherlands	1989	V	L	na	y	initials
Norway	1986	M	L, C	5	m/y	aucune
Portugal	1983	V	C	(10)	d/m/y	initials
Spain	1999	V	L, C	na	d/m/y	initials
Sweden	1985	M	C	(5)	m/y	part of SSN
Switzerland	1985	M	L, C	8	d/m/y	initials (from 1999)
United Kingdom	1984	V	L, (C from 2000)	500	d/m/y	« soundex » code

V= voluntary ; M= mandatory
L = laboratories ; C = clinicians
na = not available
d/m/y = day/month/year
SSN = social security number
* in addition to sex, all countries
** all regional systems ended in 1998
*** not standardised across regions

residence), probable date and place of infection, previous negative and positive HIV tests, reasons or circumstances of testing, and indicators of disease progression (such as CD4 lymphocyte count).

Reporting data

Data on HIV infection for 1998 were available from 11 countries, the French region of Aquitaine (2.8 million, 4.6% of the total population) and the Lazio and Trento regions of Italy (5.6 million, 10% of the population) (table 2), which together represent 201 million (52%) of the 388 million population of western Europe. A total of 8104 cases of HIV infection and 4088 AIDS cases were reported in 1998. Numbers of cases of HIV infection per million population ranged from 16 in Finland to 90 in Switzerland and 94 in the two Italian regions combined. All countries reported more cases of HIV infection than AIDS, with ratios ranging from 1.5 in Switzerland to 4.5 in Belgium. Under 5% of cases in Norway and the UK were reported without transmission category and over 30% in Greece, Italy, and Switzerland. Among the 6444 cases reported with known transmission category, 44% were homo/bisexual men, 42% heterosexuals, 10% were injecting drug users (IDUs), and 2% had acquired infection vertically. As data from countries in southern Europe with large epidemics mainly among IDUs are very limited, these data are not representative of the situation in Europe as a whole.

Discussion

HIV infection reporting systems are an established part of HIV surveillance in most countries in western Europe. In the three countries that account for two thirds of the cumulative total of reported AIDS cases, however, HIV reporting either began only recently (Spain, 1999) or has yet to be implemented at national level (France, Italy). Differences exist in the organisation of reporting and in the type and format of information collected. As with AIDS, reports of HIV infection from clinicians provide detailed epidemiological and clinical information. Since diagnosis of HIV infection is less concentrated in specialised centres, HIV infection reporting by

Table 2

HIV reporting data in western Europe – end 1998

	Cumulative HIV cases reported to end 1998		Cases reported in 1998		
Country	Data from	total number	Nr	HIV rate per million	Ratio HIV:AIDS
Belgium	1986	11 067	740	73	4.5
Denmark	1990	2482	179	34	2.5
Finland	1986	945	801	16	4.0
Aquitaine (France)	1988	3719	217*	78	3.7
Germany (labs)	1993	13 359	2247	27	2.4
Greece	1998	1917 **	278*	26	1.9
Iceland	1985	121	8	29	4.0
Lazio + Trento (Italy)	1985	18 019	535	94	1.7***
Luxembourg	1985	397	301	71	3.0
Norway	1986	1869	981	22	2.5
Portugal	1983	10 012	na	-	-
Sweden	1985	4911	246	28	3.9
Switzerland	1985	23 821	657	90	1.5
United Kingdom	1984	33 329	2789	48	2.9
Total		125 968	8104	40	2.0

* Data by year of diagnosis

** Includes retrospective reporting before 1997

*** AIDS data by year of diagnosis, not adjusted for reporting delays

na : not available

clinicians may be less complete than AIDS reporting, for which 75% to 100% of cases are estimated to be reported (3). For this reason, in most countries cases of HIV infection are (also) reported from laboratories, which are usually few in number and may provide a more exhaustive count of diagnosed cases (e.g., higher than 95% in Denmark (4))

The elimination of duplicate reports and the ability to match reports of HIV infection with other data sets are essential requirements of an effective HIV reporting system. This implies the collection of personal information which, in turn, creates a potential risk for breaches of confidentiality. Among the measures taken to ensure data security, most western European countries exclude names from the collected personal information in HIV reports. In the UK, the use of 'soundex' codes (based on the surname) and date of birth provides efficient identification of duplicates and linkage with AIDS case reports (5). The use of initials, date of birth, and sex in a simulation made on the nominal AIDS data set in Spain resulted in a very low proportion of truly new cases being erroneously classified as duplicates (0.1%) (I Noguer, personal communication). When non nominal HIV reporting is implemented, the efficiency of matching and removal of duplicates also depends on the completeness of the identifying information collected (6), however, and on the (increasing) size of data sets. Further evaluations of this issue are needed in Europe.

Reporting of HIV infection must be interpreted with caution, taking into account other available epidemiological data, because these reports do not provide a direct measurement of the incidence or prevalence of HIV infection. The proportion of HIV infected individuals who are diagnosed and reported varies according to the phase of the epidemic (4), HIV testing patterns (7), and characteristics of surveillance systems. In countries where HIV infection reporting began early, the cumulative number of HIV reports can provide a minimum estimate of prevalence if mortality data are also available or can be estimated. Numbers of cases of HIV infection reported in 1998 are higher than numbers of AIDS reports. HIV reporting is helping to improve assessment of the scale and extent of recent HIV transmission in the population. Annual numbers of HIV infections reported in the 1990s were relatively stable in some countries and decreased in others (not shown here) (8). The comparison of HIV and AIDS reporting data suggests that the level of HIV transmission has remained relatively stable in recent years and that the sudden decline of AIDS incidence has been due mainly to the effect of treatments. Overall, sexual transmission accounts for the vast majority of reported HIV infections, but the countries with the largest numbers of HIV infected IDUs are poorly represented in these data. HIV trends by transmission group are difficult to interpret in some countries because the proportion of cases with unknown mode of transmission is high and has changed over time

Four countries (Belgium, Greece, Luxembourg, United Kingdom) already provided to the European system complete individual data on cases reported since 1997, which include clinical stage at diagnosis (around 80% of cases reported in 1998 were diagnosed before AIDS), the probable year of infection (estimated for around 10% of cases) and follow-up information on AIDS and death. These data should contribute to a better description of recent HIV transmission trends, of disease progression and of care at the population level.

HIV reporting has been recently introduced, expanded, or improved in most western European countries and further developments are underway. In particular, new and planned systems in southern countries should contribute to a more representative surveillance picture of the HIV epidemic in Europe. The widespead participation in the European HIV reporting system reflects strong motivation for the collaborative development of this surveillance tool. The current momentum of change should facilitate better standardisation of surveillance definitions and practices, which remains a major challenge for international surveillance.

* The European Centre for the Epidemiological Monitoring of AIDS (EuroHIV) is supported by the European Commission (contract VS/1999/5227 (99CVVF4-023))

References

1. Hamers FF, for the group of experts and national coordinators of HIV/AIDS surveillance from the countries of WHO European Region. Recommendations for HIV surveillance in Europe. Eurosurveillance 1998; 3: 51.2. European Centre for the Epidemiological Monitoring of AIDS. HIV testing and case reporting in Europe. HIV/AIDS surveillance in Europe: Report No. 56. St Maurice: CESES, 1997: 40-7.
2. European Centre for the Epidemiological Monitoring of AIDS. Completeness of AIDS reporting in Europe. HIV/AIDS surveillance in Europe: Report No. 49. St Maurice: CESES, 1996: 30-33.
3. Smith E, Jensen L, Wachmann CH. Patterns and trends in clinically recognized HIV seroconversions among all newly diagnosed HIV-infected homo-/bisexual men in Denmark, 1991-1994. AIDS 1996; 10: 765-70.
4. Mortimer JY, Salathiel JA. Soundex codes of surnames provide confidentiality and accuracy in a national HIV database. Commun Dis Rep CDR Rev 1995; 5: R183-6.
5. CDC. Guidelines for national human immunodeficiency virus case surveillance, including monitoring for human immunodeficiency virus infection and acquired immunodeficiency syndrome. MMWR Morb Mortal Wkly Rep 1999; 48(RR-13): 1-31.
6. De Cock KM, Johnson AM. From exceptionalism to normalisation: a reappraisal of attitudes and practice around HIV testing. BMJ 1998; 316: 292-5.
7. European Centre for the Epidemiological Monitoring of AIDS. HIV/AIDS surveillance in Europe. Report No. 61. Saint Maurice: CESES, 1999.

Bioterrorism preparedness and response in European public health institutes

Bruno Coignard[1]
1. Institut de Veille Sanitaire, Saint-Maurice, France, on behalf of the members of the Eurosurveillance editorial board

Correspondence: S van den Hof (susan.van.den.hof@rivm.nl)

Citation style for this article:
Coignard B. Bioterrorism preparedness and response in European public health institutes. Euro Surveill. 2001;6(11):pii=383. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=383

Article published on 01 November 2001

The terrorist attacks on 11 September 2001 and the deliberate release of anthrax in the United States had consequences for public health not only there, but also in Europe. Europe's public health systems had to manage numerous postal materials possibly contaminated with anthrax. Our survey aimed to document the response of European public health institutes to recent bioterrorist events to identify the gaps that need to be addressed; 18 institutes from 16 countries participated in this Euroroundup. Bioterrorist threats in Europe were hoaxes only, and should be considered as a "preparedness exercise" from which three lessons can be drawn. Firstly, because of inadequate preparedness planning and funding arrangements, Europe was not ready in October 2001 to respond to bioterrorism. Secondly, although European institutes reacted quickly and adapted their priorities to a new type of threat, they need adequate and sustained support from national governments to maintain their overall capacity. Thirdly, the recent crisis demonstrated the need for increased investment in epidemiology training programmes and the establishment of a technical coordination unit for international surveillance and outbreak response in the European Union.

Introduction

Within 24 hours of the terrorist attacks on 11 September, the Centers for Disease Control and Prevention (CDC) deployed epidemiologists to assess the consequences of the disaster and to reinforce surveillance for potential acts of bioterrorism (BT) (1). Less than a month later, on 4 October 2001, CDC reported a fatal case of inhalation anthrax in Florida (2). Subsequently, a total of 22 cases of anthrax have been identified in five different states; five of the patients died. All but two have been directly associated with the deliberate release of anthrax. More than 100 epidemiologists were deployed by CDC during one of its most challenging investigations, which is still in progress (3-9).

Although the terrorist events took place in the United States (US), European armed forces were put on heightened alert, and public health systems in European countries had to manage numerous letters containing powders suspected to be contaminated with Bacillus anthracis spores. Neither terrorist attacks nor anthrax cases occurred in the following weeks; all bioterrorist threats seemed to be hoaxes. The pressure on European countries, however, was high, as they quickly had to devote public health resources to face a new type of threat.

The objective of our survey was to document the role of European public health institutes in BT preparedness and response (P&R) in the year before and the month after 4 October 2001, with special emphasis on their recent response to possible bioterrorist threats.

Population and Methods

For each European public health institute, key contacts in the area of BT P&R were identified with the help of Eurosurveillance editorial board members. Seventeen institutes – in the 15 countries of the European Union, Norway, and Estonia – were included.

The survey was conducted using a self administered questionnaire, which addressed the following issues on BT P&R: description of the national public health institute, response to recent threats, P&R in the year before and the month after 4 October 2001, communication, and plans for the future. Eurosurveillance editorial board members reviewed the questionnaire, which was mailed electronically to all participants on 22 November. Participation in the survey was voluntary, and the questionnaires had to be sent back by 14 December. Data were entered and analysed using Epi Info version 6.04d and EpiMap version 2.

Results

Respondents

Of 17 countries contacted, all but one participated in the survey. Answers from the United Kingdom were received separately for England and Wales, Northern Ireland, and Scotland. Our results therefore include answers from 18 institutes in 16 countries.

All institutes were in charge of communicable disease surveillance and control in their country. Other areas of expertise included microbiology in 14 institutes and environmental health in 12 institutes. Seven institutes reported additional activities, such as vaccinology, vaccine production, chronic disease and injury, occupational health, or toxicology.

Eight institutes reported having performed in 2000 at least one communicable disease outbreak investigation, by deploying a national team to help trial investigations of an outbreak.

Response to recent bioterrorist threats

First actions

All institutes but one organised specific meetings on BT immediately after, or even before 4 October: two organised their first BT meeting the week before, nine one week after, and six two weeks after. Among the first actions taken by institutes, 14 reinforced communicable disease surveillance systems, and all created or updated BT related guidelines. One third reported other actions, such as increasing laboratory capacity, organising collaboration with authorities, delivering information to the public, media, or professionals, or setting up a hospital based epidemiological correspondent network. The duty system was reinforced in 14 institutes: one third had epidemiologists on call 24 hours a day and seven days a week; two had extra laboratory technicians on call.

Importance of threats according to countries

European countries experienced the first threats of contaminated letters four to 13 days after 4 October. Thirteen countries were able to provide a summary figure of these threats. They managed from 50 to 2790 threats from 4 October to 3 November; the total number of threats was 7622. The number of threats per 100 000 inhabitants ranged from 0.2/100 000 to 31.4/100 000 (median 3.6/100 000). Three countries (Denmark, Portugal, and Luxembourg) had the highest cumulative incidences of mail threats (18.9, 20.3, and 31.4, respectively) while three (Austria, Italy, and Spain) reported lower incidences ranging from 0.2 to 1.1 (figure). The proportion of mail threats that required laboratory testing ranged from 22 to 100% (median 57%). The proportion of threats that required follow up (remotely or on site) by national institutes ranged from 0 to 90% (median 17%) (table 1). Four institutes conducted on site investigations of some of the threats.

The number of people put on antibiotic prophylaxis during the same period (reported by 12 countries) ranged from 0 to 1500 (median 128); total number for reporting countries was 2237. Two countries reported false alerts of contaminated water works.

Laboratory capacity

Of 16 countries, 12 reported a national laboratory capacity for all four biological agents most likely to be involved in a deliberate release (anthrax, botulism, plague, and smallpox); all countries had capacity for anthrax and botulism, 12 for smallpox, and 14 for plague. Ten countries also reported laboratory capacity for other agents including tularaemia (eight) and viral haemorrhagic fever (five).

All countries but one used Gram stains, cultures, and polymerase chain reaction (PCR) for diagnosis of B. anthracis from human samples. Scientific methods for environmental sampling and testing were not in the public domain and rarely reported; three countries reported having tested suspected letters for agents other than B. anthracis.

Impact of the events of 4 October on bioterrorism preparedness and response efforts in European countries

Preparedness

Seven countries reported having a BT P&R plan ready in the year before 4 October; six had appointed a national BT P&R team. In the month after 4 October, all reported implementing such a plan; 12 countries had appointed a national team. Partners in this team included ministries of health in 12 countries, local health departments in nine, reference laboratories in 11, armed forces in eight, civil defence agencies in 10, ministries of internal affairs in 10, ministries of justice in four, hospitals in 12, or primary care physicians in three.

Institute mandate

Eight institutes had officially been given a mandate by their governments for BT P&R in the year before 4 October; this number increased to 11 one month after this date. Two institutes coordinated their country's BT activities. The number of formal BT meetings organised by these institutes ranged from 0 to 28 (median 1) in the year before 4 October. It dramatically increased and ranged from 1 to 80 (median 15) in the month after.

Resources

In the year before 4 October, one institute had a specific budget for BT P&R, and two reported having a BT P&R unit or core team. A total of two full time equivalent (FTE) scientific personnel were specifically devoted to BT related activities in two European institutes during the same time period; 29 FTEs from five institutes could have been included as a «surge» capacity. One month after 4 October, seven institutes had a specific budget for BT P&R, and 11 had constituted a BT P&R unit or core team. In October 2001, a total of 33 FTEs were specifically devoted to BT related activities in

nine institutes; 113 FTEs from 16 institutes could have been included as a surge capacity. Two fellows from the European Programme for Intervention Epidemiology Training (EPIET) were involved in BT related activities in the year before 4 October; six were involved one month after.

Epidemiological procedures

All European institutes created or updated guidelines and recommendations related to BT prevention and control in the month after 4 October (table 2). When writing guidelines related to specific biological agents, 14 institutes used national case definitions, 11 European case definitions, 10 CDC case definitions, and seven World Health Organization (WHO) case definitions. Five institutes shared definitions they used with other European institutes.

In the year to 4 October, four institutes reported sharing these guidelines outside their own institution. This number increased to 17 after this date: four institutes published some of them in medical journals; 14 posted them on their web site; 13 provided advice to decision makers; and 13 organised press releases or conferences. No institute organised training sessions on BT P&R in the year before 4 October, but five organised a total of nine training sessions in the month after.

Laboratory procedures

Laboratory testing of suspected letters was reorganised shortly after 4 October when countries experienced numerous threats. The year before this date, six countries reported dedicating one national or a few regional laboratories to this purpose. The month after 4 October, 12 reported dedicating one national laboratory, six several regional laboratories, and two using any laboratory available in their country.
Laboratory testing of persons potentially exposed to biological agents was reorganised also. In the year before 4 October, four countries reported dedicating one national laboratory for that purpose, four several regional laboratories, and three reported using any laboratory available. In the month after 4 October, eight reported dedicating one national laboratory, seven several regional laboratories, and five using any laboratory available in their country.

National pharmaceutical stockpiles

European countries increased their stockpiles of pharmaceuticals shortly after 4 October 2001: 11 countries reported stocking ciprofloxacin in October. The total number of 60 day courses available in the six countries that reported this information was 87 540, that is, one course for 816 inhabitants. Nine countries reported having a smallpox vaccine stockpile in October; the total number of doses available in six countries that reported this information was 13 400 000 – one dose for 11 inhabitants.

Communication between countries for bioterrorism preparedness and response

Of 18 institutes contacted, 17 contacted other public health institutions about recent BT events in October 2001. Fifteen contacted other European institutes, 12 CDC, seven the WHO headquarters in Geneva, four the European Commission Directorate-General for Health and Consumer Protection (DG-SANCO), and three the WHO Regional Office for Europe in Copenhagen.

The objectives of these contacts were for 17 institutes to share information on BT threats in the United States or in Europe, for 11 to get epidemiological expertise, and for 10 to get laboratory expertise. Seventeen institutes received the information they requested from these institutions.

In addition to contacting public health institutions, all European institutes used other means to get information on recent BT events: all accessed the internet and browsed the web, 17 read the ProMed mailing-list, 17 reviewed the medical literature, and 15 reviewed the press.

Plans for the future

Of 18 institutes contacted, 16 plan to improve their BT P&R capacity in the next year. At the time of the survey, five institutes already had a budget for this activity in 2002.

Three institutes planned to create a specific BT P&R unit, and seven to recruit additional personnel: one to 18 (median 5) FTE personnel will be recruited next year in each of those institutes; zero to 16 (median 1) will be specifically devoted to this activity. According to this survey, a total of 47 FTE personnel will be recruited in Europe next year, 26 of them being exclusively involved in BT related activities. Lastly, 13 institutes planned to organise training in BT P&R in the next year.

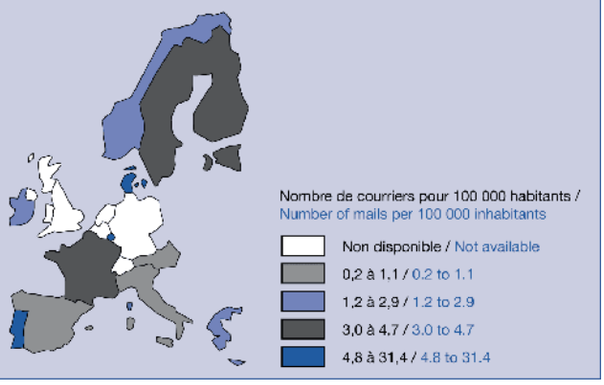
Discussion

In October 2001, BT P&R plans were set up or updated by individual European countries. They often were classified, however, and thus prevented, at least in the beginning, effective and true discussions between European institutes. This was a major problem and we believe that such activities should be made public. We thank all respondents for the information they shared, even if individual countries could not be identified in this report. This survey is not an inventory: it is a first attempt to document the response of European public health institutes to recent BT events in order to identify the gaps that need to be addressed. We encourage national institutes to complete this picture and submit to Eurosurveillance any additional information about their recent experience.

The deliberate release of B. anthracis spores through the postal service inspired copycats, and countries had to manage numerous potentially contaminated letters. All but four countries reported the total number of mail

Figure

Incidence cumulée des menaces postales par pays, Europe, 4 octobre au 3 novembre 2001 / Cumulative incidence of mail threats by country, Europe, 4th October to 3rd November 2001



threats they managed. It varied greatly by country and was not related to their size; such differences may be related to differences in case definitions or data collection procedures.

Most European countries were not prepared to face possible bioterrorist threats: less than half reported having a BT P&R plan ready in the year before 4 October, and European institutes were not systematically a part of it. When institutes were associated, resources and funding specifically devoted to this activity were scarce. Logically, BT related efforts were rare. In the US, CDC's efforts in BT P&R began in 1998 (10). The US Department of Health and Human Services spent $158 million in 1999 for BT P&R and $230 million in 2000 (11). This budget increased in 2001 and will most likely do so again in 2002. Even if BT P&R cannot be summarised only on budget, this proves that the US administration had an early and strong commitment to public health as a response to bioterrorism. To date, most of these funds were used to improve the capacities of state and local health departments. CDC's goals are to improve public health infrastructure not only to respond to a bioterrorist event but also to any infectious disease outbreak (10).

One can argue that communicable diseases resulting from bioterrorist acts only differ from «normal» ones in the nature of their source: deliberate release as opposed to natural occurrence. Europe's public health institutes therefore have the expertise to respond to both. Adequate resources, however, are needed. We wanted to assess resources in personnel and tried to get the total number of epidemiologists working on communicable disease surveillance and control in European countries. However, we could not obtain reliable estimates, as the definition of an epidemiologist varies from one country to another. Another way to assess capacity of European public health institutes was to ask if national institutes had performed at least one outbreak investigation in 2000. Less than half reported such an activity; some institutes reported not being given a mandate to carry out outbreak investigation, or only providing remote support to local health departments. What we know for certain is that existing communicable disease surveillance and control personnel in European institutes were assigned new duties. The total number of FTE involved in BT related activities in European institutes dramatically increased in October 2001; EPIET fellows participated in this effort and would have been available for outbreak response (12).

The management of possible bioterrorist threats requires standardised procedures. As only a few countries had a BT P&R plan ready before 4 October, most of the necessary guidelines were prepared during the crisis. They first addressed priorities such as management of potential exposures to biological agents. They also addressed investigation and control of the four biological agents (anthrax, botulism, plague, and smallpox) most likely to be involved in a bioterrorist attack. Bioterrorist threats are, however, not limited to this short list and a covert release of biological agents may be difficult to recognise. Syndrome based investigation guidelines are needed, but only one third of institutes had these prepared. Regarding surveillance, most of the institutes reported having reinforced relevant communicable disease surveillance systems. Respondents did not, however, describe procedures: guidelines may have been written, but we do not know if and how surveillance systems were enhanced.

Laboratory testing also was a crucial element in the management of possible bioterrorist threats. Before 4 October, only a few countries had identified specific laboratories (national or regional) for mail testing, and only half did so for patient testing. A more centralised approach was progressively adopted after this date. The proportion of mail testing varied greatly by country. Highest proportions were observed among countries managing the lowest number of threats. In countries facing a greater number of threats, national laboratory capacities were probably exceeded, and specific, more rational testing strategies were implemented. Lastly, although all countries reported laboratory capacity for anthrax, four and two countries did not have laboratory capacity for smallpox and plague, respectively. Additional surveys will be needed to assess more precisely the capacity of European laboratories. For rare organisms that could be involved in a bioterrorist act – for example, anthrax, Francisella tularensis, or smallpox viruses – it may be better to establish good reference laboratories at the European Union level than to disperse scarce resources in multiple countries. European cooperation in BT will require sharing of laboratory resources.

Multiple partners participated in the management of possible bioterrorist threats. National public health institutes were only one element of the response, alongside local health departments, reference laboratories, ministries of health, healthcare organisations, primary care physicians, justice departments,

Table 1

Mail threats and letters that required laboratory test or follow up by national institutes, by country, Europe, October 4th to November 3rd 2001

Country	Mail threats		Laboratory tests		Follow up by national institute	
	n	(per 100 000 inhabitants)	N	(%)	n	(%)
Austria*	60	(0.7)	60	(100)	5	(8)
Belgium	Unknown	-	761	-	Unknown	-
Denmark*	1 000	(18.9)	220	(22)	Unknown	-
Finland	235	(4.5)	67	(29)	40	(17)
France	2 790	(4.7)	1400	(50)	Not applicable†	-
Germany	Unknown	-	Unknown	-	0	-
Greece	220	(2.1)	86	(39)	66	(30)
Ireland*	100	(2.7)	82	(82)	50	(50)
Italy	142	(0.2)	142	(100)	0	(0)
Luxembourg	135	(31.4)	68	(50)	54	(40)
Portugal	2 000	(20.3)	700	(35)	0	(0)
Spain	450	(1.1)	400	(89)	0	(0)
Sweden*	350	(3.9)	200	(57)	10	(3)
UK, England & Wales	Classified	-	Classified	-	200	-
UK, Northern Ireland	Classified	-	Classified	-	3	-
UK, Scotland*	Classified	-	Classified	-	20	-
Norway	90	(2.0)	65	(72)	50	(56)
Estonia	50	(3.6)	45	(90)	45	(90)
Total	7622	(3.5)	4296	(46)‡	543	(8)‡

* Evaluations / Estimates † Suivi réalisé principalement par les départements de santé locaux / Follow-up primarily done by local health departments
‡ Seulement pour les pays ayant déclaré le nombre total de menaces postales / Only includes those countries having reported the total number of threats

and police and armed forces. None were involved in BT P&R teams. Local health departments and primary care physicians especially were underrepresented. These personnel may be at the forefront of an outbreak, especially in the case of a covert release, and efforts should be made to involve and train them adequately. Lastly, national BT P&R teams had very few meetings the year before 4 October. Some respondents reported issues in information sharing, action coordination, or identification of responsibilities. Those issues could have been avoided if all personnel had met previously and knew each other.

All countries that did not have a BT P&R plan implemented one immediately after 4 October. However, one can imagine that such «emergency» plans are still preliminary and will need further development. Almost all institutes added BT activity to their usual duties without additional resources, and reallocated personnel were not available for regular communicable disease control activities. Responses from institutes therefore had limits. Most of the institutes want to improve their BT P&R capacity in 2002, and reinforcing existing communicable disease surveillance and control departments seems to be the favourite, integrated approach. Some countries, however, plan to create a specific BT P&R unit. At the time of our survey, only a few institutes already had a specific budget for BT related activities in 2002. Increased and sustained support from governments will be required if European institutes want to develop their BT P&R.

Support from the European Commission may help, especially to coordinate actions and avoid duplicated efforts. Input from the Commission in the recent crisis, however, was late and respondents to the survey emphasised the need for anticipation, coordination, and support at the European level. The need for close liaison and timely communication with international agencies and other national institutes was underlined also. As a matter of fact, only a few institutes exchanged information with DG-SANCO or the WHO Regional Office for Europe in October 2001. Consequently, all institutes had similar activities: they followed events in the US,

Table 2

European institutes (N=18) having prepared guidelines for bioterrorism prevention & control, before and after October 4th 2001, by topic

Topic of guidelines	Year before 04/10/01		Month after 04/10/01	
	n	(%)	N	(%)
Alert procedures	4	(22)	17	(94)
Management of exposures	4	(22)	17	(94)
Agent specific investigation guidelines	2	(11)	17	(94)
- Anthrax	1	(6)	17	(94)
- Smallpox	1	(6)	11	(61)
- Botulism	1	(6)	10	(56)
- Plague	2	(11)	10	(56)
- Other agent*	2	(11)	5	(28)
Syndrome based investigation guidelines	0	(0)	6	(33)
Clinical management guidelines	1	(6)	9	(50)
Recommendations for the public / media	2	(11)	13	(72)
Autres recommandations / Other guidelines†	0	(0)	1	(6)

* Tularémie, fièvre virale hémorragique, entérotoxine B du staphylocoque, diphtérie, ricine, saxitoxine, fièvre Q / Tularaemia, viral haemorrhagic fever, staphylococcus enterotoxin B, diphtheria, ricin, saxitoxine, Q fever

† Procédures de tests pour les prélèvements cliniques / Laboratory testing of clinical samples

managed potentially contaminated postal materials, wrote guidelines, and provided advice to multiple partners. Only a few institutes shared the case definitions they used in guidelines; most of them were national definitions. The absence of common European BT related epidemiological procedures may be an issue in the case of an outbreak involving more than one country. Europe is currently debating the creation of a technical coordination unit (TCU) for communicable disease surveillance and outbreak response (13, 14); such a unit would provide a formal structure with a high level of scientific expertise in communicable disease control. In addition to an increased investment in EPIET and related national training programmes (12), it would greatly enhance the future response capacity of Europe to bioterrorist attacks.

Conclusion

Bioterrorist threats in Europe were only hoaxes fortunately, but should be considered as a «preparedness exercise» from which lessons have to be drawn.

A recent report identified several critical control points in the European response to communicable disease outbreaks involving more than one country (15). Some of the conclusions of this report – inadequate preparedness planning and inadequate funding arrangements – are consistent with the findings of our survey.

First lesson: in October 2001, Europe was not ready to respond to bioterrorism. Nevertheless, European public health institutes quickly adapted their priorities and reallocated limited resources to manage possible bioterrorist threats. National institutes have the necessary expertise but may have lacked the resources needed to implement all the necessary procedures. Second lesson: European public health institutes may benefit from specific funding for BT, but first they need an adequate and sustained support to maintain their overall capacity.

Lastly, anticipation, coordination, and support for communicable disease control, including BT P&R, is needed at the European level.

Third lesson: the recent bioterrorist events demonstrated again the need for increased investment in epidemiology training programmes and the establishment of a TCU for international surveillance and outbreak response in the EU.

List of respondents

The following countries, institutes, and individuals contributed to this survey:

Austria, Federal Minister for Social Security And Generations (Reinhild Strauss)

Belgium, Scientific Institute of Public Health (Frank Van Loock)

Denmark, Statens Serum Institute (Niels Frimodt-Moller)

Finland, National Public Health Institute (KTL) (Pekka Nuorti)

France, Institut de Veille Sanitaire (Philippe Malfait and Jean-Claude Desenclos)

Germany, Robert Koch Institute (Michael Kramer)

Greece, Hellenic Centre for Infectious Disease Control (George Saroglou)

Ireland, National Disease Surveillance Centre (Paul Mckeown)

Italy, Istituto Superiore Di Sanita (Donato Greco and Stefania Salmaso)

Luxembourg, Directorate of Health (Danielle Hansen-Koenig and Nicolas Rume), National Service of Infectious Diseases (Robert Hemmer), National Health Laboratory (François Schneider)
General Directorate of Health & National Institute of Health (Francisco George)

Spain, National Epidemiologie Centre - Public Health Institute Carlos III (Salvador De Mateo)

Sweden, Swedish Institute For Infectious Disease Control – SMI (Anders Tegnell)

UK - England & Wales, Communicable Disease Surveillance Centre – CDSC (Dilys Morgan and Mark Reacher)

UK - Northern Ireland, CDSC Northern Ireland (Brian Smyth)

UK - Scotland, Scottish Centre for Infection & Environmental Health (Peter Christie)

Estonia, Health Protection Inspectorate (Kuulo Kutsar)

Norway, National Institute of Public Health (Hans Blystad)

Acknowledgements

In addition to the members of the Eurosurveillance editorial board, the author thanks the following individuals for their input: Brigitte Helynck, Alain Moren, Christophe Paquet, and Mina Vilayleck.

References

1. Notice to Readers: New York City Department of Health response to terrorist attack, September 11, 2001. MMWR Morb Mortal Wkly Rep 2001; 50: 821-2.
2. Notice to Readers: Ongoing investigation of anthrax - Florida, October 2001. MMWR Morb Mortal Wkly Rep 2001; 50: 877.
3. Update: Investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. MMWR Morb Mortal Wkly Rep 2001; 50: 889-93.
4. Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. MMWR Morb Mortal Wkly Rep 2001; 50: 909-19.
5. Update: Investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. MMWR Morb Mortal Wkly Rep 2001; 50: 941-8.
6. Update: Investigation of bioterrorism-related anthrax and adverse events from antimicrobial prophylaxis. MMWR Morb Mortal Wkly Rep 2001; 50: 973-6.
7. Update: Investigation of bioterrorism-related anthrax, 2001. MMWR Morb Mortal Wkly Rep 2001; 50:1008-10.
8. Update: Investigation of Bioterrorism-Related Inhalational Anthrax - Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001; 50:1049-51.
9. Update: Investigation of Bioterrorism-Related Inhalational Anthrax - Connecticut, 2001. MMWR Morb Mortal Wkly Rep 2001; 50:1077-9.
10. McDade JE, Franz D. Bioterrorism as a public health threat. Emerg Infect Dis 1998; 4: 493-4.
11. Shalala DE. Bioterrorism: how prepared are we? Emerg Infect Dis 1999; 5: 492-3.
12. Catchpole M, Moren A. Responses to terrorism. Lancet 2001; 358: 1911.
13. Petersen LR, Catchpole M. Surveillance for infectious diseases in the European Union. A small European centre may have an important coordinating role. BMJ 2001; 323: 818-9.
14. Van Loock F, Wallyn S. A EU rapid response system for threats to public health: appraising options. Final report to the European Commission. Agreement n°SI2.225063 (2000 CFV4-006)
15. MacLehose L, Brand H, Camaroni I, Fulop N, Gill ON, Reintjes R, et al. Communicable disease outbreaks involving more than one country: systems approach to evaluating the response. BMJ 2001; 323: 861-3.

European Commissioner again pledges European centre for disease control by 2005

L Pritchard[1]
1. Eurosurveillance editorial office

Correspondence: Laura Pritchard (eurowkly@phls.org.uk)

Citation style for this article:
Pritchard L. European Commissioner again pledges European centre for disease control by 2005. Euro Surveill. 2002;6(40):pii=1888. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=1888

Article published on 03 October 2002

David Byrne, Europe's Commissioner for Health and Consumer Protection, reiterated the European Commission's commitment to the creation of a European centre for disease control by 2005 at the 2002 meeting of the European Health Forum Gastein (EHFG, http://www.ehfg.org/) (1,2). The centre will bring together the expertise in member states and will act as a reference and coordination point both in routine and crises situations. The Commission also plans to establish a health portal for online information by 2004.

The EHFG, which was launched in 1997, holds an annual event at Gastein, Austria, and assembles experts, interest groups, politicians, administrators, as well as decision makers representing patients and consumers, business and industry, and science and academia to debate key health issues. The main objective of the EHFG is to facilitate the establishment of a framework for advising and developing European health policy while recognising the importance of national and regional authorities and decision making bodies.

The theme of this year's meeting was common challenges for health and care. In the context of a widening European Union (EU), whose citizens are aware of the increasing influence that EU and global events have on health systems of individual states, the Commissioner pointed to the major health challenges of combating communicable diseases, ensuring the safety of sensitive products, such as foodstuffs or blood, and the functioning of health systems within the single market.

The Commission has a responsibility to protect EU citizens against health threats and adequate protection can no longer be achieved by health authorities in member states acting alone. Under the new public health framework programme, which begins in January 2003 , investment has been committed to finding ways to respond effectively to health threats (2). It is hoped that the review of the EU treaty framework and structures, currently being undertaken by the European Convention, will consider issues such as the powers needed by the Community to address health problems that transcend borders, such as communicable diseases and environmental threats.

As many EU citizens become active partners in managing their own health, a health portal, which will direct patients to authoritative online information, will play an important role in the dissemination of information on health and care. Other priorities expressed by the Commissioner are improving cooperation between health systems across Europe, and bringing together health and other policies in order to address health issues with the full potential of the powers and instruments at the community's disposal.

If these aims are to be achieved, it was argued, support from the member states is vital, as is the development of cooperation with specialised organisations such as the World Health Organization, which will be a principal partner in planning and developing future EHFG activities.

References:

1. Byrne D. Future priorities in EU health policies. Speech/02/426 to the European Health Forum on "common challenges for health and care", Gastein, 26 September 2002. (http://europa.eu.int/rapid/start/cgi/guesten.ksh?p_action.gettxt=gt&doc=SPEECH/02/426|0|RAPID&lg=EN&display=)
2. Commission of the European Communities. Opinion of the commission pursuant to Article 251 (2), third subparagraph, point (c) of the EC Treaty, on the European Parliament's amendments to the Council's Common Position regarding the proposal for a decision of the European Parliament and of the Council adopting a programme of Community action in the field of public health. 2000/0119 (COD). 23 January 2002. (http://europa.eu.int/eur-lex/pri/en/lip/latest/doc/2002/com2002_0029en01.doc)
3. Gill N. European Community action to enhance the capacity to tackle communicable diseases takes a major step forward. Eurosurveillance Weekly 2001; 5: 010621 (http://www.eurosurveillance.org/ew/2001/010621.asp)

SARS: Retrospective cohort study among German guests of the Hotel 'M', Hong Kong

D. Radun [1] , M. Niedrig [2], A. Ammon [1], K Stark [1]
1. Department for Infectious Disease Epidemiology, Robert Koch-Institut, Berlin, Germany
2. Centre for Biological Safety, Robert Koch-Institut, Berlin, Germany

Citation style for this article:
Radun D, Niedrig M, Ammon A, Stark K. SARS: Retrospective cohort study among German guests of the Hotel 'M', Hong Kong. Euro Surveill. 2003;8(12):pii=436. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=436

Article published on 01 December 2003

Hong Kong played a pivotal role in the international spread of the Severe Acute Respiratory Syndrome (SARS): a doctor who spent the night of 21-22 February 2003 at Hotel 'M' was identified as the index case for four national and international clusters of SARS. In parallel to the international collaborative study led by WHO and United States, a retrospective study on the cohort of German persons staying at Hotel 'M' was conducted. The inclusion criteria covered a period from 21 February to 3 March 2003 to allow the detection of cases possibly due to environmental contamination. In the twenty-one German guests traced as having stayed at Hotel "M" during this period, one case of laboratory confirmed SARS was found. The case history suggests that close vicinity to the index patient may have played a role in transmission. In line with findings of environmental investigations in the hotel, environmental contamination should be considered as a possible source of infection.

The Severe Acute Respiratory Syndrome (SARS) epidemic, which caused over 8000 reported cases, caused worldwide concern between February and June 2003, and claimed over 700 lives. A novel coronavirus was identified as the causative agent (1-3). Hotel 'M' played a pivotal role in the international spread of the new disease: on 21-22 February 2003, a doctor from Guangdong Province, China, spent one night on the ninth floor of this particular hotel in the Kowloon district of Hong Kong. On the day of his arrival, the doctor had been suffering from a respiratory illness for at least five days. After spending the night in the hotel, he was transferred to a hospital the next morning with worsening symptoms. Despite treatment, he died on 4 March 2003. Retrospectively, this man was identified as the primary index case for four national and international clusters of SARS as well as cases in two countries without documented secondary transmission (4). According to information from the Hong Kong health authorities, health talks and screening of sick leave records did not reveal any cases among hotel staff.

The United States, the United Kingdom, Canada and Germany conducted a collaborative study, led by the World Health Organization (WHO) and the United States. The objectives were to identify risk factors for development of SARS at Hotel 'M' and to identify modes of transmission. Although it is still unknown which specific modes of transmission played a role in spreading the infection within the hotel, the exposure history of secondary cases indicates that sharing the same floor at Hotel 'M' may have been a risk factor for SARS infection. In this article we report the findings of the German cohort.

Methods

To enable contact tracing, consulates were provided with a list of names of their citizens who had stayed at Hotel 'M' between mid-February and the beginning of March. The lists were passed on to the respective national health authorities. In Germany, the list was sent to the Robert Koch-Institut (RKI).

The RKI conducted a retrospective cohort study to assess whether any Germans staying at Hotel 'M' between 21 February and 3 March 2003 had contracted SARS. In contrast to the international study, we used extended inclusion criteria for the German cohort: any adult guest who had stayed at Hotel 'M' between 21 February and 3 March 2003 was included, instead of focusing on the night of 21-22 February only. One reason for this was to decrease the likelihood of missing later cases that might have occurred due to lingering hazards like environmental contamination. A standardised questionnaire was administered by telephone collecting information on symptoms compatible with SARS that had occurred within fourteen days after staying at the hotel. Furthermore, other information on possible risk factors for transmission was collected, for

example, the frequency with which the hotel elevator was used, contact with ill people within the hotel and other variables potentially associated with infection. In addition, efforts were made to obtain blood specimens from all participants to test for the SARS-CoV IgG-antibodies using immunofluorescence and ELISA techniques. Serological tests were performed after a time interval of at least eight weeks following the stay at the hotel. These assays had been validated extensively beforehand based on nearly 200 SARS positive and more than 500 SARS negative sera. Cross reactions or unspecific reactions due to other coronaviruses were not observed.

Results

In total, 21 German guests stayed at Hotel 'M' between 21 February and 3 March 2003. All of them agreed to be interviewed. Among them, 10 had stayed overnight on 21-22 February in the hotel, the same night as the index patient. Overall, 15 (71.4%) serum specimens were tested for SARS coronavirus antibodies, including 6 (60%) specimens from the 10 people whose stay had coincided with the index patient's stay.

Retrospectively, a female traveller from Germany was identified as having had SARS. She had spent the night of 21-22 February at Hotel 'M' in Hong Kong in a room on the same floor as the index patient but said she had had no contact with him. The following day, the woman travelled to Australia, her final destination. On 26 February, she experienced a febrile illness with sudden onset of symptoms including dry cough, runny nose, severe myalgia and general malaise. On 6 March, she was seen by a general practitioner who prescribed antibiotic treatment. By the time she returned to Germany on 12 March, the patient had recovered completely. Serologic tests on day 72 and 104 after onset of disease showed IgG antibodies to the SARS-CoV with a titre of 1:500 obtained by immunofluorescence and 1:800 and 1:400 by ELISA, respectively. Since her illness was confined to her stay in Australia, the case was reported to the WHO SARS surveillance system retrospectively in July by the Australian health authorities. Remarkably, the patient's travelling companion, who had experienced similar exposure, remained healthy and had negative serology for SARS-CoV. We did not find any cases in Germans who stayed at the hotel after the departure of the Chinese index patient. One other German guest who stayed on the ninth floor of Hotel 'M' during the night of 27-28 February did not become ill and had negative serology for SARS coronavirus. Two hotel guests who had stayed on the eighth floor (27 February - 2 March) and the eleventh floor (18 - 25 February) reported having had a non-febrile illness with cough. Both tested negative for SARS-CoV antibodies. The other 16 cohort members remained free of symptoms and sera investigated from 10 of these were negative for SARS coronavirus antibodies.

Discussion

We found one case of SARS in our cohort that was laboratory confirmed by highly positive antibody titres in serial tests. Quantitative risk estimation was not possible. The case history suggests that close vicinity to the index patient may have played a role in transmission. However, it is unclear why the companion of the German secondary case remained healthy despite having had similar exposure. Many questions remain concerning transmission modes and the role of host susceptibility. As indicated in the recently published WHO consensus document on the epidemiology of SARS (4), environmental samples taken from the carpet outside of the room three months after the index case had stayed there revealed SARS-CoV RNA. Environmental contamination should therefore be considered as a possible source of infection in the German traveller. It is not known how long the infectious virus persists in the surroundings of a SARS patient. In case of a resurgence of SARS, further research should be undertaken to evaluate the role of environmental contamination as well as biological factors that might determine the degree of host susceptibility to SARS. We expect that the publication of the findings of the international study will shed more light on risk factors and modes of transmission of SARS in the hotel setting.

References

1. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003; 361(9366):1319-25
2. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med; 2003. 348(20): 1953-66
3. Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, van Riel D et al. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 2003; 362(9380):263-70
4. Geneva World Health Organization, 2003: Consensus document on the epidemiology of severe acute respiratory syndrome (SARS) http://www.who.int/entity/csr/sars/en/WHOconsensus.pdf

West Nile outbreak in horses in Southern France: September 2004

Hervé Zeller[1] (zeller@cervi-lyon.inserm.fr), Stéphan Zientara[2], Jean Hars[3], Jérôme Languille[4], Alexandra Mailles[5], H. Tolou[6], Marie-Claire Paty[7], Francis Schaffner[8], Alexis Armengaud[9], Pierre Gaillan[10], Jean-François Legras[11], Pascal Hendrikx[12]

1. Centre National de Référence des Arbovirus;
2. Agence Française de Sécurité Sanitaire des Aliments;
3. Office National de la Chasse et de la Faune Sauvage;
4. Direction Générale de l'Alimentation;
5. Institut National de Veille Sanitaire;
6. Laboratoire des Arbovirus des Services de Santé des Armées;
7. Direction Générale de la Santé;
8. Entente Interdépartementale de Démoustication Méditerranée;
9. Cellule Inter-Régionale d'Épidémiologie Sud;
10. Agence française de sécurité sanitaire des produits de santé;
11. Etablissement Français du Sang;
12. Direction Départementale des Services Vétérinaires du Gard.

Correspondence: Hervé Zeller (zeller@cervi-lyon.inserm.fr)

Citation style for this article:
Zeller H, Zientara S, Hars J. West Nile outbreak in horses in Southern France: September 2004. Euro Surveill. 2004;8(41):pii=2564. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=256

Article published on 07 October 2004

On 28 August 2004 (week 35), two suspected clinical cases of West Nile virus (WNV) infection in horses were identified by veterinarians in Saintes-Maries de la Mer, in the Camargue region of southeastern France (Figure 1). ELISA tests were performed on blood specimens from these horses by the Agence Française de Sécurité Sanitaire des Aliments (the French food safety agency), and WNV IgM and IgG antibodies were detected on 10 September. An alert was sent to the national authorities on 13 September 2004.

By 30 September 2004 (week 40), 37 suspected cases in horses, including 4 fatalities or euthanasia, were reported. Fourteen of the 18 horses tested were positive for WNV (WNV IgM detection or positive RT-PCR) (Figure 2). The most common clinical symptoms were fever, prostration, anorexia, ataxia, paresis and irritability. The Centre National de Référence des Arbovirus (national reference centre for arboviruses) in Lyon confirmed the presence of specific neutralising antibodies in 3 cases (PRNT80 titre >160).

The suspected cases were distributed over an area extending about 35km west and north from the initial focus, Saintes-Maries de la Mer. Saintes-Maries de la Mer is situated in the Rhône delta where migrating and resident birds are numerous. The infected area covered around the same region where a previous WNV outbreak in horses occurred in 2000 (131 suspected cases/76 confirmed cases from late August until early November) [1]. No human cases were reported in 2000 and none in 2004 by week 39.

After the 2000 outbreak, an integrated programme of WNV surveillance involving partners from the ministries of agriculture, public health and the environment, as well as local agencies, was initiated. It covered 3 départements: Hérault, Gard and Bouches du Rhône [2]. Sentinel birds (chicken and ducks) were tested for WNV antibody detection on a regular basis. Suspected cases in horses and humans were tested for WNV infection. Dead wild birds were collected for WNV testing. Because of the limited WNV outbreak in Frejus (in the Var department, 200 km east of the Camargue) in 2003 which involved 7 human cases (3 encephalitis and 4 cases of febrile illness) and 4 equine cases, the 2004 sentinel bird surveillance programme was extended along the Mediterranean coast to cover 6 départements from the eastern Pyrénées to the Var, as well as the report of suspected cases in humans and horses [3].

A low level of WNV activity was reported in the Camargue region in sentinel birds: one seroconversion in 2001, one in 2002 and none in 2003. In late July 2004, a WNV seroconversion was reported in a sentinel chicken from Saintes-Maries de la Mer, and a second seroconversion was reported in mid-August at the same location. On 6 September 2004, two thirds of the sentinel birds from this flock were positive for

Figure 1

Location of the West Nile outbreaks in France in 2003 and 2004.

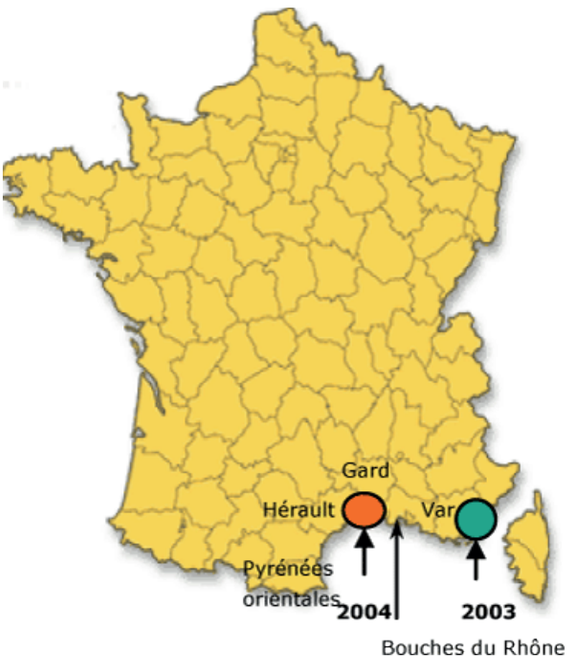
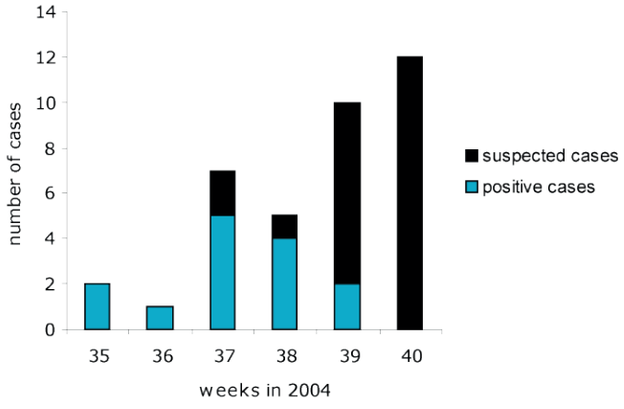


Figure 2

Suspected and confirmed equine cases of West Nile infection in the Camargue region, France, reported from 27 August (week 35) to 30 September 2004 (week 40).



WNV antibodies. A sentinel duck was reported to be positive for WNV on 16 August (infection confirmed on 7 September 2004) in Saint-Just, Hérault.
Following the alert on 13 September several measures were taken:

- Increased surveillance for detection of suspected cases in human and equine populations
- Entomological studies at areas where infected horses have been found
- A restriction on blood donations from individuals living in or with history of travel to the infected area until the end of October 2004

An absence of WNV viral genome was reported in a retrospective study on 789 blood donations collected from donors in the infected region from the beginning of August 2004 to mid-September.

References:

1. Murgue B, Murri S, Zientara S, Labie J, Durand B, Durand JP, et al. West Nile outbreak in horses in southern France, 2000: the return after 35 years. Emerg Inf Dis 2001; 7(4):692-6. (http://www.cdc.gov/ncidod/eid/vol7no4/murgue.htm)
2. Hars J, Pradel J, Auge P, Chavernac D, Gerbier G, Roger F, et al. Programme de surveillance de l'infection de l'avifaune par le virus West Nile en 2003 dans la Petite et la Grande Camargue. Rapport ONCFS/DGAl. 2004; 23
3. Mailles A, Dellamonica P, Zeller H, Durand JP, Zientara S, Goffette R et al. Human and equine West Nile virus infections in France, August-September 2003. Eurosurveillance Weekly 2003; 7(43):23/10/2003 (http://www.eurosurveillance.org/ew/2003/031023.asp#1)

Surveillance of listeria infections in Europe

H de Valk[1], C Jacquet[2], V Goulet1, V Vaillant[1], A Perra1, F Simon[1], JC Desenclos[1] , P Martin[2] on behalf of the Listeria surveillance feasibility study participants
1. Institut de Veille Sanitaire, Saint-Maurice, France
2. Centre National de Référence des Listeria , Institut Pasteur, Paris

Citation style for this article:
de Valk H, Jacquet C, Goulet V, Vaillant V, Perra A, Simon F, Desenclos JC, Martin P. Surveillance of listeria infections in Europe. Euro Surveill. 2005;10(10):pii=572. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=572

Article published on 01 Ocotber 2005

In addition to the economic consequences and threats associated with outbreaks, listeriosis remains of great public health concern, as it has one of the highest case fatality rates of all the foodborne infections (20%-30%), and has common source epidemic potential. Changes in the way food is produced, distributed and stored have created the potential for diffuse and widespread outbreaks involving many countries. In 2002, a survey was carried out to assess the need for and the feasibility of a European network on listeria infections in humans. Data on surveillance systems and laboratory methods were collected through two postal surveys sent to the national Centres for communicable disease surveillance and to the listeria reference laboratories. Surveillance systems for listeria infections were in operation in 16 out of the 17 countries surveyed, and 16 countries had a national reference laboratory (NRL). All countries based their case definition of listeriosis on the isolation of *Listeria monocytogenes*. Fourteen NRLs performed at least one typing method on human strains. At least 13 countries already carried out or expressed willingness to carry out characterisation of isolates by pulsed field gel electrophoresis (PFGE) of *L. monocytogenes* strains isolated from human cases following a standard protocol. The participants concluded that there was a clear added value to having a European surveillance network for listeria infections, particularly for outbreak detection and investigation, and that a surveillance network based on the existing national surveillance systems was feasible.

Introduction

Listeria monocytogenes causes invasive illness, mainly in certain well-defined high-risk groups, including immunocompromised people, pregnant women and neonates. Listeriosis can, however, occur in otherwise healthy individuals, particularly in an outbreak setting. *L. monocytogenes* primarily causes abortion, septicaemia or infections of the central nervous system, with a case fatality ratio of 20%-30 % [1]. It has only recently been recognised that foodborne transmission of L. monocytogenes can also cause a self-limiting acute gastroenteritis in immunocompetent persons [2]. The public health importance of listeriosis is not always recognised, particularly since listeriosis is a relatively rare disease compared with other common foodborne illnesses such as salmonellosis. Most countries within the European Union have an annual incidence between 2-10 reported cases per million population per year. However, because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to foodborne illness: it ranks second, after salmonellosis, in the United States (US) and France; and fourth in England and Wales [3-5].

Epidemiological investigations during the past 20 years have shown that listeriosis is a foodborne disease [6]. Discovery of *L. monocytogenes*, mainly in raw and ready-to-eat meat, poultry, seafood, and dairy products, has prompted numerous product recalls which have led to large financial losses for the food industry and numerous health scares. Effective prevention and control measures exist, as documented in France and the US, where a threefold and a twofold reduction respectively in incidence over the past decade was attributed to increased regulatory activity, implementation of Hazard Analysis and Critical Control Points (HACCP) programmes throughout the food industry, and specific recommendations to high-risk groups [7,8]. However, several countries still have relatively high incidence,and many countries do not have a surveillance system that allows them to estimate incidence or evaluate incidence trends. Moreover, its common source epidemic potential presents a real threat and persists even in countries with a decreasing or low incidence. Changes in the way food is produced and distributed have further increased the potential for diffuse and widespread outbreaks involving many countries. Because these outbreaks can be dispersed

with a limited number of cases in each country, they are likely to go undetected if information from these countries is not pooled. Improved surveillance, coordinated at a European level, combining rapid subtyping methods, cluster identification, and collaborative epidemiological investigation, can identify and halt these potentially large, outbreaks.

Because of the potential benefits of collaborative European surveillance described above, this project was initiated with the aim of defining the feasibility and scope of a European network on listeria infections, and to develop common methodologies for surveillance of listeriosis in Europe.

Methods

The project was coordinated by the Institut de Veille Sanitaire (InVS) and the French National Reference Centre for Listeria at the Institut Pasteur, assisted by an expert panel of microbiologists and epidemiologists from nine countries. Data for the inventory were collected through two postal surveys and, when necessary, completed through telephone interviews. One questionnaire, sent to epidemiologists in charge of surveillance of communicable diseases at the national level, collected information on surveillance systems, other data sources, information flow, case definitions, data collected, frequency of reporting and analysis, outbreak detection mechanisms, reported cases and outbreaks. A second questionnaire, sent to the national reference centre (NRL), collected information about their tasks as reference laboratory, the origin of isolates, identification and typing methods and practices, antibiotic resistance surveillance, and quality assurance and control. A third questionnaire was sent out to assess the acceptability, capacity and possibility that the NRL could to routinely perform typing of *L. monocytogenes*, or at regular intervals, and with a specific common protocol. During a meeting with epidemiologists and microbiologists from each participating country, the results of the inventory were presented, different scenarios for European surveillance were discussed, and recommendations for a European listeriosis surveillance network were formulated.

Results

In total, 17 countries participated. This included 14 EU countries: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, the Netherlands, Portugal, Spain, Sweden, and the United Kingdom (England & Wales and Scotland only) and Norway, Iceland and Switzerland. We present the results of Scotland separately from England & Wales, but count England & Wales and Scotland as a single country within the United Kingdom (UK).

Surveillance systems

All countries except Portugal had at least one surveillance system for listeriosis, and 12 countries had more than one system. In several countries, notification of foodborne illness (e.g., Austria and Ireland) or foodborne illness outbreaks (e.g., Belgium, the Netherlands and France) was statutory, and in theory, listeria infections could be notified through these systems. In practice, however, listeriosis cases were not notified through these systems. In this inventory, therefore, we do not consider notification of foodborne illness and outbreaks to be the same thing as a surveillance system for listeriosis. Listeriosis was statutorily notifiable in 10 countries, four countries had universal voluntary reporting, 11 countries had listeria surveillance based on their NRL, two countries had sentinel surveillance, and five countries had syndrome based surveillance of infections of the central nervous system and blood stream infections that covered listeria infections among other infections.

In 15 countries, diagnostic laboratories were involved in reporting to at least one of the surveillance systems. In addition, physicians were involved in the reporting in 13 countries. In Italy, physicians were the only notifying partners.

Listeriosis surveillance data were available at the national level in 16 countries, either at the national surveillance centre (five countries), at the NRL (one country) or at both (10 countries). These data at the national level were available as single case reports in all countries. Data transmission to the national level was immediate or weekly in all countries with the exception of Italy, where it was done quarterly.

All countries based their case definition of listeriosis on the isolation of L. monocytogenes, with or without specific requirements regarding site of isolation and the presence of clinical symptoms. Two countries also considered the presence of serum antibodies as laboratory confirmation of a case, but in practice, only cases with an isolate were reported. None of the countries had a specific definition for acute listeria gastroenteritis. Theoretically, in countries with a case definition based on the isolation of L. monocytogenes from any site, these patients should be reported. In practice, none of the countries had acute listeria gastroenteritis cases reported, although outbreaks of acute listeria gastroenteritis had occasionally been identified and reported to the national level: in Italy in 1993 and 1997, in Denmark in 1996, and in Belgium in 2001.

In general, countries with listeriosis surveillance collected at least basic demographic data (age/date of birth and sex), contact details for the reporting institute, laboratory confirmation (date of isolation of L. monocytogenes or date first positive specimen received in diagnostic laboratory), and the type of investigated material. Additional information such as principal diagnosis, associated pregnancy, outcome, and travel and food history, were available in between five to 10 countries.

National Reference Laboratories

All countries except for Ireland had an NRL. The tasks of these 16 NRLs were: microbiological surveillance (16 countries); detection of outbreaks (14 countries); provision of microbiological expertise (13 countries); research on listeria (12 countries); training (nine countries); and provision of reference material such as strains, sera, DNA profiles, protein extracts, phages, or guidelines for laboratory diagnosis (eight countries). Strains isolated from patients were sent to the NRL: in seven countries this was done systematically, and in eight countries this was done according to the will of the laboratory, or in specific situations such as outbreak or suspected outbreak settings. In Sweden and Switzerland, the sending of isolates to the NRL was statutory. In Spain, about half of the 16 autonomous communities sent their isolates to the NRL.

The NRLs also received information along with strains. This information concerned the site of isolation of the bacteria (13 countries), clinical data (11 countries), epidemiological data (10 countries), and strain characteristics (eight countries). In most countries (11 out of 17), the NRLs for human listeria also received listeria strains isolated from food, and in three countries, the NRLs received information on food strains.

Identification

Fifteen NRLs carried out identification of listeria strains. Only four countries performed a Gram stain and a catalase test. Biochemical characterisation was performed using API-Listeria in eight countries, API-coryne in one, while four countries used home made sugars. Nine countries looked for haemolysis, six for motility. Two countries also used polymerase chain reaction (PCR) for diagnosis, and one country also used an automated system of bacterial identification.

Characterisation of strains

Fourteen NRLs performed at least one typing method on human strains, either on an ongoing basis or at regular intervals. 13 NRLs routinely performed serotyping, either on an ongoing basis or at regular intervals. Seven countries used home made antisera, six used commercially available sera, and two used both. Thirteen countries had developed the capacity to perform DNA macrorestriction and pulsed field gel electrophoresis (PFGE) on human strains of *L. monocytogenes,* and performed it either routinely, for specific investigations or for ad hoc studies. All used the CHEF (contour-clamped homogeneous electric field) system for PFGE, and most used two enzymes, AscI and ApaI. Twelve countries said they would be willing to set up routine PFGE with image analysis, at least weekly or immediately after receiving a strain, in order to participate in a common surveillance system of human strains. Several countries, including one country not willing to carry out PFGE routinely, said they would be willing to send strains to another European laboratory to be typed by PFGE. Thirteen countries were willing to use a common standardised protocol for PFGE and to send profiles or strains to contribute to a European database. European surveillance including results of harmonised characterisation of isolates by PFGE of L. monocytogenes strains isolated from human cases could therefore cover at least 13 countries.

All countries who were performing or intended to perform PFGE said they would be willing to send PFGE profiles to a common European laboratory under the following conditions: access to common information (six countries), confidentiality (four), access restricted to participants only (one), and provided that strains were not distributed and profiles used only for the purpose of surveillance (one).

Antimicrobial susceptibility testing

Ten out of 17 laboratories (including Ireland) reported performing antimicrobial susceptibility testing. Three countries used the E test method for testing, and seven countries used agar dilution breakpoints. Two countries also used the Clinical and Laboratory Standards Institute (formerly NCCLS) method and one country also used a disk diffusion method. The antimicrobial agents tested varied between countries. Laboratories most frequently tested the susceptibility of listeria for gentamicin and trimethoprim-sulfamethoxazole (seven countries); ampicillin, tetracycline and erythromycin (six countries); ciprofloxacin (five countries); or chloramphenicol, streptomycin and vancomycin (four countries).

Quality control and quality assurance, accreditation

The NRLs in 14 countries reported having internal quality control for their identification procedures (nine countries) and/or typing procedures (nine countries).

Seven countries participated in an external quality control. Six of the seven countries used NEQAS from the Public Health Laboratory Service (PHLS) in the UK for identification procedures, and three also used another external quality control.

Seven NRLs were engaged in a quality assurance system, and five intended to be so in the near future. Six NRLs said that they were ISO/UE 17025 accredited and two more were accredited on an other standard: PHLS in the UK (Clinical Pathology Accreditation Ltd) and the NRL in the Netherlands (accredited by CCKL-test). One NRL is ISO 9001 certified.

Outbreak detection

Real-time reporting and analysis, high sensitivity, results of typing of strains available in real time for surveillance, and the existence of outbreak detection criteria or thresholds are all surveillance system characteristics that contribute to efficient outbreak detection. Eight countries have developed outbreak detection mechanisms and thresholds. Real time reporting and analysis characterised the surveillance systems of 15 and 11 countries respectively. The estimated or

Table 1

Observed number of cases and incidence of listeriosis, by country, by surveillance system (latest year available), Listernet

Country	Year	System	Observed cases	Observed incidence* (1 000 000)
Austria	2000	Reference laboratory	14	1.7
Belgium (Flanders)	1999	Statutory notification	26	4.4
Belgium	2000	Sentinel + reference laboratory	48	4.7
Denmark	2000	Syndromic surveillance (meningitis)	6	1.1
	2001	Statutory notification	38	7.2
	2001	Reference laboratory	38	7.2
England and Wales	2001	Universal voluntary reporting and reference laboratory	144	2.7
	2000	Reference laboratory	81	1.5
Finland	2001	Statutory notification	29	5.5
France	2001	Statutory notification+ reference laboratory	187	3.2
	2000	Syndromic surveillance (CNS+blood stream infections)	148	2.5
Germany	2001	Statutory notification	220	2.7
Greece	2001	Universal voluntary reporting	3	0.3
	2001	Syndromic surveillance (meningitis)	2	0.2
Iceland	2001	Statutory notification + NRL	0	0.0
Ireland	2001	Universal voluntary reporting	6	1.6
Italy	1999	Reference laboratory	11	0.2
	1999	Statutory notification	40	0.7
	2001	Syndromic surveillance (meningitis)	31	0.5
Netherlands	2001	Sentinel surveillance	17	1.1
	2000	Syndromic surveillance (meningitis)	26	1.7
Norway	2001	Statutory notification	17	3.8
	2000	Reference laboratory	11	2.5
Portugal		No surveillance		
Scotland	2001	Universal voluntary reporting	15	2.9
Spain	2000	Universal voluntary reporting	35	0.9
	2000	Reference laboratory	60	1.5
Sweden	2001	Statutory notification	67	7.5
	2001	Reference laboratory	12	1.4
Switzerland	2000	Statutory notification	54	7.4
	2000	Reference laboratory	46	6.3

* The observed incidence reflects both the real incidence and the sensitivity of the surveillance system. Therefore, data cannot be compared between countries without taking into account the differences in sensitivity of these surveillance systems

assumed sensitivity was reasonably high or high in at least 10 countries. For outbreak detection, 12 countries had results of strain typing available, routinely and on a real time or weekly basis: serotyping (12 countries), biotyping (four countries), ribotyping (three countries), PFGE analysis (six countries), and phagetyping (one country).

Reported listeria infections and outbreaks

The incidence of reported cases varied between 0.3 and 7.5 cases per million per year. The mean incidence of reported cases was 3.4 per million inhabitants (data from 16 countries, latest year available) [TABLE 1]. Five countries reported an incidence of more than four cases per million, and three of these five countries reported an incidence of more than six per million population. These figures mostly reflect the sensitivity of the surveillance systems, as well as the incidence of the disease. However, few countries have formal evaluations or studies allowing estimation of sensitivity, geographical coverage and representativeness of their surveillance systems. In general, the surveillance systems described above covered, in principal, the entire country, except for Spain, where approximately half of the autonomous communities transmitted their data direct to the national level.

Between 1991 and 2002, a total of 19 outbreaks of invasive listeriosis were reported in nine different countries, with a total of 526 outbreak related cases) [TABLE 2]. While the number of reported outbreaks increased gradually over time, from seven outbreaks detected in the period 1992-1996 to 11 in the period 1997-2001, the mean number of cases related to these outbreaks decreased from 57 to 11 over the same period. This suggests more efficient outbreak detection, investigation

TABLE 2

Reported outbreaks of listeriosis and of *Listeria* gastroenteritis in Europe 1990-2002

Year	Country	Number of cases*	Transmission	Incriminated food	Potential international implication
1992	France	279	foodborne	Pork tongue in jelly (11)	Exported product
1992	Spain	24	foodborne	Unknown	
1992	Norway	6	foodborne	Sliced cold meat	
1993	France	38	foodborne	Rillettes (pork meat) (12)	Exported product
1993	Italy	18 gastroenteritis	foodborne	Rice salad (2)	
1994-95	Sweden	9	foodborne	Gravad trout (13)	
1995	France	36	foodborne	Cheese (raw cows' milk) (14)	
1995	Iceland	5	unidentified	Unidentified	
1996	Denmark	3 gastroenteritis	unidentified	Unidentified (15)	
1997	France	14	foodborne	Cheese (raw cows' milk)	Exported product
1997	Finland	5	foodborne	Cold-smoked rainbow trout (16)	
1997	Italy	1566 gastroenteritis	foodborne	Corn salad (17)	
1998-99	Finland	25	foodborne	Butter (18)	
1999	England and Wales	2	foodborne	Cheese/cheese salad/ sandwiches (19)	
1999	France	3	foodborne	Cheese (raw cow's milk)	Possible cases in Germany?
1999	France	10	foodborne	Rillettes (processed pork meat) (20)	Exported product
1999-00	Finland	10	foodborne	Vacuum-packed fish products (21)	Exported?
2000	France	32	foodborne	Pork tongue in jelly (20)	Exported ?
2000	Portugal	1	foodborne	Cheese	
2000	Spain	15	foodborne	Undetermined	
2001	Belgium	1 + 2 gastroenteritis	foodborne	Ice cream cake	Invasive illness of Belgian case diagnosed in France
2002	France	11	foodborne	Spreadable raw sausage (22)	Export to Germany, Belgium and Luxembourg

* Cases refer to invasive listeriosis unless otherwise specified

and control. In addition, four outbreaks of acute listeria gastroenteritis were reported: two outbreaks in Italy in 1993 (18 cases) and 1997 (1566 cases); an outbreak in Denmark in 1996 (3 cases); and an outbreak in Belgium in 2001 (2 cases of acute gastroenteritis and one case of invasive listeriosis).

The incriminated food at the origin of the invasive listeriosis outbreaks was processed meat products (six outbreaks), cheese (five outbreaks), processed fish products (three outbreaks), butter (one outbreak) and undetermined (three outbreaks). The incriminated products for at least six of these outbreaks were known to have been exported, creating the potential for the occurrence of outbreak related cases in other countries. Moreover, cases related to one outbreak in one country were diagnosed in a neighbouring country. The outbreaks of gastroenteritis were linked to the consumption of contaminated rice salad and corn salad respectively, while the Belgian outbreak of gastroenteritis and invasive listeriosis was linked to a contaminated ice cream cake. The origin of one outbreak of gastroenteritis remained undetermined.

Conclusions and recommendations

Based on the inventory, it appears that there is an appropriate basic infrastructure for a European surveillance network for listeria infections, and that the necessary harmonisation of methods is feasible considering the infrastructure already in place and the expressed willingness of countries to adapt or set up methodologies for European surveillance.

It was recommended by the representatives of the participating countries/the working group to set up a European network for the surveillance of listeria infections, with, as the main objectives, providing comparative data, monitoring trends of international importance, and rapidly detecting and investigating international outbreaks more efficiently. The network should also contribute to the strengthening of national surveillance in participating countries. In its initial phase the network should concentrate on surveillance of human cases of listeria infection and not yet actively seek to collect data on food isolates. Once the network is well established and surveillance of human cases is operational, the possibilities of including data from food and animal surveillance should be studied.

Common case definitions should be agreed upon as well as a common minimum dataset, which could be further developed over time to include additional data (optimal dataset). Case definitions, in line with those developed by the Community Network (under decision N° 2002/253/EC, amended by Commission Decision 2003/534EC), and a minimum and optimal dataset, for which the collection is, at present, feasible for the majority of participating countries, were proposed [9].

Because of the wide disparity in listeria outbreaks, a common European database should include results of real time characterisation of strains to reinforce the ability to detect international outbreaks. The participants concluded that, at present, characterisation by both serotype and PFGE would be the most appropriate methods and the best option to meet the objectives of outbreak detection and trend analysis. The necessary harmonisation of microbiological methods and of the type of epidemiological data collected appears feasible considering the infrastructure already in place and the expressed willingness of countries to adapt or set up methodologies in the perspective of European surveillance.

The network should encourage individual countries to strengthen national surveillance of listeria infections, and should contribute to their strengthening by providing a model and specific tools for surveillance and investigations. Each country should set up a national database which combines laboratory data and data from the notification systems. Participating countries should be encouraged to increase the sensitivity of the surveillance systems in order to reinforce the ability to detect national and international outbreaks. Countries can participate in a stepwise manner, contributing initially with the data they already have available, even if incomplete. With time, countries may wish to adapt their in-country data collection in order to cover all data fields in the database. For those countries where routine and ongoing typing of strains is difficult to carry out because of the low number of isolates, the possibility of having their strains typed in another country's NRL, should be investigated.

In addition to the harmonisation of epidemiological and microbiological methods and the creation of a common database, it was recommended that the network should develop outbreak detection algorithms and a protocol for collaborative investigation of international clusters and outbreaks. The network will need to develop principles of collaboration that should deal with access to the database by participants and by outsiders, confidentiality of country specific data, confidential and public domain reports, data protection requirements, as well as transmission to other programmes and projects. It was recommended to adapt the principles of collaboration of Enternet to listeria [10].

Finally, the participants recommended that a project proposal be developed by the coordinators of the actual feasibility study. In May 2003, an application was submitted to the European Commission under the 2003 call for proposals in the programme of community action in the field of public health (2003-2008). Although the proposal was accepted, co-funding was not proposed by the commission until August 2004. By this time, the situation of the different partners of the project had evolved, and senior staff who committed themselves to contribute to the project had taken up other commitments. However, European investment in such a project remains a priority for the years to come. In particular, it would be important to assess how such a project could be integrated into other ongoing EU surveillance projects such as Enter-net.

Acknowledgements

The listeria surveillance feasibility study was co-financed by the European Commission, DG Sanco Agreement number SI2.326491 (2001CVG4-023).

Participants in the study are: Austria: Franz Allerberger, Bundesst. Bakt.-serol. Untersuchungsanstalt; Reinhild Strauss, FM for Social Security and Generations – Belgium: Francine Matthys, Epidemiology Section, Scientific Institute of Public Health - Louis Pasteur; Mark Yde, Bacteriology section, Scientific Institute of Public Health - Louis Pasteur – Denmark: Peter Gerner-Smidt, Statens Serum Institut; Brita Bruun, Department of Clinical Microbiology –; England and Wales: Mark Reacher, Jim McLauchlin and JW Smerdon, Public Health Laboratory Service Communicable Disease Surveillance Centre and Central Public Health Laboratory – Finland: Outi Lyytikäinen and Anja Siitonen, National Public Health Institute – France: Véronique Goulet, Institut de Veille Sanitaire; Paul Martin and Christine Jacquet, Institut Pasteur – Germany: Andrea Ammon and Helmut Tschaepe, Robert Koch-Institut; Herbert Hof, Institute for Medical Microbiology and Hygiene; Jochen Bockemühl, Institute for Hygiene – Greece: I Tselentis and Takis Panagiotopoulos, Hellenic Center for Infectious Diseases Control – Iceland: Gudrun Sigmundsdottir, Directorate of Health – Ireland: Martin Cormican, University College Hospital, Galway; Paul McKeown, National Disease Surveillance Centre; Bartley Cryan, Cork University Hospital – Italy: Stefania Salmaso and Paolo Aureli, Istituto Superiore di Sanità,; – The Netherlands: Yvonne van Duynhoven and Wim Wannet, National Institute of Public Health – Norway: Line Vold and Jørgen Lassen, National Institute of Public Health – Portugal: Laura Brum and Jorge Machado, Instituto Nacional de Saude Dr Ricardo Jorge – Scotland: John M Cowden and Alison Smith-Palmer, Scottish Centre for Infection and Environmental Health – Spain: Julio A Vasquez and Luisa P Sánchez Serrano, Instituto de Salud Carlos III – Sweden: Margareta Lofdahl, Birgitta Henriques Normark, Christina Johansson and Johan Giesecke, Swedish Institute for Infectious Disease Control – Switzerland: Hans Schmid, Swiss Federal Office of Public Health; Jacques Bille, Clinical Microbiology Laboratory University Hospital, Lausanne – and Enter-net Surveillance hub: Ian Fisher.

References

1. Goulet V., Marchetti Ph. Listeriosis in 225 non-pregnant patients in 1992: clinical aspects and outcome in relation to predisposing conditions. Scand J Infect Dis 1996, 28:367-374.
2. Salamina G., Dalle Donne E., Niccolini A., et al. A food-borne outbreak of gastro-enteritis involving Listeria monocytogenes. Epidemiol Infect 1996, 117:429-36.
3. Mead SM, Slutsker L, Dietz V, et al, Food-Related Illness and Death in the United States, Emerg Infect Dis 1999; 5:607-25

4. Vaillant V, de Valk H, Baron E, et all, Foodborne Infections in France. Foodborne Pathogens and Disease (Accepted for publication in september 2005)
5. Adak GK, Long SM, O'Brien SJ. Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. Gut 2002;51:832-841
6. Schlech W.F., Lavigne P.M., Bortolussi R.A., et al. Epidemic listeriosis - evidence for transmission by food. N Engl J Med 1983; 308: 203-206.
7. Goulet V, de Valk H, Pierre O et all. Effect of prevention measures on incidence of human listeriosis, France, 1987-1997. EID 2001;7:983-989.
8. Tappero JW, Schuchat A, Deaver KA, Mascola L, Wenger JD, for the listeriosis study group. Reduction in the incidence of human listeriosis in the United States, effectiveness of prevention efforts? JAMA 1995;273:1118-22.
9. De Valk H, Jacquet Ch, Goulet V, Vaillant V, Perra A, Desenclos J-C, Martin P, & the Listeria Working Group. Feasability study for a collaborative surveillance of Listeria infections in Europe. Report to the European Commission. Institut de Veille Sanitaire, Paris 2003 : p 1-107 ; (http://www.invs.sante.fr/publications/default.htm)
10. Fisher I, Gill N. Réseaux de surveillance internationaux et principes de collaboration. Eurosurveillance 2001;6:17-21
11. Goulet V, Lepoutre A, Rocourt J, Courtieu AL, Dehaumont P, Veit P. Epidémie de llistériose en France. Bilan final et résultats de l'enquête épidémiologique. Bull. Epidémiol. Hebd. 4 :13-14
12. Goulet V, Rocourt J, Rebiere I, Jacquet Ch, Moyse C, Dehaumont P, Salvat G, Veit P, Listeriosis outbreak asociated with the consumption of rillettes in France in 1993. Scand. J. Infect. Dis. 177(1) :155-160
13. Tham W, Ericsson H, Loncarevic S, Unnerstad H, Danielsson-Tham ML. Lessons from an outbreak of listeriosis related to vacuum-packed gravad and cold-smoked fish. Int J Food Microbiol. 2000 Dec 20;62(3):173-5.
14. Goulet V, Jacquet Ch, Vaillant V, Rebiere I, Meuret E, Lorente Ch, Maillot E, Stainer F, Rocourt J. Listeriosis from consumption of raw-milk cheeses. The Lancet 1995 ;345 :1581-1582
15. Heitmann M, Gerner-Smidt P, Heltberg O. Gastro-enteritis caused by Listeria monocytogenes in a private day-care facility. Pediatr. Infect. Dis. J. 1997 ; 16(8) :827-8
16. Miettinen MK, Siitonen A, Heiskanen P, Haajanen H, Björkroth KJ, Korkeala HJ. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by Listeria monocytogenes in cold-smoked rainbow trout. J Clin Microbiol 1999;37:2358-2360.
17. Aureli P, Giovanni C, Caroli D, Marchiaro G, Novara O, Leone L, Salmaso S. An outbreak of febrile gastro-enteritis associated with corn contaminated by Listeria monocytogenes. N. Engl. J. Med. 243:1236-41
18. Lyytikäinen O, Autio T, Maijala R, Ruutu P, Honkanen-Buzalski T, Miettinen M, Hatakka M, Mikkola J, Anttila VJ, Johansson T, Rantala L, Aalto T, Korkeala H, Siitonen A. An outbreak of Listeria monocytogenes serotype 3a infections from butter in Finland. J Infect Dis 2000; 181:1838-1841.
19. Graham JC, Lanser S, Bignardi G, Pedler S, Hollyoak V. Hospital-acquired listeriosis J Hosp Infect. 2002 Jun;51(2):136-9.
20. de Valk H, Vaillant V, Jacquet C, Rocourt J, Le Querrec F, Stainer F, Quelquejeu N, Pierre O, Pierre V, Desenclos JC, Goulet V. Two consecutive nationwide outbreaks of Listeriosis in France, October 1999-February 2000. Am J Epidemiol. 2001 Nov 15;154(10):944-50.
21. Lyytikäinen O, Siitonen A, Johansson T, Hatakka M. Listeriosis cases suspected to have been caused by vacuum-packed fish products in Finland. Eurosurveillance weekly, April 13, 2000.
22. Goulet V, Martin p, Jacquet Ch. Cluster of listeriosis cases in France. Eurosurveillance Weekly. 2002; 6:27

Clostridium difficile PCR ribotype 027 outbreaks in the Netherlands: recent surveillance data indicate that outbreaks are not easily controlled but interhospital transmission is limited

S van den Hof[1], T van der Kooi[1], R van den Berg[2], EJ Kuijper[2], DW Notermans[1]
1. Centre for Infectious Disease Control, Rijksinstituut voor Volksgezondheid en Milieu (RIVM, National Institute of Public Health and the Environment), The Netherlands
2. Medical Microbiology, Leiden University Medical Center (LUMC), The Netherlands

Correspondence: S van den Hof (susan.van.den.hof@rivm.nl)

Citation style for this article:
van den Hof S, van der Kooi T, van den Berg R, Kuijper EJ, Notermans DW. Clostridium difficile PCR ribotype 027 outbreaks in the Netherlands: recent surveillance data indicate that outbreaks are not easily controlled but interhospital transmission is limited. Euro Surveill. 2006;11(4):pii=2882. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2882

Article published on 26 January 2006

In June 2005, shortly after outbreaks of *Clostridium difficile*-associated diarrhoea (CDAD) caused by PCR ribotype 027 (toxinotype III) were reported in Britain, several Dutch healthcare institutions reported outbreaks of CDAD in patients caused by the same organism [1,2].

Surveillance of CDAD

Surveillance of CDAD in the hospitals with an epidemic increase was started. All institutions that observed a rise in the incidence of CDAD, or cases with more serious symptoms or lack of response to treatment with metronidazole, were invited to send in samples to the reference laboratory in Leiden for typing to detect 027 and send monthly updates on the outbreak situation to the national Centre for Infectious Disease Control at the RIVM (http://www.rivm.nl/).

So far, type 027 has been found in 15 of 23 participating institutions. Hospitals without 027 appeared not to have an increased incidence of CDAD. Further transmission seems to have occurred in only 8 of the 15 institutions where 027 was found: 7 hospitals and 1 nursing home.

Before the outbreaks, different testing strategies were in place in the institutions. During outbreaks, hospitals tested all patients with diarrhoea of unknown cause, all patients developing diarrhoea after a minimum of three days or all patients from a specific department (eg, geriatrics).

The hospital laboratories used several different assay types: toxin A immunoassays, toxin A/B immunoassays or cell cytotoxicity assays. Almost all hospitals started to use a rapid toxin A/B immunoassay during the outbreak. Additionally, some hospitals have started culturing *C. difficile*.

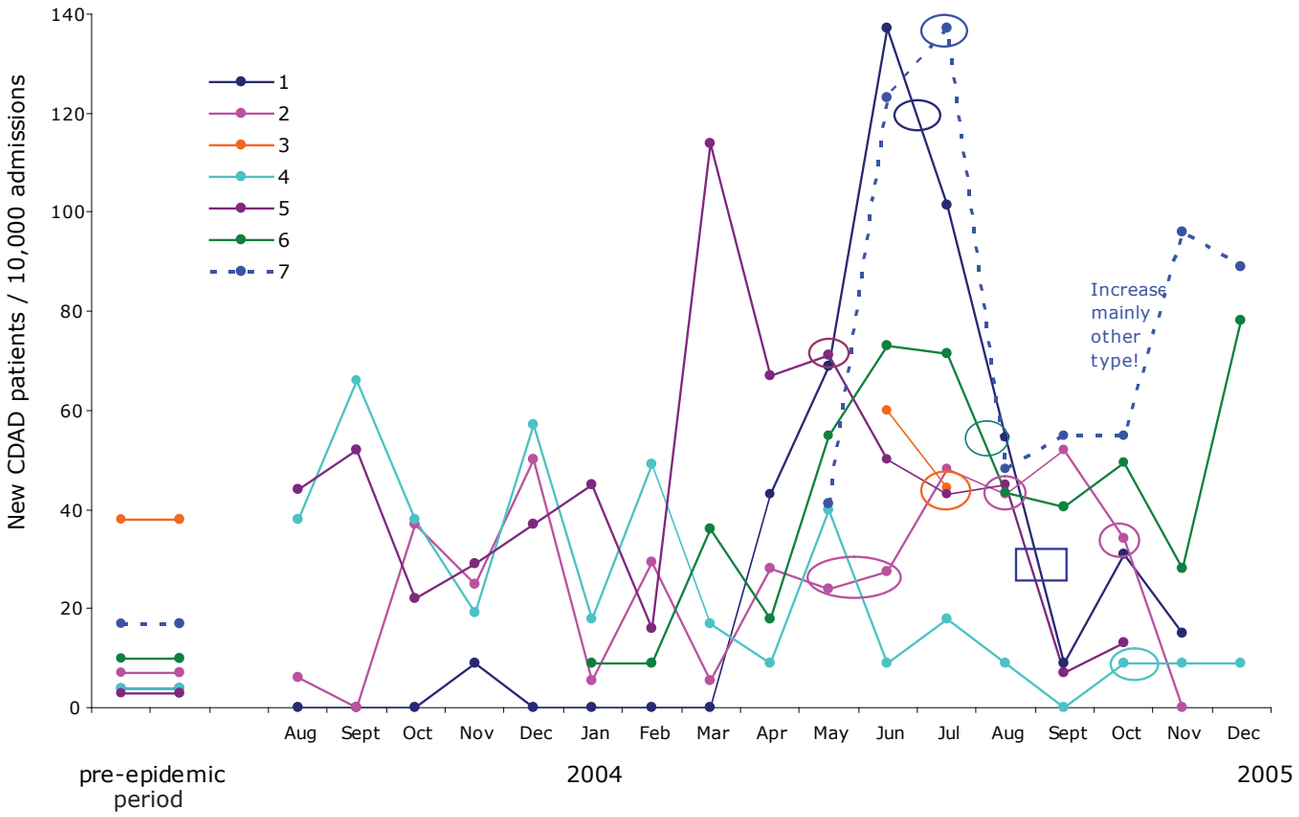
Preliminary results

The course of the epidemic differed between institutions (Figure). In one region where three hospitals use a single regional laboratory, the incidence *C. difficile* rose in 2004 or earlier. Unfortunately, no samples from that period were kept for typing. In some other hospitals, a sharp increase was seen in 2005. Not all patients with 027 had severe infection, some had mild colitis and were detected because of increased CDAD incidence. The median age was 74 years, but a wide range was observed: 13% of patients were under 50 years of age, 17% 50-64 years, 37% 65-79 years, and 35% over 80 years.

A number of guidelines for diagnosis and outbreak control of 027 were issued by a national expert group (in Dutch available at http://www.infectieziekten.info. These guidelines were produced in July-August 2005 and have been used since in all institutions, but not all measures have been followed up to the full extent in each hospital. By the end of 2005, the incidence had decreased in several institutions. However, the outbreaks are difficult to control: most hospitals have continued to have new cases for a long time.

Figure

Monthly incidence of *C. difficile*-associated diarrhoea in seven hospitals with transmission of PCR-ribotype 027*



*The dotted line represents an estimation of the incidence as admission numbers are not available yet. The ovals indicate the timing of several measures in the specific hospital, such as contact isolation and restriction of the use on fluoroquinolones. The rectangle indicates the easing of measure(s).

It appears that institutions where stricter measures were agreed on had a sharper decline in CDAD incidence. However, as numbers are small and in some hospitals guidelines were better complied with than in others, conclusions can only be drawn cautiously.

Conclusion

The transmission in 2005 to other hospitals not already affected appears to have been limited. However, to maintain vigilance into the development of CDAD (027) outbreaks in Dutch health care institutions, this surveillance will be continued for at least another half year. Updates of the results will be published on the Centre for Infectious Disease Control website (http://www.rivm.nl/gezondheid/infectieziekten/centrum_izb/).

Acknowledgements:

We would like to acknowledge all medical microbiologists and infection control professionals for their contribution in the control and surveillance of CDAD.

References:

1. van Steenbergen J, Debast S, van Kregten E, van den Berg R, Notermans D, Kuijper E. Isolation of Clostridium difficile ribotype 027, toxinotype III in the Netherlands after increase in C. difficile-associated diarrhoea. Eurosurveillance 2005; 10(7): 050714. (http://www.eurosurveillance.org/ew/2005/050714.asp#1)
2. Smith A. Outbreak of Clostridium difficile infection in an English hospital linked to hypertoxin-producing strains in Canada and the US. Eurosurveillance 2005; 10(6): 050630. (http://www.eurosurveillance.org/ew/2005/050630.asp#2)

Reasons for the sharp increase of genital chlamydia infections reported in the first months of 2007 in Sweden

I Velicko[1], S Kühlmann-Berenzon[1,2], A Blaxhult[1]
1. Department of Epidemiology, Smittskyddsinstitutet (Swedish Institute for Infectious Disease Control, SMI) Solna, Sweden
2. Stockholm Group for Epidemic Modelling

Correspondence: Velicko (inga.velicko@smi.ki.se)

Citation style for this article:
Velicko I, Kühlmann-Berenzon S, Blaxhult A. Reasons for the sharp increase of genital chlamydia infections reported in the first months of 2007 in Sweden. Euro Surveill. 2007;12(10):pii=737. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=737

Article published on 01 Ocotber 2007

After a continuous increase in the reported chlamydia incidence over the past 10 years in Sweden, the incidence decreased by 2% in 2006. A new genetic variant of *Chlamydia trachomatis* (nvCT) was discovered in Sweden in October 2006 that could not be detected by some of the commonly used diagnostic tests, which led to underreporting of chlamydia cases. This variant has also been called "swCT" by some authors. After the switch at the end of 2006 to other diagnostic tests that can detect nvCT, the reported incidence rose considerably (75 per 100,000 population) in the beginning of 2007. The objective of this study was to explore alternative explanations for this increase and to propose further action if needed. A data quality check was done in order to exclude double reporting and delayed reporting. To compare the incidence of chlamydia and the proportion of the population that was tested, we divided the Swedish counties into two groups, according to the diagnostic test used. We estimated the chlamydia incidence trend for January and February in the years from 2000 to 2005 by regression model, and predict the chlamydia incidence for the same period in 2006 and 2007. The age and sex distribution of the cases in January and February did not differ between the years 2000 to 2007. The proportion of tested people increased on average by 5% every year. If we assume that the percentage of the population that was tested had been 20% higher in 2007 than in 2006, the incidence predicted by the model for January and February 2007 is exactly the same as the incidence that was actually observed. The change of diagnostic test and an increase in the number of people tested, as well as the increase in the prevalence of CT have probably all contributed to the increased numbers of reported chlamydia cases in January and February 2007. These findings support the need for enhanced prevention campaigns in order to control spread of CT.

Introduction

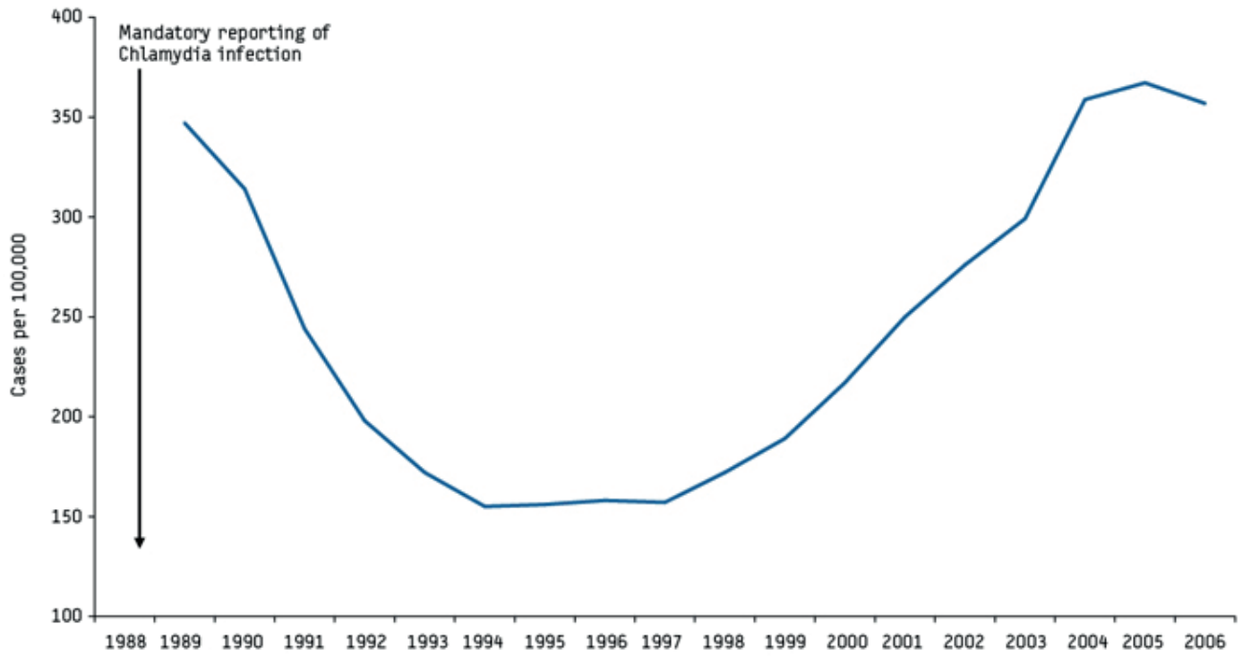
Reported *Chlamydia trachomatis* (CT) cases have increased substantially in the past 10 years and have become by far the most common sexually transmitted infection (STI) in Sweden (Figure 1) [1]. The number of cases reported to the national surveillance system increased from 13,905 (157 per 100,000) in 1997 to 32,281 cases (359 per 100,000) in 2004, representing a rise of over 120%. In 2005, the annual reported incidence increased only by 2%, and even decreased by 2% in 2006. One reason for this decrease may have been the emergence of a new genetic variant of *Chlamydia trachomatis* (nvCT) in October 2006 that can not be detected by some of the diagnostic tests commonly used in Sweden [2,3]. As a result, chlamydia diagnoses were missed and the national rates of chlamydia cases were underestimated in 2006 [4].

The nvCT was found to be widely spread in Sweden and its proportion varied between counties from 10% to 65%, leading to false negative results [3,5]. Laboratories in 13 of the 21 counties in Sweden had used diagnostic kits in 2006 that did not detect nvCT (Roche Diagnostics and Abbott Laboratories), while laboratories in eight counties had used diagnostic kits by Becton Dickinson that could detect both wild-type CT and nvCT. In order to improve diagnosis of the nvCT, the use of other PCR testing kits (Becton Dickinson or Artus) and/or culture was recommended [6]. An overview by the Swedish Institute for Infectious Disease Control showed that, by March 2007, all laboratories (except one) had switched to one of the suggested diagnostic kits. This change made it possible to diagnose chlamydia infections caused by nvCT and to perform non-interrupted contact tracing, resulting in a renewed increase in reported cases.

In the beginning of 2007, the Swedish Institute for Infectious Disease Control noticed a sharp increase in reported chlamydia cases through the electronic

FIGURE 1

Chlamydia infection incidence in Sweden, 1989-2006



reporting system. The incidence of chlamydia in the two-month period of January and February 2007 was 38% higher than the incidence during the same period in 2006. This raised the question: Can this increase be explained only by better diagnosis of nvCT infections? The objective of this study was to explore alternative explanations for the increase and propose further action if needed. Based on available surveillance data several alternative hypotheses were developed. One of the alternative hypotheses is an increase in the testing activity in the beginning of 2007. Another alternative hypothesis is a continued increase in the prevalence of chlamydia infection. Before the hypotheses were tested, we considered the data quality with regards to double reporting or delayed reporting to the system.

Methods

Surveillance system

Genital chlamydia infection is a mandatorily notifiable disease in Sweden under the Communicable Disease Act from 1988 [7]. Partner notification and contact tracing are also routinely performed [7]. The report of a chlamydia case to the national surveillance system contains an individual laboratory notification from the diagnostic laboratory and an individual clinical notification from the health care professional. Notifications do not contain the name of the patient but are coded, based on the social security number (*personnummer*). In addition, all laboratories that perform testing for CT report on a voluntary basis the number of people tested and the number found positive for CT every six months. These data are available in electronical format since 2000.

Quality check for reported cases

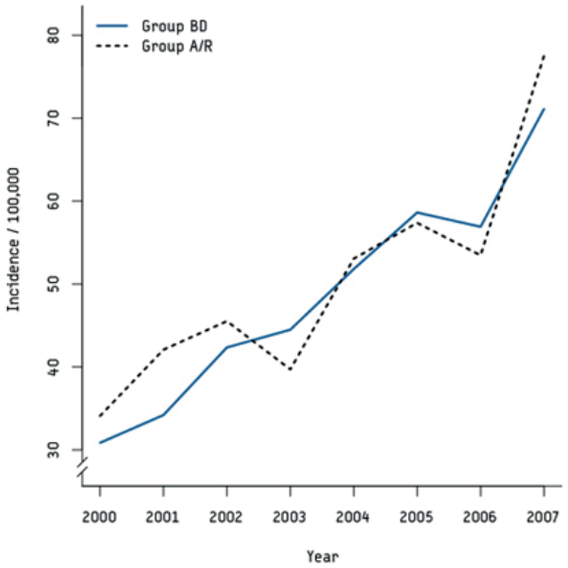
Double reporting or delayed reporting of chlamydia cases was checked for every month in 2006 and for January and February in 2007. The time between clinical diagnosis and reporting was compared. Reporting of a clinical case more than one week after diagnosis was defined as a delay. In Sweden, all positive laboratory findings for CT with the same code within a three-month period are considered as new infections.

Grouping of counties according to diagnostic methods

We divided all 21 Swedish counties into two groups based on the diagnostic kits used by their laboratories in 2006: Group A/R used Cobas Amplicor (Roche Diagnostics), Cobas TagMan48 (Roche Diagnostics) or Abbott *m*2000 (Abbott Laboratories) and were unable to detect nvCT. Group BD used the ProbecTec ET kit by Becton Dickinson that is able to detect nvCT. According to this division, 13 counties were included in Group A/R and eight counties in Group BD (including county Västra Götaland, where three out of four laboratories had used Becton Dickinson diagnostic kit and one the Roche diagnostic kit).

FIGURE 2

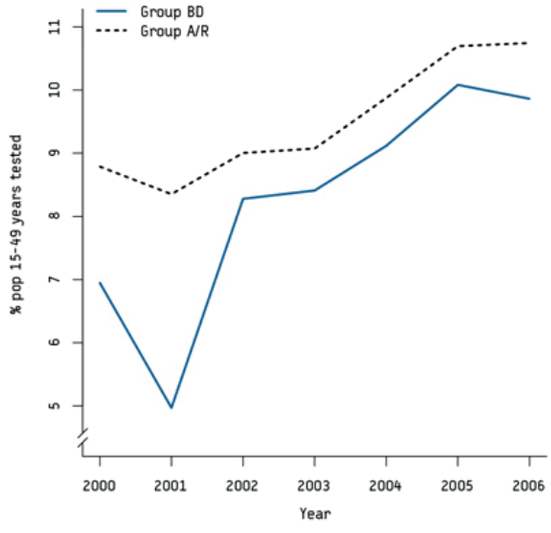
Chlamydia incidence in Sweden (grouping of counties according to diagnostic kit used in 2006#), January-February period of 2000-2007



Group BD: counties that used Becton Dickinson kit.
Group A/R: counties that used Abbot or Roche kit.

FIGURE 3

Proportion of 15- to 49-year-olds tested for *Chlamydia trachomatis* annually in Sweden by county group#, 2000-2006



Group BD: counties that used Becton Dickinson kit.
Group A/R: counties that used Abbot or Roche kit.

Chlamydia cases and incidence

Reported cases in the period of January and February were described in terms of the total number, proportion of males and females, median age, and the reporting county. The incidence of chlamydia was calculated as all reported chlamydia cases per 100,000 population during January and February in the years 2000 to 2007. The national incidence and the incidence per group (A/R and BD) were calculated as geometric means of the incidence of the respective counties.

Testing for *C. trachomatis*

In order to quantify to what degree the different counties invested in finding new chlamydia cases, we calculated the proportion of the population between 15 and 49 years of age that was tested for chlamydia. This particular age group is tested most frequently and with the highest incidence (ca. 90% of all reported cases). Since it was not possible to obtain the specific data on tests performed in January and February, the annual number of tests was used instead.

Trend estimation

A negative binomial regression model was used to study the time trend of chlamydia cases in January and February in 2000 to 2005. The year 2006 was excluded due to underreporting of nvCT. To model the incidence of chlamydia in January and February, the following variables were included in the model:

a) county group A/R or BD (according to diagnostic kits used),

b) proportion of the population in age group 15 to 49 years tested in each county,

c) year.

We also added an interaction effect of method and time, as differences between the two diagnostic kits could have been exacerbated by the spread of the nvCT over time. The initial model with i=1, ...21 (county) and j=1,...6 (year) was:

$$\log(casesij / popij) = \beta o + \beta 1 yearj + \beta 2 proportion\ testedij + \beta 3 groupi + \beta 4 groupi * yearj .$$

All calculations were based on data from individual counties. Based on the model, a prediction of cases was done for January and February 2006 and 2007. Since the proportion of tested individuals is not yet available for 2007, two scenarios were used. The proportion of persons tested in 2007 was assumed to be:
1) 5% more than in 2006 in each county, which represents the average annual increase.

2) 20% more than in 2006 in each county (extreme scenario).

The differences between the observed and predicted incidence were summarized as mean values.

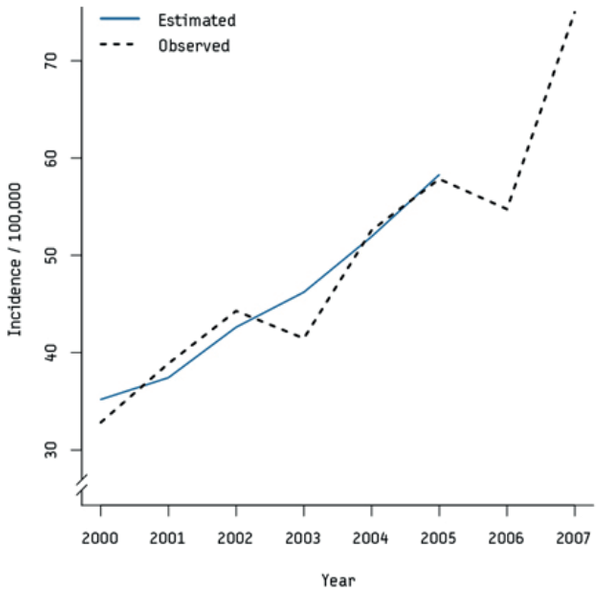
Results

Quality check

The quality check for reported chlamydia cases revealed that every month, 1-2% of cases were reported with a delay. This was consistent throughout the year

FIGURE 4

Observed chlamydia incidence in Sweden, January-February 2000-2007 and estimated chlamydia incidence in Sweden, January-February 2000-2005



2006 and also in January and February 2007. No double reporting of chlamydia cases was discovered.

Description of cases

During January and February 2007, a total of 6,903 chlamydia cases were reported to the national surveillance system. Compared to the same period in 2006, this was an increase of 38%. The distribution of the cases by sex and median age was similar to that observed in the previous years (Table 1). The median age was 21.4 years for females and 24.1 years for males.

Chlamydia incidence

Between 2000 and 2005, the trend of reported chlamydia incidence in the period of January and February was increasing in all counties (Figure 2). In 2006, however, the reported chlamydia incidence decreased both in the counties of group A/R and in those of group BD, and then increased again in 2007.

Chlamydia cases were reported in all 21 counties (Table 2). Some variation in reported incidence was observed in each county year by year (Table 2). The 2006 decrease in incidence was apparent in 13 counties and in the national incidence, while the increase in reported incidence observed in 2007, affected all counties.

Testing for *C. trachomatis*

Figure 3 shows the proportion of the population aged between 15 and 49 years that were tested in both groups of counties. From 2000 to 2006 there was, on average, 2% more testing in Group A/R than in Group BD. In both groups of counties there was an upward trend in the proportion of the population tested for chlamydia.

Model estimation

We found that neither the effect for '*group*' nor that for the interaction '*group*year*' were significant in the model, meaning there were no differences in the trend between groups of counties. However, the general trend ('*year*', p-value ‹ 0.001) and the proportion of the population tested (p-value ‹ 0.001) were highly significant. Therefore the final model included only the significant factors, with i=1, ...21 (county) and j=1,...6 (year):

$$\log(\text{cases}_{ij} / \text{pop}_{ij}) = \beta_0 + \beta_1 \text{year}_j + \beta_2 \text{proportion tested}_{ij} .$$

The model estimated an increase of 8.4% (95% confidence interval 5.8%-11.0%) in incidence per year, given a constant proportion of tested individuals. An assumed increase of 5% in testing in the same year would result in an increase in incidence of 24%. Figure 4 shows the estimated versus the reported incidence for 2000-2005 in all counties in Sweden according to this model.

We predicted the national incidence in 2006 to be 63 per 100,000 population, using a proportion of tested individuals reported in that year. The model overestimated the reported incidence in almost all counties, as well as at national level (observed incidence 55 per 100,000 population). The mean error, however, was smaller among BD counties with −3.6 compared to −10.1 in A/R counties. The incidence for 2007 was estimated using two scenarios. When it was assumed that 5% more people were tested in each county in 2007 than in 2006, the model estimated a national incidence of 70 per 100,000 population in January and February. When it was assumed that 20% more people were tested in 2007, the predicted incidence was 75 per 100,000 population. The latter gives a prediction close to what was actually observed this year (mean error per county: 2.5).

Discussion and conclusions

The emergence of the new genetic variant of *C. trachomatis* (nvCT) in 2006 led to a temporary decrease in the number of diagnosed cases. In early 2007, a renewed increase in chlamydia incidence was observed. This was expected after the change to diagnostic kits that were able to detect nvCT. The cases did not differ from previous years in terms of age and sex distribution or geographical distribution. We also excluded the possibility of delayed and double reporting as a reason for the increase. However, our comparison of counties using different diagnostic kits showed that the sharp increase in 2007 could not be solely explained by switching the diagnostic method, since rising numbers of CT were also noted in those counties that had already in 2006 used kits that can detect nvCT. This suggests that other factors could have played a role, such as a higher number of persons being tested and/or a higher CT prevalence in the population.

Table 1

Characteristics of chlamydia cases in Sweden, January-February period of 2000-2007 (n=36,339)

	2000	2001	2002	2003	2004	2005	2006	2007
Number of cases:	2,982	3,542	4,023	3,898	4,880	5,100	5,011	6,903
Female (%)	56.7	55.4	56.6	56.9	56.2	56.2	57.5	57.1
Male (%)	43.3	44.6	43.4	43.1	43.8	43.8	42.5	42.9
Median age (years):								
Female	22.3	22.1	22.2	21.9	21.7	21.9	21.7	21.4
Male	25.0	24.9	24.9	24.5	24.4	24.5	24.8	24.1

Table 2

Reported chlamydia incidence per 100,000 population in Sweden by county, January-February period of 2005-2007

County	2005	2006	2007
Group A/R*			
Dalarna	58.7	44.2	170.8
Gotland	52.2	71.6	73.3
Gävleborg	69.6	57.7	66.0
Halland	58.4	46.7	67.5
Kalmar	54.3	45.8	91.1
Kronoberg	51.6	36.2	59.6
Skåne	60.0	57.0	76.1
Stockholm	56.4	59.5	76.8
Södermanland	66.8	62.0	122.0
Värmland	63.7	61.8	65.5
Västernorrland	46.4	52.9	68.9
Örebro	51.8	48.0	56.7
Östergötland	60.5	62.7	66.0
Group BD*			
Blekinge	53.1	52.8	65.4
Norrbotten	76.7	50.4	56.4
Uppsala	68.0	58.5	80.6
Västerbotten	44.6	45.8	56.7
Västmanland	62.4	72.0	92.6
Västra Götaland	43.9	48.4	66.3
Jämtland	74.8	85.8	88.2
Jönköping	55.1	51.6	71.8
Geometric mean of Incidence (Sweden)	**57.8**	**54.8**	**75.0**

*Group BD: counties that used Becton Dickinson kit. Group A/R: counties that used Abbot or Roche kits.

In almost all counties, our statistical model predicted a higher incidence for 2006 than that actually observed. This supports an effect of underreporting due to undetected cases of nvCT already in January and February 2006. When we assumed that 20% more people were tested in 2007 than in 2006, the predicted incidence for January-February 2007 was the same as the observed incidence. The situation with the newly emerged CT variant was widely covered by mass media in Sweden by the end of 2006, contributing to better knowledge on chlamydia diagnostic problems and possible false negative results. This could have led to increased testing for CT in the beginning of 2007, induced both by health professionals and patients themselves.

An additional explanation for the higher incidence could be a continuous increase in the prevalence of chlamydia in the population, as has been described earlier in Sweden [8]. This explanation was also supported by our model.

Several limitations could influence our results. Firstly, our model did not take into consideration size of population, age distribution, testing policy, or the degree of

partner tracing in the different counties, which could influence our results. In addition, we assumed that the number of tests performed during the entire year was proportional to the number of tests performed in the period of January and February. Neither did we investigate other possible explanations such as a change in sexual behaviour that could contribute to increased spreading of CT.

The sharp increase in January and February 2007 is misleading if compared to the same period in 2006 without taking into consideration the underestimated rates in 2006. Due to the fact that the diagnostic methods failed to detect nvCT in 2006, cases remained undiagnosed and as a result the contacts of these cases were not traced. This led to an accumulation of chlamydia cases and further spread. We can expect to see this effect in those 13 counties in Sweden that had used diagnostic kits unable to detect nvCT. However, more active testing due to the reasons described above or an increase in the prevalence of CT are likely to have contributed to the increased incidence in January and February 2007.

Published reports from other European countries have so far shown limited evidence of spread of the nvCT outside of Sweden [9,10]. Sporadic cases were reported from neighbouring countries such as Denmark and Norway [11,12]. However, sexual contacts during international travels could lead to spread of this genetic variant to other countries as well. Detection of the nvCT through the surveillance system can take time, as was the case in Sweden where the decrease of chlamydia notifications in some counties was masked by the overall national rates. Therefore epidemiological and laboratory vigilance are important not only at national but also at local level. Continuous evaluation of diagnostic tests is necessary. Sexual health promotion needs to be intensified in order to effectively control the spread of sexually transmitted diseases in general. Sweden has intensified prevention campaigns with information in mass media, Internet and cinemas, condom distribution to teenagers, etc. in the summer of 2007 [13].

References

1. Smittskyddsinstitutet. Statistics on infectious diseases. Available from: http://www.smittskyddsinstitutet.se/in-english/statistics/
2. Ripa T, Nilsson PA. A variant of Chlamydia trachomatis with deletion in cryptic plasmid: implications for use of PCR diagnostic tests. Euro Surveill 2006;11(11):E061109.2. Available from: http://www.eurosurveillance.org/ew/2006/061109.asp#2
3. Ripa T, Nilsson PA. A Chlamydia trachomatis strain with a 377-bp deletion in the cryptic plasmid causing false-negative nucleic acid amplification tests. Sex Transm Dis. 2007 May;34(5):255-6.
4. Söderblom T, Blaxhult A, Fredlund H, Herrmann B. Impact of a genetic variant of Chlamydia trachomatis on national detection rates in Sweden. Euro Surveill 2006;11(12):E061207.1. Available from: http://www.eurosurveillance.org/ew/2006/061207.asp#1
5. Unemo M, Olcén P, Agné-Stadling I, Feldt A, Jurstrand M, Herrmann B, et al. Experiences with the new genetic variant of Chlamydia trachomatis in Örebro county, Sweden – proportion, characteristics and effective diagnostic solution in an emergent situation. Euro Surveill 2007;12(4)[Epub ahead of print]. Available online: http://www.eurosurveillance.org/em/v12n04/1204-223.asp
6. Smittskyddsinstitutet. Hur hanterar vi den uppkomna situationen avseende klamydiadiagnostik? [In Swedish]. EPI-Aktuellt. 2006;5(42). Available from: http://www.smittskyddsinstitutet.se/publikationer/smis-nyhetsbrev/epi-aktuellt/epi-aktuellt-2006/epi-aktuellt-vol-5-nr-42/
7. Smittskyddslag. SFS 1988:1472. Available from: http://www.notisum.se/rnp/sls/lag/19881472.HTM
8. Götz H, Lindback, Ripa T, Arbenorn M, Ramstedt K, Ekdahl K. Is the increase in notifications of Chlamydia trachomatis infections in Sweden the result of changes in prevalence, sampling frequency or diagnostic methods? Scand J Infect Dis. 2002;34(1):28-34.
9. Lynagh Y, Crowley B, Walsh A. Investigation to determine if newly-discovered variant of Chlamydia trachomatis is present in Ireland. Euro Surveill 2007;12(2):E070201.2. Available from: http://www.eurosurveillance.org/ew/2007/070201.asp#2
10. de Vries H, Catsburg A, van der Helm J, Beukelaar E, Morré S, Fennema J, Thiesbrummel H. No indication of Swedish Chlamydia trachomatis variant among STI clinic visitors in Amsterdam. Euro Surveill 2007;12(2):E070208.3. Available from: http://www.eurosurveillance.org/ew/2007/070208.asp#3
11. Moghaddam A, Reinton N. Identification of the Swedish Chlamydia trachomatis variant among patients attending a STI clinic in Oslo, Norway. Euro Surveill 2007;12(3):E070301.3. Available from: http://www.eurosurveillance.org/ew/2007/070301.asp#3
12. Statens Serum Institut. Klamydia 2006. [In Danish]. EPI-NYT. 2006;10. Available from: http://www.ssi.dk/sw49276.asp
13. Press release from Smittskyddsinstitutet och Socialstyrelsen on 31 May 2007. Klamydiaspridningen måste stoppas. [In Swedish]. Available from: http://www.socialstyrelsen.se/Aktuellt/Nyheter/2007/Q2/Press070531.htm

Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe

T M Coque[1,2], F Baquero[1,2], R Cantón[1,2]
1. Microbiology Department, University hospital Ramón y Cajal, CIBER Epidemiology and Public Health (CIBERESP), Madrid, Spain
2. Unidad de Resistencia a Antibióticos y Virulencia Bacteriana asociada al Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain

Correspondence: M Coque (mcoque.hrc@salud.madrid.org)

Citation style for this article:
Coque TM, Baquero F, Cantón R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill. 2008;13(47):pii=19044. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19044

Article published on 20 November 2008

Extended-spectrum beta-lactamases (ESBLs) have been increasingly reported in Europe since their first description in 1983. During the 1990s, they were described mainly as members of the TEM- and SHV-beta-lactamase families in *Klebsiella pneumoniae* causing nosocomial outbreaks. Nowadays, they are mostly found in *Escherichia coli* that cause community-acquired infections and with increasing frequency contain CTX-M enzymes. Dissemination of specific clones or clonal groups and epidemic plasmids in community and nosocomial settings has been the main reason for the increase in most of the widespread ESBLs belonging to the TEM (TEM-24, TEM-4, TEM-52), SHV (SHV-5, SHV-12) and CTX-M (CTX-M-9, CTX-M-3, CTX-M-14 or CTX-M-15) families in Europe. Co-selection with other resistances, especially to fluoroquinolones, aminoglycosides and sulfonamides, seems to have contributed to the problem. The emergence of epidemic clones harbouring several beta-lactamases simultaneously (ESBLs, metallo-beta-lactamases or cephamycinases) and of new mechanisms of resistance to fluoroquinolones and aminoglycosides warrants future surveillance studies.

Introduction

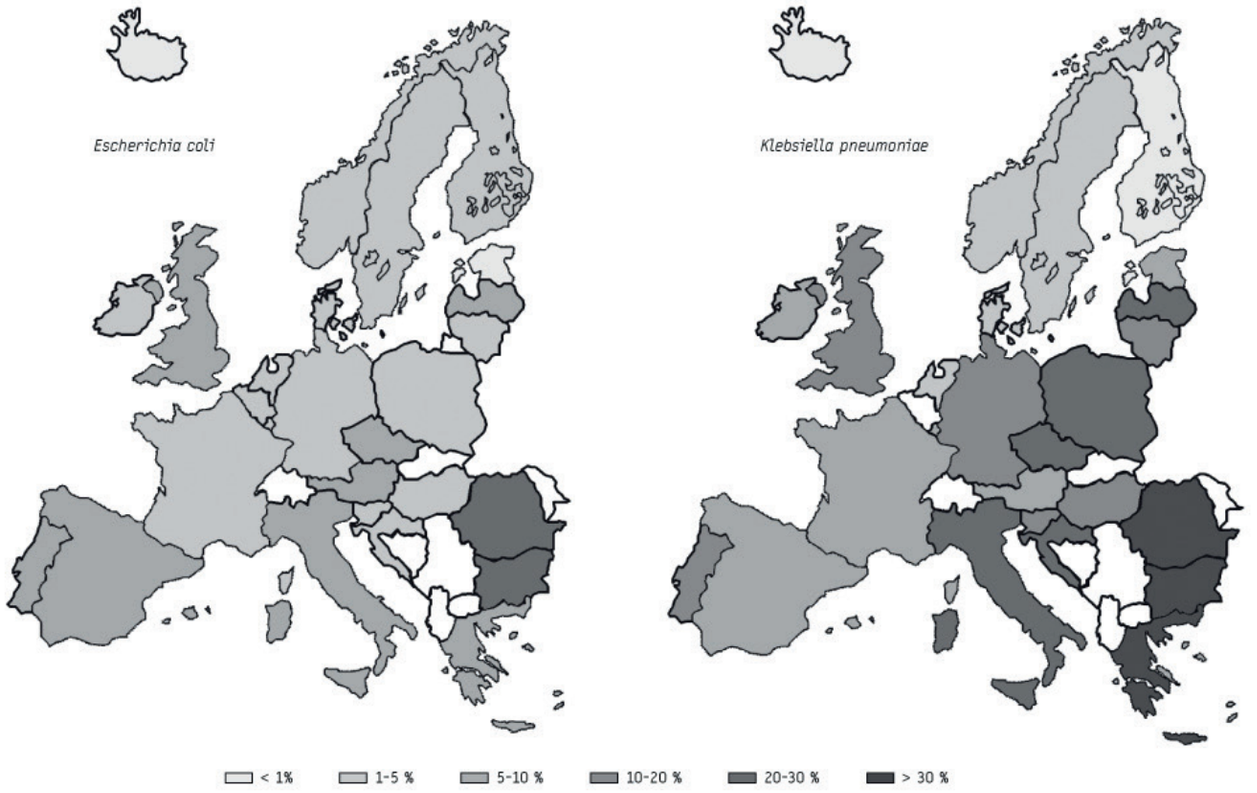
Enterobacteriaceae have become one of the most important causes of nosocomial and community acquired infections. Beta-lactams (mainly extended-spectrum cephalosporins and carbapenems) and fluoroquinolones constitute the main therapeutic choices to treat infections caused by these microorganisms. However, resistance to these compounds has been reported more and more frequently in Europe in the past years [1-5].

Acquired resistance to beta-lactams is mainly mediated by extended-spectrum beta-lactamases (ESBLs) that confer bacterial resistance to all beta-lactams except carbapenems and cephamycins, which are inhibited by other beta-lactamase inhibitors such as clavulanic acid. A shift in the distribution of different ESBLs has recently occurred in Europe, with a dramatic increase of CTX-M enzymes over TEM and SHV variants. Other non-TEM, non-SHV enzymes, such as PER, GES, IBC or certain OXA types, have also been found in some European countries [1]. Although ESBLs still constitute the first cause of resistance to beta-lactams among Enterobacteriaceae, other "new beta-lactamases" conferring resistance to carbapenems, such as metallo-beta-lactamases (MBL) and KPC carbapenemases, or to cephamycins, such as CMY enzymes, have more recently emerged and are often associated with ESBLs (see section *Epidemiology of ESBL in Europe*).

Overall data on resistance to third generation cephalosporins, mainly due to ESBL, in Europe have been provided by the European Antibiotic Resistance Surveillance System (EARSS; http://www.rivm.nl/earss/) and other international surveillance systems (Table 1). In addition to a large number of detailed molecular analyses on particular ESBL types, multicentre studies performed in hospitals, farms, or slaughterhouses, using different surveillance systems in each country, have contributed to a better understanding of the epidemiology of these enzymes at local, national and international level. The current increase in ESBL-producing bacteria in inpatients as well as outpatients at the time of hospital admission points towards a continent-wide rise, mainly in *Escherichia coli*, with great variations in the occurrence and distribution of different ESBLs among countries (see section *Epidemiology of ESBL in Europe*). A community-origin explaining this rise has been highlighted in many surveys, but the prevalence of ESBLs in this setting is difficult to ascertain accurately, as faecal colonisation surveys among humans without direct or indirect hospital exposure are scarce (see section *Faecal colonisation surveillance studies*).

Figure 1

Proportion of invasive *Escherichia coli* and *Klebsiella pneumoniae* isolates resistant to third generation cephalosporins in 2006 (EARSS study)



EARRS: European Antibiotic Resistance Surveillance System

Antibiotic overuse in humans and animals, hospital cross-infection, the food chain, trade and human migration seem to have contributed to the recent dissemination of ESBLs outside hospitals, although the role of these factors is variable and linked to particular epidemiological situations (see sections *Epidemiology of ESBL in Europe and ESBLs in non-humans hosts*). Recent studies have demonstrated the clonal expansion of certain enterobacterial clones that are able to acquire multiple ESBL plasmids (see section *Clonal expansion of ESBL-producing Enterobacteriaceae*). These successful clones seem to have favoured the expansion of ESBLs on our continent, as exemplified by the highly virulent *E. coli* O25:H4-ST131, a strain that is thought to be responsible for the pandemic dissemination of the CTX-M-15 enzyme. The origin of widespread *E. coli* clonal complexes is still unknown, although it is likely that the resistance they exhibit against trimetoprim-sulfamethoxazole or fluoroquinolones is due to a strong selection pressure prior to ESBL acquisition (see section *Clonal expansion of ESBL-producing Enterobacteriaceae*). Plasmid dissemination also plays a critical role in the wide spread of ESBL in Europe (see section *The impact of plasmid transfer on ESBL-producing Enterobacteriaceae*). The increasing description of isolates simultaneously containing ESBLs, carbapenemases, CMY or new mechanisms of resistance to fluoroquinolones and aminoglycosides is of concern (see section *Multi-resistance profiles in ESBL producing isolates*). In this review, we summarise the more recent findings on ESBL epidemiology in Europe in order to understand the recent increase in hospitals and in the community, and to implement appropriate intervention strategies to avoid their pandemic dissemination as has happened with certain Gram-positive organisms such as methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus faecium*.

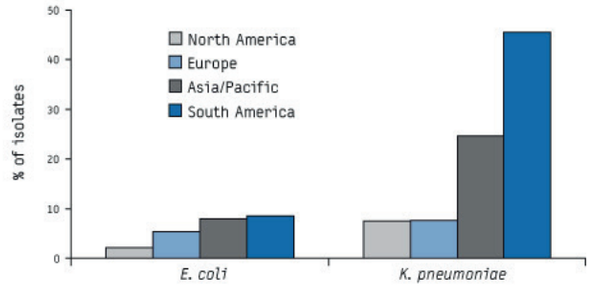
Epidemiology of ESBL in Europe

General surveillance studies

European and intercontinental surveillance studies have collected data on ESBL-producing Enterobacteriaceae in Europe, all of which consistently show a variable proportion among different geographic locations, enterobacterial species and isolates from different sources (Table 1, Figure 1). Some of them allow comparison with non-European geographic areas, such as the TEST (Tigecycline Evaluation and Surveillance Trial) or SMART (Study for Monitoring Antimicrobial Resistance Trends) [4], which showed that ESBL were far less frequent in Europe than in Latin America and Asia/Pacific regions but more common than in North America (Figure 2). However, these studies have not addressed potential differences between hospital and community isolates.

Figure 2

Frequency of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates reported in the TEST surveillance study (2004-2006) in different geographic areas [27]



ESBL: extended-spectrum beta-lactamases; TEST: Tigecycline Evaluation and Surveillance Trial.

A recent multicentre European study performed in 2005 in settings with a high antibiotic selection pressure such as intensive care units (ICU) gave results similar to those collected by EARSS [7]. That study had been designed to monitor the association between specific antibiotic consumption and antimicrobial resistance, but no clear correlation was found between the two. This was probably due to differences in the prevalence of patients who were colonised with resistant pathogens at admission, and to the different efforts put in place in different ICUs to avoid cross-transmission of these bacteria.

To date, there have not been any specific European multicentre studies addressing the prevalence of ESBL among community isolates, although there have been different efforts at national and local levels. A study performed in Turkey showed a prevalence of 21% ESBL producers among *E. coli* causing community-acquired urinary tract infection (UTI) during 2004 and 2005 [8]. This percentage was higher than the 5.2% observed in a Spanish multicentre study covering 15 microbiology laboratories in 2006 [9]. Moreover, the rate of community-acquired bacteraemias caused by ESBL-producing *E. coli* was 6.5% in Spain, whereas it ranged from 12.9% to 26.8% for *K. pneumoniae* in studies performed in Spain and the United Kingdom (UK) [10-12].

Faecal colonisation surveillance studies

There are no multicentre studies to address faecal colonisation rates with ESBL-producing isolates in Europe, although this is a common practice in the hospital setting for implementing epidemiological measures to curtail or control their spread. Nevertheless, the rate of inpatients, outpatients and healthy volunteers colonised by ESBL producers has been addressed in a few national studies and provided interesting observations. A Spanish analysis demonstrated that the frequency of faecal carriers had increased from under 1% to 5% among outpatients and from under 1% to 12% among hospitalised patients between 1991 and 2003, with a prevalence of 4% in healthy volunteers during 2004 [13]. It is of interest to note that the ESBL characterised among isolates obtained from faecal carriers was similar to the one obtained in the clinical setting in Spain at the time these studies were performed. This could prove useful for monitoring ESBL trends [14,15]. Nevertheless, these proportions are in contrast with what was found in a study performed among 322 healthy volunteers in the Paris area that did not detect any carriers of ESBLs. However, the same study frequently observed colonisation with prevalent clones that are associated with particular ESBLs but did not actually contain these enzymes [16].

Two other Spanish studies showed that the faecal carriage rate of ESBL-producing *E. coli* in community patients who had UTIs caused by this pathogen was around 70%, which is much higher than that of individuals with infections not associated with ESBLs [17,18]. Interestingly, faecal carriage in the household contacts of infected patients with ESBL-producing *E. coli* ranged from 16.7% to 27.4% in these two studies. This led to the suggestion that faecal colonisation with ESBL-producing bacteria is a risk factor for acquisition of UTI caused by these pathogens and a potential source for transmission among households.

Geographic differences and ESBL types circulating in European hospitals

The last EARSS report from 2006, covering over 800 laboratories from 31 countries, showed a continuous increase since 2000 in invasive *E. coli* and *K. pneumoniae* isolates resistant to third generation cephalosporins, with prevalences higher than 10% for half of the enrolled countries (Figure 1). In addition, it shows important geographical differences, ranging from a percentage of under 1% (Estonia) to 41% (Romania) for *E. coli* and from 0% (Iceland) to 91% (Romania) for *K. pneumoniae*. Although these proportions are generally associated with the production of ESBL, they might be somewhat overestimated due to the inclusion of isolates with a greater susceptibility to beta-lactams when EUCAST breakpoints are used, or due to isolates overproducing AmpCs which represent about 1-2% of isolates resistant to third generation cepholosporins.

All published studies have confirmed that in most northern European countries, the prevalence of ESBL isolates is still low compared to southern and eastern European countries. Unfortunately, not all publications indicate precise frequency rates, since most of them were designed to establish the molecular epidemiology of circulating ESBLs, but not to ascertain the prevalence of these isolates.

Northern European countries

In Denmark (www.danmap.org), Norway (www.antibiotikareistens.no) and Sweden (www.strama.se), yearly national surveillance and published studies show continuous rising trends of ESBLs. In the Copenhagen area of Denmark, the occurrence of ESBL producers was below 1% in isolates received at a national reference

Table 1

Global surveillance studies covering Europe and including ESBL-producing bacterial isolates

Surveillance Study	Date (Year)	Countries (no.)	Centres (no.)	Sample Origin	Overall frequency (%)	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*P. mirabilis*	*Enterobacter* spp.	Reference
SENTRY	1997-98	15	25	Blood, urine, respiratory tract, wounds,	4.9	1.3	18.4	12.6	5.3	n.a.	[3]
SMART	2004	9	31	Intra-abdominal		6.4	8.8	n.a.	n.a.	11.8	[4]
TEST	2004-06	19	62	Blood, urine, respiratory tract, wounds, sterile fluids		7.6	13.3	n.a.	n.a.	n.a.	[5]
MYSTIC	2006	12	40	Blood culture, urine, sputum, sterile fluids, wounds	5.6	8.2	9.8	n.a.	1.4	n.a.	[6]
EARSS	2006	31	ca. 800	Blood		<1-41	0-91	n.a.	n.a.	n.a.	-

ESBL: Extended-spectrum beta-lactamases; SMART: Study for Monitoring Antimicrobial Resistance Trends; TEST: Tigecycline Evaluation and Surveillance Trial; MYSTIC: Meropenem Yearly Susceptibility Test Information Collection; EARSS: European Antibiotic Resistance Surveillance System (http://www.rivm.nl./earss/).
n.a.: not available.

laboratory, with dominance of CTX-M and SHV enzymes [19]. In Norway, a prospective survey of clinical *E. coli* isolates with reduced susceptibility to oxyimino-cephalosporins demonstrated the dominance of CTX-M-15 (46%) and CTX-M-9-like (30%) enzymes among ESBL-positive *E. coli* and of SHV-5 (47.4%) and SHV-2 (21.0%) among ESBL-positive *K. pneumoniae* isolates [20]. This ESBL distribution is similar to that encountered in Sweden during the period from 2001 to 2006, when 92% of consecutive non-duplicate ESBL-positive *E. coli* isolates expressed a CTX-M-type enzyme, CTX-M-1 being the predominant group [21]. Similar results were found in multicenter studies performed between 2002 and 2004 in Finland [22]. More recently, clonal outbreaks caused by CTX-M-15 *K. pneumoniae* have been reported in Scandinavia [23].

Southern countries

The prevalence of ESBL producers in Spain and Portugal has increased over time, with a predominance of CTX-M-producing *E. coli* causing community acquired UTIs [14,24-26]. In Spain, a shift in the proportion of ESBL-producing *Klebsiella* isolates recovered from outpatients (7% to 31%) and ICU patients (41% to 25%) was observed between the periods 1989 to 2000 and 2001 to 2004 [27]. Although a high diversity of ESBLs are reported in most Spanish studies, high local prevalence of CTX-M-9, CTX-M-14, CTX-M-10 and TEM-4 enzymes is observed among inpatients, outpatients and healthy individuals [13,15,17]. In Portugal, nationwide surveys are not available. Studies of individual hospitals reflect a common spread of CTX-M-14, TEM-52, and GES [24,26]. TEM-24, CTX-M-15, CTX-M-32 and SHV-12 are frequently detected in both Spain and Portugal [15,24].

In Italy, the prevalence of ESBL producers among clinical isolates has also increased over the past ten years [28]. The most prevalent ESBL-positive species are *E. coli* among hospitalised patients and *Proteus mirabilis* among outpatients. A predominance of TEM enzymes (45.4%), SHV-12, and the emergence of non-TEM, non-SHV enzymes (CTX-M-type in *E. coli* and *K. pneumoniae*, and PER-type in *P. mirabilis*) has been described. More recent studies performed in single institutions showed the frequent recovery of CTX-M-15-producing *E. coli* and other variants from this group such as CTX-M-1 and CTX-M-32 [29-31].

In France, the prevalence of ESBL production in Enterobacteriaceae reported in different multicentre studies is under 1%, with a progressive increase in the occurrence of CTX-M enzymes linked to *E. coli* expansion [32]. The frequency of certain ESBL producers in 2005 was far lower than reported in previous years including *P. mirabilis* (3.7% versus 1.3%), *Enterobacter aerogenes* (53.5% versus 21.4%) and *K. pneumoniae* (9.4% versus 3.71%), but had increased for *E. coli* (0.2% versus 2%). In addition, ESBLs have frequently been observed in the community setting, linked to nosocomial acquisition [33]. CTX-M-variants were predominant and belonged primarily to the CTX-M-1 (85%) and CTX-M-9 (11.3%). A variety of TEM enzymes has been identified both in hospitals and in the community, although TEM-3 and TEM-24 remain the more common types, they have persistently been recovered since the late 1990s and have often been associated with clonal outbreaks [32,33].

United Kingdom

A recent dramatic increase in ESBL-producing organisms is being observed both in hospitals and in the community, mainly caused by the CTX-M-15 enzyme [2]. This enzyme, first reported in the UK in 2003, initially co-existed with CTX-M-9, CTX-M-14, SHV-variants (mainly SHV-12), and to a lesser extent with TEM derivatives both in the hospital and in the community. It has now become the most prevalent enzyme in both settings [2,34].

Eastern countries

The occurrence and distribution of ESBLs in this area differs from that in other countries. The prevalence of ESBLs is over 10% in Hungary, Poland, Romania, Russia and Turkey. *K. pneumoniae* is the most frequent ESBL-producing species in Hungary and Russia, and an increase in the percentage of ESBL producers among *K. pneumoniae* isolates has been reported from Poland, Turkey, Bulgaria, and Romania [35-40]. CTX-M-3, SHV-2 and SHV-5 are usually widely spread in eastern European countries.

Table 2

Plasmids involved in the wide dissemination of specific ESBLs in European countries

ESBL	Country	Year	Inc Group	Origin	Species	Reference
CTX-M-1[a]	France (10 slaughterhouses, 5 districts)	2005	IncI1	Animals	*E. coli*	[64]
CTX-M-2	Belgium, France	2000-2003	IncHI2	Poultry flocks, poultry meat, humans	*S. enterica* serovar.Virchow	[68, 98]
CTX-M-3[b]	Poland	1996-2005	IncL/M	Hospitals	*K. pneumoniae, Serratia marcescens, E. coli*	[35, 41, 99]
	Bulgaria, Poland, France		IncL/M	Hospitals	*Different species*	[94]
CTX-M-9	Spain, UK[c]	1996-2006	IncHI2	Hospitals	*E. coli, Salmonella*	[73, 95, 98]
	Spain	1998-2003	IncP1-α	Hospitals	*E. coli*	[86, 95]
	France	2003	IncHI2	Poultry	*S. enterica* serovar.Virchow	[69, 98]
CTX-M-14	Spain UK	1996-2006 2004-2005	IncK IncK	Hospitals Poultry	*E. coli* *E. coli*	[47] [75]
CTX-M-15[d]	Spain, Portugal, Italy, Turkey, Switzerland, France, Norway, Canada, Kuwait, India	2000-2007	IncFII	Hospitals	*E. coli, Klebsiella*	[30, 73, 78, 88]
CTX-M-32	Spain, Portugal, UK	2000-2006	IncN	Hospitals	*E. coli*	[86, 87]
TEM-24	Spain, Portugal, France, Belgium		IncA/C_2	Hospitals	*Enterobacter aerogenes, Proteus mirabilis, K.oxytoca*	[51]
TEM-52[e]	Spain, Portugal, France, The Netherlands, Belgium	2001-05	IncI1	Hospitals, animals	*E. coli, Salmonella*	[65, 70, 76]
SHV-5	Poland	1996-	IncFII	Hospitals	*E. coli*	[100]
	Hungary	1998-2003	Not determined	Hospitals	*K.pneumoniae*	[38]
SHV-12	Italy	2005	IncI1	Poultry	*E. coli*	[89]
	Spain	2005	IncI1	Humans	*E. coli, Klebsiella*	[Valverde, unpublished]

ESBL: Extended-spectrum beta-lactamases.
(a) The $bla_{CTX-M-1}$ gene has been located on plasmids of incompatibility groups N (among *E. coli* from humans and swine in Spain and Denmark, respectively) and A/C (from Spanish inpatients) [86,98].
(b) Relationship among these two plasmids has not been published.
(c) Associated with travel to Spain [73].
(d) CTX-M-15 plasmids of the group IncI1 have been described among human *Salmonella* Typhimurium isolates in the UK, although their distribution is unknown [73].
(e) This IncI plasmid has also been associated with bla_{TEM-20} in *E. coli* from Norway and *Salmonella* Paratyphi B dT from the Netherlands [65].

In Poland, the proportion of ESBL producers in hospitals (11.1%) varied for different species from 2.5% for *E. coli*, 40.4% for *K. pneumoniae* and 70.8% for *Serratia marcescens*, the latter two having a higher prevalence due to outbreak situations. ESBL types were dominated by CTX-Ms (82%, CTX-M-3) and SHV types (17%, SHV-2, SHV-5, and SHV-12), while TEM-like enzymes (‹1%, TEM-19 and TEM-48) were found only sporadically. In contrast to other countries, CTX-M-15 was rarely recovered in Poland [35]. The current scenario in Poland differs from that in the late 1990s, when there was a dominance of TEM ESBLs and spread of CTX-M-3 producers all over the country [41,42].

In Bulgaria, hospital outbreaks caused by CTX-M-3, CTX-M-15 and SHV-12 are described, often with an involvement of *S. marcescens* in addition to *K. pneumoniae* [40]. In Hungary, a recent eruptive and extensive spread of highly ciprofloxacin-resistant CTX-M-15 *K. pneumoniae* epidemic clones has been detected [36]. Nosocomial outbreaks involving SHV-2a-producing *K. pneumoniae* are also frequent [38]. In Turkey, CTX-M-15 is widely distributed [8,39], and epidemic strains of *K. pneumoniae* isolates producing the carbapenemase OXA-48 and the ESBLs SHV-12 or CTX-M-15 have emerged [43].

Predominant ESBLs circulating in Europe

The emergence and wide spread of the CTX-M-15 enzyme in most European countries, including those with previous low rates of ESBLs, is one of the most relevant findings associated with the current epidemiology of ESBL in Europe [8,14,23,36,44,45]. This enzyme is increasingly being associated with isolates from the community setting, including healthcare centres, as documented in studies from France, Spain, Turkey and the UK, [2,8,14,32,46, see also section *Clonal expansion of ESBL-producing Enterobacteriaceae*].

Other CTX-M variants are amplified locally, such as CTX-M-9 and -10 in Spain [15,25], CTX-M-14 in Portugal and Spain [15,24,47] , CTX-M-3 in eastern countries [35,40] and CTX-M-5 in Belarus and Russia [37]. The SHV-12 enzyme is one of the most prevalent enzymes associated with nosocomial *K. pneumoniae* isolates in Italian, Polish and Spanish hospitals and is also increasingly reported in *E. coli* isolates from community patients [13,31,48]. SHV-5, widely disseminated in Europe, is especially abundant in Bosnia and Herzegovina, Croatia, Greece, Hungary and Poland [35,38,48,49,50].

In addition, particular TEM types deserve special attention as they were traditionally associated with the ICU setting, TEM-3 and TEM-4, are associated with epidemic clones of *K. pneumoniae* in France and Spain, while TEM-24 is associated with epidemic *E. aerogenes* strains in Belgium, France, Portugal and Spain [24,32,33,51]. Nowadays, these enzymes have been also characterised in *E. coli* and *P. mirabilis* recovered in the community [24,33,51]. Finally, TEM-52, first identified in *Salmonella* spp. isolates from animal origin, is currently found among different Enterobactereriaceae species involved in human infections [24,33].

Co-production of different ESBLs is increasingly reported in European countries. Clinical isolates expressing SHV (SHV-5 or SHV-12) or TEM-24 and also other ESBL (CTX-M-9 or CTX-M-14) or carbapenemases (KPC, OXA, or VIM) have been described, sometimes associated with clonal outbreaks [43,49,52-54].

ESBLs in non-humans hosts

ESBL-producing *E.coli* and non-typhoidal *Salmonella* species have been isolated from farm animals, wild animals, food, pets and from environmental samples in different European countries [55-59]. The variability in the date of emergence and in the proportion of ESBL producers among animals seem to be due to differences between European countries in cephalosporin usage, and detection method, and to the importation of resistant strains through travellers or trade [59-62].

Different national surveys performed in Italy [63], France [64], the UK [http://www.defra.gov.uk/], Denmark [60], Norway [65] and Spain [57,66] demonstrated that the resistance to broad-spectrum cephalosporins is still low among zoonotic pathogens. However, a recent study performed in Denmark showed that veterinary beta-lactams (amoxicillin, ceftiofur, cefquinome) select for indigenous ESBL-producing *E. coli* in the intestinal flora of pigs and favour the emergence of strains that acquire ESBL genes by horizontal transfer. This selective effect persists for a period longer than the withdrawal time required for these antimicrobials [67]. Although the transmission of ESBL-producing bacteria through the food chain or direct contact between humans and animals has seldom been proven [66-68], animals should be considered as an important reservoir of ESBL-strains and highly transmissible plasmids.

ESBLs isolated from animals include different variants belonging to the CTX-M (-1,-2,-3,-8, -9,-13,-14,-15,-24,-28,-32), SHV (-2,-5,-12), and TEM (-52,-106,-116) families. CTX-M-1, TEM-52 and SHV-12 are the ones most commonly found to date. Their dissemination among non-human hosts seems to have been facilitated mainly by mobile conjugative elements [55; Table 2]. The epidemiology of the most prevalent variants in European countries exemplifies different transmission routes and is therefore briefly revised in this section.

The CTX-M-1-like-enzymes (CTX-M-1, -15 and -32) are widely distributed among animals from western European countries and mainly associated with epidemic plasmid spread among clonally unrelated *E. coli* [57,58,62,64,67]. CTX-M-1 is widespread among healthy and sick farm animals (poultry, swine) and pets in Belgium, Denmark, France, Italy, the Netherlands, Portugal and Spain [56-58,62,64,67,71]. It was also the most frequent ESBL in a Belgium survey, representing 27.4% of ESBL producers, some of which were also producing CMY-2 [62]. CTX-M-32 has been detected among healthy and sick animals in Greece, Portugal and Spain [57,58,72]. CTX-M-15, frequently recovered among clinical isolates, has been sporadically identified from pets and farm animals in different countries in the European Union (EU), although it is associated with different strains and plasmids than the ones that are responsible for the wide distribution of this ESBL in hospitals [73].

The CTX-M-9-like enzymes (CTX-M-9 and CTX-M-14) have been linked directly or indirectly with animals in different countries. CTX-M-9 producers have been detected among healthy and sick animals in Spain since 1997 [57,66]. In France, it was found in unrelated poultry isolates of *Salmonella enterica* serotype Virchow collected by the Agence Française de Sécurité Sanitaire des Aliments network in 2003 in a single hatchery located in the southwest of France that supplied different farms with chicks [69]. CTX-M-9 producers have also been linked to food-borne disease outbreaks or colonisation of food handlers in Spain, travellers returning to the UK from Spain and quails imported by Denmark from France [55,67,74]. CTX-M-14-producing *E. coli* or *Salmonella* on the other hand were identified from different slaughter animals in Belgium, Denmark, France, Spain and the UK. It was also linked to travellers returning to the UK from Thailand and to imported chickens in the UK [59,62,67,75].

Epidemic strains of *S. enterica* serotype Virchow producing CTX-M-2 have been isolated from poultry and poultry products in Belgium, France, and the Netherlands since 2000 [61,62,68]. The recent recovery in the UK of *E. coli* producing CTX-M-2 from imported raw chicken meat from Brazil suggests a transmission route from areas where this enzyme is endemic [59].

TEM-52-producing *E. coli* and *Salmonella* isolates have been detected in sick and healthy farm animals, pets, and beef meat food in, Belgium, Denmark, France, Greece, the Netherlands, Spain and the UK [61,70,72]. In Portugal, TEM-52 was widely disseminated among different enterobacterial species recovered from humans, pets, wild animals and livestock [56,58]. In Belgium and France, TEM-52 producers have frequently been isolated from *Salmonella* isolates of different serovars recovered from poultry and humans [70]. It is noteworthy that multidrug-resistant isolates of the serovars Agona (widely distributed in Belgian poultry) and Typhimurium phagotype DT104 (disseminated globally) have been detected which carry both SGI1 and a plasmid-borne ESBL [70]. Not only has clonal transmission involving *Salmonella* Blockey and Hadar been demonstrated within the Netherlands [61], but the joint spread of two epidemic plasmids between countries has been shown in two different studies [70,76]. Importation of animals or meat was the potential source of *bla*TEM-52 in some areas in the EU [61,77].

SHV-12 producers in animals were detected in Italy during 2005 and 2006, and they were genetically related clones of *Salmonella* Livingstone, scattered on different farms in the northeast of the country, the main region for poultry production [http://www.istat.it; 63]. In Spain, the Netherlands and the UK, SHV-12-positive *Salmonella* and/or *E. coli* isolates have been identified from faecal samples from poultry and pigs [35,57,61,66]. Surprisingly, SHV-12 from animal origin has rarely been described in other European countries.

Clonal expansion of ESBL-producing Enterobacteriaceae

One of the major factors involved in the current prevalence of ESBL-producing Enterobacteriaceae is clonal spread. The most representative example linked to ESBL-producing Enterobacteriaceae is the recent and fast global dissemination of the highly virulent ciprofloxacin-resistant clone B2-*E. coli* O25:H4-ST131 that causes UTI and is associated with the CTX-M-15 pandaemia. This clone has been detected in the majority of European countries, e.g. France, Greece, Italy, Norway, Portugal, Spain, Switzerland, Turkey, and the UK [8,22,44,45,78]. Interestingly, B2-*E. coli* ST131 is able to acquire multiple resistance mechanisms, and this strain was identified repeatedly, harbouring different CTX-Ms, AmpC or SHV-12 recovered in recent British (2004-2005) and Spanish (2004) multicentre hospital surveys [44, Oteo *et al.*, personal communication]. It was also frequently identified among quinolone-resistant non-ESBL UTI-causing *E. coli* strains in clinical isolates from 10 different countries included in the last ARESC study (2004-2005) as well as in healthy volunteers in the Paris area (2007) [16,46,79]. Other widely distributed quinolone-resistant *E. coli* clones in the EU are responsible for the spread of specific ESBLs, such as A-*E. coli* ST10 or B1-*E. coli*-ST359, ST155*, which are mainly identified among CTX-M-14 producers in the central area of Spain [16,47]. These findings suggest that the acquisition of ESBL plasmids by widespread continental fluoroquinolone-resistant *E. coli* clones may have contributed to the dissemination, amplification and persistence of ESBL on our continent.

Nationwide dissemination of particular multidrug-producing *K. pneumoniae* clones has been observed in several countries. In Greece, an endemic SHV-5-producing strain that emerged in the 1990s has recently acquired plasmid-borne VIM-1. This clone is currently spread among Greek hospitals and has also been identified in France [49,80]. Clonal outbreaks caused by *K. pneumoniae* producing SHV-5 and VIM-1 have also been detected in Italy, although a possible link with the Greek clone has not been investigated [54]. A predominance of SHV-type (SHV-5 and SHV-2a)-producing *K. pneumoniae* susceptible to ciprofloxacin is responsible for major clonal outbreaks in Hungarian neonatal ICUs, but endemic or inter-hospital dissemination of these local epidemic clones has not been addressed [38]. Dissemination of ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-producing *K. pneumoniae* clones has recently been reported from the ICUs of 35 hospitals in 13 counties across Hungary, representing 97% of all CTX-M producers in this country [36,38]. The ST15 *K. pneumoniae* clone has also been identified in ESBL-producing isolates from France, Poland and Portugal, although the real dissemination impact of this clone in these countries is unknown [51]. Long-term persistence (>2 years) of ESBL-producing *K. pneumoniae* has been documented in single institutions in France (TEM-24), Greece (SHV-5), Hungary (SHV-2a), Portugal (GES-1) and Spain (TEM-4, SHV-12) [27,38,81,82]. Only a few sporadic cases of international exchange of epidemic *K. pneumoniae* clones are reported in the literature [80].

Representative examples of clonal expansion in other enterobacterial species include a multidrug-resistant *E. aerogenes* strain widely disseminated in EU hospitals since the 1990s, which is responsible for the spread of TEM-24 in Belgium, France, Portugal and Spain [24,51,83]. This clone can simultaneously carry *bla*TEM-24 and plasmids encoding different ESBLs (*bla*SHV-12, *bla*SHV-5, *bla*TEM-20) and MBLs (*bla*IMP-1, *bla*VIM-2) [84]. An aminoglycoside-resistant *Enterobacter cloacae* clone containing a conjugative plasmid carrying the *qnrA1*, *bla*CTX-M-9, and *aad*B genes has been detected in 11 of 15 Dutch hospitals and has caused outbreaks in at least four of them [85]. ESBL-producing *P. mirabilis* (TEM-24), *Shigella sonnei*, *S. marcescens* and *Klebsiella oxytoca* have caused clonal outbreaks in different EU countries, although it remains to be elucidated whether they are of more than local significance [24,51,62].

The increasingly frequent description of endemic bacterial strains that are able to acquire genes coding for ESBLs, carbapenemases (VIM, OXA), and AmpC highlights the need to identify and successfully follow up the clones occurring in Europe [43,44,49,53,80,83].

The impact of plasmid transfer on ESBL-producing Enterobacteriaceae

Currently, the high prevalence of all *bla*ESBL genes in different European regions is caused by horizontal transfer of plasmids among clonally unrelated clones and also among local or international epidemic clones. Plasmid transmission has played a significant role in the persistence of CTX-M-3 in Poland from the late 1990s until today [35,41], the persistence of TEM-4, CTX-M-10, CTX-M-9 and CTX-M-14 in Spanish hospitals since the first description of each enzyme [27,86], and the spread of SHV-5 in hospitals in Greece, Hungary and Poland [38]. Spread of plasmids between countries has been reported for CTX-M-2 (Belgium and France), CTX-M-15 (10 countries), CTX-M-32 (Mediterranean area), TEM-24 and TEM-52 (Belgium, France, Portugal and Spain) [51,68,70,76,78,87,88]. Plasmid-mediated horizontal transfer of *bla*CTX-M-2 and *bla*CTX-M-9 genes has been demonstrated between poultry and human *S. enterica* and *E. coli* strains isolated in very different geographical regions [67,68,89]. The predominant plasmids circulating in Europe in both hospitals and the community are listed in Table 2.

The emergence of epidemic strains that simultaneously carry several plasmids encoding distinct ESBLs, AmpC and MBLs is of concern and deserves further follow-up (see above, section *Clonal expansion of ESBL-producing Enterobacteriaceae*).

Multidrug-resistance profiles in ESBL-producing isolates

ESBL producers are commonly resistant to different antibiotic families including – besides beta-lactams – fluoroquinolones, aminoglycosides and trimetoprim-sulfametoxazole, which contribute to the selection and persistence of multidrug-resistant ESBL strains and plasmids in both clinical and community settings [1,91]. The proportion of ESBL-producing isolates resistant to fluoroquinolones has increased over time, initially in *K. pneumoniae* and later also in *E. coli* [1,89,90]. This increase has apparently occurred in parallel to the increase in plasmid-mediated resistance mechanisms including Qnr proteins (*qnrA*, *qnrB* or *qnrS*), acetylases that can affect the action of certain fluroquinolones (*aac(6')-Ib-cr*) or systems pumping fluoroquinolones out of the bacteria (*qepA*) [92,93].

Very recent studies indicate that the *aac(6')-Ib-cr* gene seems to be confined to *E. coli* ST131 and thus has mainly been linked to CTX-M-15 isolates in different surveys, whereas *qnr* genes are mostly associated with enzymes from the CTX-M-9 or CTX-M-1 groups, which reflects the fact that genes coding for resistance to beta-lactams and quinolones are located on the same plasmid and thus passed on together among different enterobacterial species [79,92]. A high level of fluoroquinolone resistance is often due to additional loss of outer membrane proteins or efflux pump overexpression in clones that already contain *gyrA* and *parC* chromosomal mutations and plasmid-mediated mechanisms [79]. Genes that encode resistance to aminoglycosides (different modifying enzymes and ArmA methylase), trimetoprim or sulfonamides and are located on a wide range of genetic elements such as class 1, 2 and 3 integrons or transposable elements have been associated with different multidrug-resistant ESBL plasmids from human and animal origin [93-96; Curiao *et al.*, unpublished results].

Finally, the recent recovery of plasmids coding for ESBLs that express a low level of resistance to beta-lactams [65] or contain multiple silenced antibiotic resistance genes [97] is of particular concern, as they may serve as reservoirs of antibiotic resistance determinants in bacteria that we are unaware of and that cannot be detected by phenotype.

Concluding Remarks

Increased prevalence of Enterobacteriaceae resistant to extended spectrum beta-lactamases has been reported all over Europe, albeit with a great variability in the occurrence and distribution of ESBL enzymes among different geographic areas. Nordic European countries still show the lowest rates of ESBL prevalence in clinical isolates and have not reported any isolates in animals, while southern and eastern countries present high and increasing frequencies of ESBL-producing strains in both nosocomial and community settings. However, some general epidemiological features such as:

1. the wide representation of CTX-M enzymes, particularly among *E. coli* isolates that cause community-acquired infections,
2. the wide spread of particular successful clones and multidrug-resistant plasmids,
1. and the increasing number of Enterobacteriaceae with ESBLs that also contain MBLs or AmpCs and other new mechanisms of resistance to fluoroquinolones or aminoglycosides indicate that the recent increase of ESBL producers in Europe constitutes a complex multifactorial problem of high public health significance that deserves a deep analysis and the implementation of specific interventions at different levels.

Firstly, the use of broad spectrum cephalosporins and fluoroquinolones in humans and animals should be urgently limited to cases in which other therapeutic alternatives according to evidence-based guidelines are not possible. Limiting antimicrobial use may curtail the selection and persistence of predominant ESBL clones and the probable dissemination of conjugative plasmids among strains, thus decreasing not only the number of potential ESBL donors but also the accumulation of antibiotic resistance genes on common genetic elements.

Secondly, and in accordance with the former recommendation, methods should be improved to efficiently detect and track those bacterial clones and plasmids that constitute the major vehicles for the spread of ESBL-mediated resistance. Ideally, such methods of detection should be accessible to medium-level diagnostic microbiology laboratories, to assure the possibility of performing interventions in real time.

Thirdly, the importation of ESBL-producing bacterial strains through food animals and pets has the potential to cause the wide dissemination of antibiotic resistance among countries and their spread to humans. It highlights the need for national and supra-national public health efforts to implement surveillance, epidemiologic, environmental health, and policy-making components.

Fourth, the implementation of ecological surveillance of ESBL-producing organisms, including environmental (particularly water environments, as sewage) and faecal colonisation surveillance studies in community-based individuals and animals is urgently needed to address the "colonisation pressure" outside hospitals, to detect circulation of highly epidemic clones and to monitor ESBL trends. These ecological studies could be useful as biosensors of modifications in the ESBL landscape.

Fifth, an improvement is needed in the methods for detecting multidrug-resistant ESBL producers that express a low level of resistance to beta-lactams or might contain silenced antibiotic resistance genes

not detectable by standard phenotype. Also strongly suggested is a standardisation of beta-lactam breakpoints recommended by the different agencies and committees.

Finally, the scientific and public health community should be aware that the potential interventions directed to control the world-wide spread of ESBL-producing organisms have a limited time-window for effective action. Once a number of thresholds were crossed (critical absolute number of ESBL-genes in the microbial world, critical associations of these genes with wide-spread genetic platforms, critical dissemination of ESBLs among different bacterial species and clones), the control will be simply impossible by applying the standard measures. We should act now, and be prepared for the uncertain future, by promoting innovative ways of controlling ESBL-producing organisms.

Acknowledgements:

We are deeply grateful to our collaborators in the ESBL field – Ângela Novais, Aránzazu Valverde, Tania Curiao, Marta Tato and Elisabete Machado – for their permanent input and enthusiasm. We are also in depth to Ângela Novais for help in the preparation of the manuscript, Luísa Peixe for critical review of this paper. Research on ESBLs in our laboratory is supported by grants from the European Commission LSHM-CT-2003-503335 (COBRA) and LSHM-CT-2005-018705 (DRESP), from the Ministery of Science and Technology of Spain (SAF 03-09285), and from the Fondo de Investigaciones Sanitarias, Ministry of Health from Spain (PI 07/1441).

*Author's correction:

On request of the authors, B1-E. coli-ST45 was changed to read B1-E. coli-ST359, ST155. This correction was made on on 22 November 2008.

References

1. Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, et al. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect. 2008;14 Suppl 1:144-53.
2. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al. CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother. 2007;59(2):165-17.
3. Nijssen S, Florijn A, Bonten MJ, Schmitz FJ, Verhoef J, Fluit AC. Beta-lactam susceptibilities and prevalence of ESBL-producing isolates among more than 5000 European Enterobacteriaceae isolates. Int J Antimicrob Agents. 2004;24(6):585-91.
4. Bochicchio GV, Baquero F, Hsueh PR, Paterson DL, Rossi F, Snyder TA, et al. In vitro susceptibilities of Escherichia coli isolated from patients with intra-abdominal infections worldwide in 2002-2004: results from SMART (Study for Monitoring Antimicrobial Resistance Trends). Surg Infect (Larchmt). 2006;7(6):537-45.
5. Reinert RR, Low DE, Rossi F, Zhang X, Wattal C, Dowzicky MJ. Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. J Antimicrob Chemother. 2007;60(5):1018-29.
6. Turner PJ. Meropenem activity against European isolates: report on the MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) 2006 results. Diagn Microbiol Infect Dis. 2008;60(2):185-92.
7. Hanberger H, Arman D, Gill H, Jindrák V, Kalenic S, Kurcz A, et al. Surveillance of microbial resistance in European Intensive Care Units: a first report from the Care-ICU programme for improved infection control. Intensive Care Med. 2008 Aug 1. [Epub ahead of print]
8. Yumuk Z, Afacan G, Nicolas-Chanoine MH, Sotto A, Lavigne JP. Turkey: a further country concerned by community-acquired Escherichia coli clone O25-ST131 producing CTX-M-15. J Antimicrob Chemother. 2008;62(2):284-8.
9. Andreu A, Planells I; Grupo Cooperativo Español para el Estudio de la Sensibilidad Antimicrobiana de los Patógenos Urinario. Etiology of community-acquired lower urinary infections and antimicrobial resistance of Escherichia coli: a national surveillance study. Med Clin (Barc). 2008;130(13):481-6.
10. Rodríguez-Baño J, Navarro MD, Romero L, Muniain MA, de Cueto M, Ríos MJ, et al. Bacteremia due to extended-spectrum beta-lactamase-producing Escherichia coli in the CTX-M era: a new clinical challenge. Clin Infect Dis.2006; 43(11):1407-14.
11. Melzer M, Petersen I. Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing E .coli compared to non-ESBL producing E. coli. J Infect. 2007;55(3):254-9.
12. Nicolas-Chanoine MH, Jarlier V; 'La Collégialé de Bactériologie-Virologie-Hygiène Hospitalière de l'Assistance Publique, Hôpitaux de Paris, France. Extended-spectrum beta-lactamases in long-term-care facilities. Clin Microbiol Infect. 2008 Jan;14 Suppl :111-6.
13. Valverde A, Coque TM, Sánchez-Moreno MP, Rollán A, Baquero F, Cantón R. Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae during nonoutbreak situations in Spain. J Clin Microbiol. 2004;42(10):4769-75.
14. Oteo J, Navarro C, Cercenado E, Delgado-Iribarren A, Wilhelmi I, Orden B, et al. Spread of Escherichia coli strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. J Clin Microbiol. 2006;44(7):2359-66.
15. Hernández JR, Martínez-Martínez L, Cantón R, Coque TM, Pascual A; Spanish Group for Nosocomial Infections (GEIH). Nationwide study of Escherichia coli and Klebsiella pneumoniae producing extended-spectrum beta-lactamases in Spain. Antimicrob Agents Chemother. 2005;49(5):2122-5.
16. Leflon-Guibout V, Blanco J, Amaqdouf K, Mora A, Guize L, Nicolas-Chanoine MH. Absence of CTX-M enzymes but a high prevalence of clones, including clone ST131, among the fecal Escherichia coli isolates of healthy subjects living in the Paris area. J Clin Microbiol. 2008 Oct 8. [Epub ahead of print]
17. Valverde A, Grill F, Coque TM, Pintado V, Baquero F, Cantón R, et al. High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. J Clin Microbiol. 2008;46(8):2796-9.
18. Rodríguez-Baño J, López-Cerero L, Navarro MD, Díaz de Alba P, Pascual A. Faecal carriage of extended-spectrum beta-lactamase-producing Escherichia coli: prevalence, risk factors and molecular epidemiology. J Antimicrob Chemother. 2008;62(5):1142-9.
19. Kjerulf A, Hansen DS, Sandvang D, Hansen F, Frimodt-Møller N. The prevalence of ESBL-producing E. coli and Klebsiella strains in the Copenhagen area of Denmark. APMIS 2008;116(2):118-24.
20. Tofteland S, Haldorsen B, Dahl KH, Simonsen GS, Steinbakk M, Walsh TR, et al. Effects of phenotype and genotype on methods for detection of extended-spectrum-beta-lactamase-producing clinical isolates of Escherichia coli and Klebsiella pneumoniae in Norway. J Clin Microbiol. 2007;45(1):199-205.
21. Fang H, Ataker F, Hedin G, Dornbusch K. Molecular epidemiology of extended-spectrum beta-lactamases among Escherichia coli isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. J Clin Microbiol. 2008;46(2):707-12.
22. Nyberg SD, Osterblad M, Hakanen AJ, Huovinen P, Jalava J, The Finnish Study Group For Antimicrobial Resistance. Detection and molecular genetics of extended-spectrum beta-lactamases among cefuroxime-resistant Escherichia coli and Klebsiella spp. isolates from Finland, 2002-2004. Scand J Infect Dis. 2007;39(5):417-24.
23. Lytsy B, Sandegren L, Tano E, Torell E, Andersson DI, Melhus A. The first major extended-spectrum beta-lactamase outbreak in Scandinavia was caused by clonal spread of a multiresistant Klebsiella pneumoniae producing CTX-M-15. APMIS. 2008;116(4):302-8.
24. Machado E, Coque TM, Cantón R, Novais A, Sousa JC, Baquero F, et al. High diversity of extended-spectrum beta-lactamases among clinical isolates of Enterobacteriaceae from Portugal. J Antimicrob Chemother. 2007;60(6):1370-4.
25. Romero L, López L, Rodríguez-Baño J, Ramón Hernández J, Martínez-Martínez L, Pascual A. Long-term study of the frequency of Escherichia coli and Klebsiella pneumoniae

isolates producing extended-spectrum beta-lactamases. Clin Microbiol Infect. 2005;11(8):625-31.

26. Mendonça N, Leitão J, Manageiro V, Ferreira E, Caniça M. Spread of extended-spectrum beta-lactamase CTX-M-producing Escherichia coli clinical isolates in community and nosocomial environments in Portugal. Antimicrob Agents Chemother. 2007; 51(6):1946-55.
27. Valverde A, Grill F, Coque TM, Pintado V, Baquero F, Cantón R, et al. High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. J Clin Microbiol. 2008;46(8):2796-9.
28. Luzzaro F, Mezzatesta M, Mugnaioli C, Perilli M, Stefani S, Amicosante G, et al. Trends in production of extended-spectrum beta-lactamases among enterobacteria of medical interest: report of the second Italian nationwide survey. J Clin Microbiol. 2006;44(5):1659-64.
29. Mugnaioli C, Luzzaro F, De Luca F, Brigante G, Perilli M, Amicosante G, et al. CTX-M-type extended-spectrum beta-lactamases in Italy: molecular epidemiology of an emerging countrywide problem. Antimicrob Agents Chemother. 2006;50(8):2700-6.
30. Carattoli A, García-Fernández A, Varesi P, Fortini D, Gerardi S, Penni A, et al. Molecular epidemiology of Escherichia coli producing extended-spectrum beta-lactamases isolated in Rome, Italy. Clin Microbiol. 2008;46(1):103-8.
31. Caccamo M, Perilli M, Celenza G, Bonfiglio G, Tempera G, Amicosante G. Occurrence of extended spectrum beta-lactamases among isolates of Enterobacteriaceae from urinary tract infections in southern Italy. Microb Drug Resist. 2006;12(4):257-64.
32. Galas M, Decousser JW, Breton N, Godard T, Allouch PY, Pina P; et al. Nationwide study of the prevalence, characteristics, and molecular epidemiology of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in France. Antimicrob Agents Chemother. 2008;52(2):786-9.
33. Arpin C, Coulange L, Dubois V, André C, Fischer I, Fourmaux S, et al. Extended-spectrum-beta-lactamase-producing Enterobacteriaceae strains in various types of private health care centers. Antimicrob Agents Chemother. 2007;51(9):3440-4.
34. Yates CM, Brown DJ, Edwards GF, Amyes SG. Detection of TEM-52 in Salmonella enterica serovar Enteritidis isolated in Scotland. J. Antimicrob. Chemother. 2004;53(2):407-8.
35. Empel J, Baraniak A, Literacka E, Mrówka A, Fiett J, Sadowy E, et al. Molecular survey of beta-lactamases conferring resistance to newer beta-lactams in Enterobacteriaceae isolates from Polish hospitals. Antimicrob Agents Chemother 2008;52(7):2449-54.
36. Damjanova I, Tóth A, Pászti J, Hajbel-Vékony G, Jakab M, Berta J, et al. Expansion and countrywide dissemination of ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-type {beta}-lactamase-producing Klebsiella pneumoniae epidemic clones in Hungary in 2005--the new 'MRSAs'?. J Antimicrob Chemother. 2008;62(5):978-85.
37. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L. Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in Russian hospitals. Antimicrob Agents Chemother. 2003;47(12):3724-32.
38. Damjanova I, Tóth A, Pászti J, Jakab M, Milch H, Bauernfeind A, et al. Epidemiology of SHV-type β-lactamase-producing Klebsiella spp. from outbreaks in five geographically distant Hungarian neonatal intensive care units: widespread dissemination of epidemic R-plasmids. Int J Antimicrob Agents. 2007;29(6):665–71
39. Korten V, Ulusoy S, Zarakolu P, Mete B; Turkish MYSTIC Study Group. Antibiotic resistance surveillance over a 4-year period (2000-2003) in Turkey: results of the MYSTIC Program.Diagn Microbiol Infect Dis. 2007;59(4):453-7.
40. Markovska R, Schneider I, Keuleyan E, Sredkova M, Ivanova D, Markova B, et al. Extended-spectrum beta-lactamase-producing Enterobacteriaceae in Bulgarian hospitals. Microb Drug Resist. 2008;14(2):119-28.
41. Baraniak A, Fiett J, Sulikowska A, Hryniewicz W, Gniadkowski M. Countrywide spread of CTX-M-3 extended-spectrum beta-lactamase-producing microorganisms of the family Enterobacteriaceae in Poland. Antimicrob Agents Chemother. 2002;46(1):151-9.
42. Baraniak A, Fiett J, Mrówka A, Walory J, Hryniewicz W, Gniadkowski M. Evolution of TEM-type extended-spectrum beta-lactamases in clinical Enterobacteriaceae strains in Poland. Antimicrob Agents Chemother. 2005;49(5):1872-80.
43. Carrër A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. Spread of OXA-48-positive carbapenem-resistant Klebsiella pneumoniae isolates in Istanbul, Turkey. Antimicrob Agents Chemother. 2008;52(8):2950-4.
44. Lau SH, Kaufmann ME, Livermore DM, Woodford N, Willshaw GA, Cheasty T, et al. UK epidemic Escherichia coli strains A-E, with CTX-M-15 {beta}-lactamase, all belong to the international O25:H4-ST131 clone. J Antimicrob Chemother. 2008;62(6):1241-4.
45. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Caniça MM, et al. Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother. 2008;61(2):273-81.
46. Cagnacci S, Gualco L, Debbia E, Schito GC, Marchese A. European emergence of ciprofloxacin-resistant Escherichia coli clonal groups O25:H4-ST 131 and O15:K52:H1 causing community-acquired uncomplicated cystitis. J Clin Microbiol. 2008;46(8):2605-12.
47. Valverde A, Cantón R, Novais A, Gal,an JC, Baquero F, Coque TM. Local Spread of CTX-M-14 in Madrid (Spain) is linked to plasmids of the IncI-complex disseminated among different Escherichia coli genetic backgrounds. 2008 (submitted)
48. Oteo J, Garduño E, Bautista V, Cuevas O, Campos J; Spanish members of European Antimicrobial Resistance Surveillance System. Antibiotic-resistant Klebsiella pneumoniae in Spain: analyses of 718 invasive isolates from 35 hospitals and report of one outbreak caused by an SHV-12-producing strain. J Antimicrob Chemother. 2008;61(1):222-4.
49. Psichogiou M, Tassios PT, Avlamis A, Stefanou I, Kosmidis C, Platsouka E, et al. Ongoing epidemic of blaVIM-1-positive Klebsiella pneumoniae in Athens, Greece: a prospective survey. J Antimicrob Chemother. 2008;61(1):59-63.
50. Uzunovic-Kamberovic S, Bedenic B, Vranes J. Predominance of SHV-5 beta-lactamase in enteric bacteria causing community-acquired urinary tract infections in Bosnia and Herzegovina. Clin Microbiol Infect. 2007;13(8):820-3.
51. Novais A, Cantón R, Machado E, Curiao T, Baquero F, Peixe L, Coque TM. International dissemination of a multi-resistant IncA/C2 plasmid containing blaTEM-24, Tn21 and Tn1696 among epidemic and non-epidemic Enterobacteriaceae species. 18th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) Barcelona, April 2008. Abstract available from: http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=62501&XNSPRACHE_ID=2&XNKONGRESS_ID=73&XNMASKEN_ID=900
52. Tsakris A, Kristo I, Poulou A, Markou F, Ikonomidis A, Pournaras S. First occurrence of KPC-2-possessing Klebsiella pneumoniae in a Greek hospital and recommendation for detection with boronic acid disc tests. J Antimicrob Chemother. 2008;62(6):1257-60.
53. Tato M, Coque TM, Ruíz-Garbajosa P, Pintado V, Cobo J, Sader HS, et al. Complex clonal and plasmid epidemiology in the first outbreak of Enterobacteriaceae infection involving VIM-1 metallo-beta-lactamase in Spain: toward endemicity? Clin Infect Dis. 2007;45(9):1171-8.
54. Cagnacci S, Gualco L, Roveta S, Mannelli S, Borgianni L, Docquier JD, et al. Bloodstream infections caused by multidrug-resistant Klebsiella pneumonia producing the carbapenem-hydrolysing VIM-1 metallo-beta-lactamase: first Italian outbreak.J Antimicrob Chemother. 2008;61(2):296-300.
55. Carattoli A. Animal reservoirs for extended spectrum beta-lactamase producers. Clin Microbiol Infect. 2008;14 Suppl 1:117-23.
56. Costa D, Poeta P, Sáenz Y, Vinué L, Rojo-Bezares B, Jouini A, et al. Detection of Escherichia coli harbouring extended-spectrum beta-lactamases of the CTX-M, TEM and SHV classes in faecal samples of wild animals in Portugal. J. Antimicrob. Chemother. 2006;58(6):1311-2.
57. Briñas L, Moreno MA, Teshager T, Sáenz Y, Porrero MC, Domínguez L, et al. Monitoring and characterization of extended-spectrum β-lactamases in Escherichia coli strains from healthy and sick animals in Spain in 2003. Antimicrob. Agents Chemother. 2003;49(3):1262-4.
58. Machado E, Coque TM, Cantón R, Sousa JC, Peixe L. Antibiotic resistance integrons and extended-spectrum {beta}-lactamases among Enterobacteriaceae isolates recovered from chickens and swine in Portugal. J Antimicrob Chemother. 2008;62(2):296-302.
59. Warren RE, Ensor VM, O'Neill P, Butler V, Taylor J, Nye K, et al. Imported chicken meat as a potential source. J Antimicrob Chemother. 2008;61(3):504-8.
60. Aarestrup FM, Hasman H, Agersø Y, Jensen LB, Harksen S, Svensmark B. First description of blaCTX-M-1-carrying Escherichia coli isolates in Danish primary food production. J Antimicrob Chemother. 2006;57(6):1258-9.
61. Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. β-Lactamases among extended-spectrum β-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and

human patients in The Netherlands. J. Antimicrob. Chemother. 2005;56(1):115-21.

62. Smet A, Martel A, Persoons D, Dewulf J, Heyndrickx M, Catry B, et al. Diversity of extended-spectrum beta-lactamases and class C beta-lactamases among cloacal Escherichia coli Isolates in Belgian broiler farms. Antimicrob Agents Chemother. 2008;52(4):1238-43.
63. Chiaretto G, Zavagnin P, Bettini F, Mancin M, Minorello C, Saccardin C, et al. 2008. Extended spectrum beta-lactamase SHV-12-producing Salmonella from poultry.Vet Microbiol. 2008;128(3-4):406-13.
64. Girlich D, Poirel L, Carattoli A, Kempf I, Lartigue MF, Bertini A, et al. Extended-spectrum beta-lactamase CTX-M-1 in Escherichia coli isolates from healthy poultry in France. Appl Environ Microbiol. 2007;73(14):4681-5
65. Sunde M, Tharaldsen H, Slettemeås JS, Norström M, Carattoli A, Bjorland J. Escherichia coli of animal origin in Norway contains a blaTEM-20-carrying plasmid closely related to blaTEM-20 and blaTEM-52 plasmids from other European countries. J Antimicrob Chemother. 2008 Oct 29. [Epub ahead of print].
66. Riaño I, Moreno MA, Teshager T, Sáenz Y, Domínguez L, Torres C. Detection and characterization of extended-spectrum beta-lactamases in Salmonella enterica strains of healthy food animals in Spain.J Antimicrob Chemother. 2006;58(4):844-7.
67. Cavaco LM, Abatih E, Aarestrup FM, Guardabassi L. Selection and persistence of CTX-M-producing Escherichia coli in the intestinal flora of pigs treated with amoxicillin, ceftiofur, or cefquinome. Antimicrob Agents Chemother. 2008;52(10):3612-6.
68. Bertrand S, Weill FX, Cloeckaert A, Vrints M, Mairiaux E, Praud K, et al. Clonal emergence of extended-spectrum beta-lactamase (CTX-M-2)-producing Salmonella enterica serovar Virchow isolates with reduced susceptibilities to ciprofloxacin among poultry and humans in Belgium and France (2000 to 2003).J Clin Microbiol. 2006;44(8):2897-903.
69. Weill FX, Lailler R, Praud K, Kérouanton A, Fabre L, Brisabois A, et al. Cloeckaert. Emergence of extended-spectrum-ß-lactamase (CTX-M-9)-producing multiresistant strains of Salmonella enterica serotype Virchow in poultry and humans in France. J. Clin. Microbiol. 2004;42(12):5767-73.
70. Cloeckaert A, Praud K, Doublet B, Bertini A, Carattoli A, Butaye P, et al. Dissemination of an extended-spectrum-β-lactamase blaTEM-52 gene-carrying IncI1 plasmid in various Salmonella enterica serovars isolated from poultry and humans in Belgium and France. Antimicrob. Agents Chemother. 2007;51(5):1872-5.
71. Vo AT, van Duijkeren E, Fluit AC, Gaastra W. Characteristics of extended-spectrum cephalosporin-resistant Escherichia coli and Klebsiella pneumoniae isolates from horses. Vet Microbiol. 2007;124(3-4):248-55.
72. Politi L, Tassios PT, Lambiri M, Kansouzidou A, Pasiotou M, Vatopoulos AC, et al. Repeated occurrence of diverse extended-spectrum beta-lactamases in minor serotypes of food-borne Salmonella enterica subsp. enterica. J Clin Microbiol. 2005;43(7):3453-6.
73. Hopkins KL, Liebana E, Villa L, Batchelor M, Threlfall EJ, Carattoli A. Replicon typing of plasmids carrying CTX-M or CMY beta-lactamases circulating among Salmonella and Escherichia coli isolates. Antimicrob Agents Chemother. 2006;50(9):3203-6.
74. Lavilla S, González-López JJ, Miró E, Domínguez A, Llagostera M, Bartolomé RM, et al. Dissemination of extended-spectrum beta-lactamase-producing bacteria: the food-borne outbreak lesson. J Antimicrob Chemother. 2008;61(6):1244-51.
75. Liebana E, Batchelor M, Hopkins KL, Clifton-Hadley FA, Teale CJ, Foster A, et al. Longitudinal farm study of extended-spectrum beta-lactamase-mediated resistance. J Clin Microbiol. 2006;44(5):1630-4.
76. Pedrosa A, Novais A, Machado E, Cantón R, Peixe L, Coque TM. Recent dissemination of blaTEM-52 producing Enterobacteriaceae in Portugal is caused by spread of IncI plasmids among Escherichia coli and Klebsiella clones. XVIII European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) Barcelona, April 2008. Abstract available from: http://registration.akm.ch/einsicht.php?XNABSTRACT_ID=66706&XNSPRACHE_ID=2&XNKONGRESS_ID=73&XNMASKEN_ID=900
77. Jensen LB, Hasman H, Agersø Y, Emborg HD, Aarestrup FM. First description of an oxyimino-cephalosporin-resistant, ESBL-carrying Escherichia coli isolated from meat sold in Denmark. J. Antimicrob. Chemother. 2006;57(4):793-4.
78. Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related Escherichia coli strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerg Infect Dis. 2008;14(2):195-200.
79. Jones GL, Warren RE, Skidmore SJ, Davies VA, Gibreel T, Upton M. Prevalence and distribution of plasmid-mediated quinolone resistance genes in clinical isolates of Escherichia coli lacking extended-spectrum {beta}-lactamases.J Antimicrob Chemother. 2008;62(6):1245-51.
80. Kassis-Chikhani N, Decré D, Gautier V, Burghoffer B, Saliba F, Mathieu D, et al. First outbreak of multidrug-resistant Klebsiella pneumoniae carrying blaVIM-1 and blaSHV-5 in a French university hospital. J Antimicrob Chemother. 2006;57(1):142–5.
81. Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J Clin Microbiol. 2005;43(8):4178–82.
82. Duarte A, Boavida F, Grosso F, Correia M, Lito LM, Cristino JM, et al. Outbreak of GES-1 beta-lactamase-producing multidrug-resistant Klebsiella pneumoniae in a university hospital in Lisbon, Portugal. Antimicrob Agents Chemother. 2003;47(4):1481-2.
83. Dumarche P, De Champs C, Sirot D, Chanal C, Bonnet R, Sirot J. TEM derivative-producing Enterobacter aerogenes strains: dissemination of a prevalent clone. Antimicrob Agents Chemother. 2002;46(4):1128-31.
84. Biendo M, Canarelli B, Thomas D, Rousseau F, Hamdad F, Adjide C, et al. Successive emergence of extended-spectrum beta-lactamase-producing and carbapenemase-producing Enterobacter aerogenes isolates in a university hospital. J Clin Microbiol. 2008;46(3):1037-44.
85. Paauw A, Verhoef J, Fluit AC, Blok HE, Hopmans TE, Troelstra A, et al. Failure to control an outbreak of qnrA1-positive multidrug-resistant Enterobacter cloacae infection despite adequate implementation of recommended infection control measures. J Clin Microbiol. 2007;45(5):1420-5.
86. Diestra K, Juan C, Curiao T, Moyá B, Miró E, Oteo J, et al. Characterisation of plasmids encoding blaESBL and surrounding genes in Spanish clinical isolates of Escherichia coli and Klebsiella pneumoniae. J Antimicrob Chemother. 2008 Nov 6. [Epub ahead of print].
87. Novais A, Cantón R, Moreira R, Peixe L, Baquero F, Coque TM. Emergence and dissemination of Enterobacteriaceae isolates producing CTX-M-1-like enzymes in Spain are associated with IncFII (CTX-M-15) and broad-host-range (CTX-M-1, -3, and -32) plasmids. Antimicrob Agents Chemother. 2007;51(2):796-9.
88. Gonullu N, Aktas Z, Kayacan CB, Salcioglu M, Carattoli A, Yong DE, et al. Dissemination of CTX-M-15 beta-lactamase genes carried on Inc FI and FII plasmids among clinical isolates of Escherichia coli in a university hospital in Istanbul, Turkey. J Clin Microbiol. 2008;46(3):1110-2.
89. García-Fernández A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A, et al. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum beta-lactamases in Escherichia coli and Salmonella of human and animal origin. J Antimicrob Chemother. 2008;61(6):1229-33.
90. Lautenbach E, Strom BL, Bilker WB, Patel JB, Edelstein PH, Fishman NO. Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae. Clin Infect Dis. 2001;33(8):1288-94.
91. Morosini MI, García-Castillo M, Coque TM, Valverde A, Novais A, Loza E, et al. Antibiotic co-resistance in extended-spectrum-beta-lactamase-producing Enterobacteriaceae and in vitro activity of tigecycline. Antimicrob Agents Chemother. 2006;50(8):2695-9.
92. Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. J Antimicrob Chemother. 2005;56(3):463-9.
93. Cattoir V, Poirel L, Nordmann P. Plasmid-mediated quinolone resistance pump QepA2 in an Escherichia coli isolate from France. Antimicrob Agents Chemother. 2008;52(10):3801-4.
94. Galimand M, Sabtcheva S, Courvalin P, Lambert T. Worldwide disseminated armA aminoglycoside resistance methylase gene is borne by composite transposon Tn1548.Antimicrob Agents Chemother. 2005;49(7):2949-53.
95. Novais A, Cantón R, Valverde A, Machado E, Galán JC, Peixe L, et al. Dissemination and persistence of blaCTX-M-9 are linked to class 1 integrons containing CR1 associated with defective transposon derivatives from Tn402 located in early antibiotic resistance plasmids of IncHI2, IncP1-alpha, and IncFI groups. Antimicrob Agents Chemother. 2006;50(8):2741-50.
96. Machado E, Ferreira J, Novais A, Peixe L, Cantón R, Baquero F, et al. Preservation of integron types among Enterobacteriaceae producing extended-spectrum beta-lactamases in a Spanish hospital over a 15-year period (1988 to 2003). Antimicrob Agents Chemother. 2007;51(6):2201-4.
97. Enne VI, Delsol AA, Roe JM, Bennett PM. Evidence of antibiotic resistance gene silencing in Escherichia coli.Antimicrob Agents Chemother. 2006;50(9):3003-10.

98. García Fernández A, Cloeckaert A, Bertini A, Praud K, Doublet B, Weill FX, et al. Comparative analysis of IncHI2 plasmids carrying blaCTX-M-2 or blaCTX-M-9 from Escherichia coli and Salmonella enterica strains isolated from poultry and humans. Antimicrob Agents Chemother. 2007;51(11):4177-80.

99. Gołebiewski M, Kern-Zdanowicz I, Zienkiewicz M, Adamczyk M, Zylinska J,Baraniak A, et al. Complete nucleotide sequence of the pCTX-M3 plasmid and its involvement in spread of the extended-spectrum beta-lactamase gene blaCTX-M-3. Antimicrob Agents Chemother. 2007;51(11):3789-95.

100. Zienkiewicz M, Kern-Zdanowicz I, Gołebiewski M, Zyliñska J, Mieczkowski P,Gniadkowski M, et al. Mosaic structure of p1658/97, a 125-kilobase plasmid harboring an active amplicon with the extended-spectrum beta-lactamase gene blaSHV-5.Antimicrob Agents Chemother. 2007;51(4):1164-71.

New influenza A(H1N1) virus infections in Spain, April-May 2009

Surveillance Group for New Influenza A(H1N1) Virus Investigation and Control in Spain[1,2,3,4]
1. Coordinating Centre for Health Alerts and Emergencies, Spanish Ministry of Health and Social Policy, Madrid, Spain
2. National Centre for Epidemiology and National Centre for Microbiology, Instituto de Salud Carlos III, Madrid, Spain
3. Regional Surveillance and Alert Teams from the Autonomous Communities in Spain
4. National Influenza Laboratory Network, Spain

Correspondence: P Santa-Olalla (psantaolalla@msc.es)

Citation style for this article:
Surveillance Group for New Influenza A(H1N1) Virus Investigation and Control in Spain. New influenza A(H1N1) virus infections in Spain, April-May 2009. Euro Surveill. 2009;14(19):pii=19209. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19209

Article published on 14 May 2009

An outbreak of infections with a new influenza A(H1N1) virus that was first detected in the United States and Mexico is currently ongoing worldwide. This report describes the initial epidemiological actions and outbreak investigation of the first 98 laboratory confirmed cases of infection with this new virus in Spain.

Background

On 25 April 2009, the World Health Organization (WHO) declared the outbreak of swine-origin influenza A(H1N1) virus infections, first reported by the United States (US) [1] and Mexico [2], as a 'Public Health Event of International Concern' (PHEIC) under the International Health Regulations (2005) [3]. The pandemic alert level was raised from level 3 to level 4 on 27 April, and to level 5 on 29 April, after verification of sustained community-level outbreaks in at least two countries from the same WHO region.

On 26 April, epidemiological and laboratory investigations on three persons returning from Mexico were initiated in Spain. On 27 April, Spain reported the first laboratory-confirmed case of the new influenza A(H1N1) virus infection in Europe, in a traveller returning from Mexico. Since then, the number of confirmed cases in Spain has risen continuously and reached a total of 98 as of 11 May 2009.

Enhanced surveillance

On 24 April, in response to alarming reports from the US of swine-origin influenza A(H1N1) virus infection in several patients [1,4] and media news of a possibly related outbreak of severe respiratory illness in Mexico, the Coordinating Centre for Health Alerts and Emergencies (CCAES) at the Spanish Ministry of Health and Social Policy, issued a national epidemiologic alert. The alert asked public health authorities at national and regional level to enhance surveillance and to report urgently any case of fever and severe respiratory illness among people with history of travel to Mexico or history of previous contact with a confirmed case of influenza virus A(H1N1) infection (Table 1).

On 25 April, following WHO's declaration of a PHEIC, the National Pandemic Influenza Preparedness and Response Plan was activated. A case definition as well as protocols for case and contact management and for infection control were developed and distributed to the National Health Service through regional health authorities and other involved institutions (Table 2).

No increase in seasonal influenza activity has been reported so far. Routine seasonal influenza surveillance will continue beyond week 20. Data analysis of mortality for all causes since 1 May has not shown an increase or change of patterns in mortality.

Since 24 April, the outbreak of new influenza A(H1N1) has been monitored by the Ministry of Health and Social Policy (Centro de Coordinación de Alertas y Emergencias Sanitarias, CCAES) jointly with the National Centre for Epidemiology (Instituto de Salud Carlos III) and in coordination with all the Regional Surveillance and Alert Teams from the Autonomous Communities in Spain. This new influenza A(H1N1) investigation and control group also discusses and recommends prevention and control measures.

Confirmed cases of new influenza virus A(H1N1)

As of 11 May, 98 laboratory-confirmed cases of infection with the new influenza virus A(H1N1) have been reported in Spain out of 640 possible cases investigated. The geographical distribution of reported cases by region is shown in Figure 1.

Seventy-six confirmed cases (78%) acquired the infection abroad; all these cases had a history of travel to Mexico. Of the 45 cases for whom this information was available, 16 (36%) were symptomatic during

Figure 1

Geographical distribution of cases of laboratory-confirmed new influenza virus A(H1N1) infection, Spain, as of 11 May 2009

Figure 2

Cases of laboratory-confirmed new influenza virus A(H1N1) infection, by date of travel return to Spain, as of 11 May, 2009 (n=70)

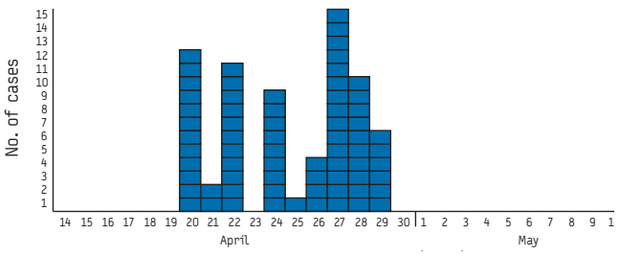
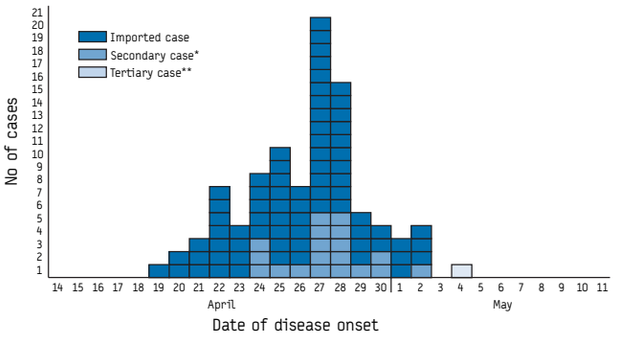


Figure 3

Cases of laboratory-confirmed new Influenza virus A(H1N1) infection, by date of disease onset, Spain, as of 11 May 2009 (n=93)



* contact of a confirmed imported case

** contact of a confirmed secondary case

the inbound flight from Mexico. Dates of return from affected areas were available for 70 confirmed cases and ranged from 20 to 29 April (Figure 2).

Information on disease onset was available for 93 cases. The first of the 93 cases reported onset of

Table 1

Timeline of key events in detection and response to the new influenza A(H1N1) virus outbreak, Spain, 24 April-11May 2009

Date	Event
24 April	Alert issued to enhance surveillance at the public health services and national health system
24 April	Information for the public and recommendations for travellers going to and returning from Mexico published on the website of the Spanish Ministry of Health and Social Policy
25 April	National pandemic influenza preparedness and response plan activated.
25 April	Case definition, case and contact management, and infection control protocols distributed
26 April	Notification of the first three cases under investigation
27 April	First laboratory-confirmed case of new influenza A(H1N1) virus infection reported.
27 April	Ministry of Health recommends avoiding non-essential travel to Mexico
27 April	World Health Organization raises pandemic alert to phase 4
29 April	World Health Organization raises pandemic alert to phase 5
29 April	First secondary case of new influenza A(H1N1) virus reported
1 May	Regional influenza laboratories to start initial testing; National reference laboratory to confirm
7 May	New case definition approved, including the United States as an affected area, reducing incubation period (seven days) and establishing fever cut off at 38ºC
11 May	First laboratory-confirmed tertiary case
11 May	Status: 98 laboratory confirmed cases of new Influenza virus A(H1N1) infection

Table 2

Case definition and case classification, new influenza A(H1N1) infection, Spain, 25 April-7 May, 2009

	Incubation period 10 days
Clinical criteria	Any person with ONE of the following: • Fever (≥ 37.5 °C)* AND signs or symptoms of acute respiratory infection • Pneumonia • Death from an unexplained acute respiratory illness
Epidemiological criteria	At least ONE of the following in the 10 days* prior to disease onset: • Travel to an area where there are confirmed cases of new influenza A(H1N1) (Mexico*) • Close contact to a confirmed case of new influenza A(H1N1) virus infection • Recent history of contact with an animal with confirmed or suspected swine influenza A(H1N1) virus infection (This criterion was substituted on 27 April for: "A person employed at a laboratory and manipulating potentially contaminated samples").
Laboratory criteria	At least ONE of the following tests: • RT-PCR • Four-fold rise in new influenza A(H1N1) virus-specific neutralizing antibodies (implies the need for paired sera, at least from acute phase illness and then at convalescent stage 10-14 days later) • Viral culture
Case classification	A. Case under investigation Any person meeting clinical AND epidemiological criteria B. Probable case Any person meeting clinical AND epidemiological criteria AND with a positive influenza A infection of an unsubtypable type C. Confirmed case Any person with laboratory confirmation*

* Differences to proposed case from the European Centre for Disease Prevention and Control.

Table 3

Clinical features of confirmed cases for new influenza virus A(H1N1) infection, Spain, as of 11 May 2009

Symptom	Cases with symptom/ cases for whom information is available	Percentage
Fever (≥37.5 °C)	87 / 91	96%
Cough	83 / 87	95%
Headache	27 / 44	61%
Coryza	24 / 41	59%
Sore throat	29 / 48	60%
Myalgia	29 / 49	59%
Shortness of breath	18 / 70	26%
Malaise	23 / 38	61%
Diarrhoea	17 / 41	41%
Vomiting	4 / 32	13%

illness (any symptom) on 19 April, and the most recent case reported onset on 4 May (Figure 3).

More than 2,000 contacts have been traced and followed. Of these, 39% were household members of cases and 45% friends of cases. Twenty-one confirmed secondary cases and one tertiary case have been reported. Secondary cases were family or close contacts of cases with history of travel to Mexico. Five secondary cases were infected by primary cases that did not meet clinical criteria. The tertiary case was a family contact of a secondary case. Analysis of secondary transmission is ongoing.

Four secondary cases had received prophylaxis with oseltamivir before being diagnosed as cases.

From the analysis of disease onset for primary and secondary cases, the median of the serial interval was estimated to be 3.5 days, ranging from one to six days. The estimation for the maximum incubation period ranged from one to seven days, with a median of three days.

Demographic and clinical features

Cases ranged in age from 14 to 55 years, with an average of 24 years (standard deviation (SD) 6.3) and a median of 22; 50 (51%) cases were male.

The most frequently reported symptoms were fever (96%) and cough (95%). Four cases did not have fever. Among 41 cases for whom this information was available, 17 (41%) reported diarrhoea (Table 3).

No deaths have been reported. Disease presentation has been described as a mild influenza-like illness with full recovery in all cases. Some cases were hospitalised at the beginning of the outbreak for respiratory isolation following the national pandemic preparedness plan, this procedure having no association with illness severity.

No differences in disease presentation have been described for secondary cases. No pregnancies among confirmed cases have been reported.

Information on seasonal influenza 2008-9 vaccine status is available for 52 cases (53%); of these, only five cases had history of vaccination.

Laboratory confirmation

Nose and throat swabs from cases who met clinical and epidemiological criteria were taken and referred to the national influenza reference laboratory (WHO National Influenza Centre) at the Instituto de Salud Carlos III for confirmation. Two independent assays have been used for diagnosis; a reverse transcription (RT)-nested PCR designed for typing the nucleoprotein gene and another one for subtyping the haemagglutinin gene. An alternative RT-PCR was done in case the first two PCR gave contradictory results. Amplified products were sequenced and a phylogenetic analysis was done to identify the new A (H1N1) virus. The strain identified in all cases was confirmed as genetically similar to viruses previously isolated from cases in California (A/California/04/2009).

Detailed information on co-infection with other respiratory viruses is pending. Virological studies on antiviral sensitivity and on molecular-level indicators of severity are ongoing.

Discussion

Spain was the first country in Europe to report a laboratory-confirmed case of new influenza A(H1N1) virus infection. Several factors may have contributed: intense air traffic and contacts with Mexico [5] but also a timely alert with high media coverage that raised early awareness among public health and healthcare professionals, as well as among the public.

An extremely efficient surveillance system and a sensitive case definition that was distributed early in the event made it possible to detect cases at the very beginning of the outbreak and to trace more than 2,000 close contacts. Secondary cases have been identified among close contacts of the first reported cases. However, they are still only a minor percentage of all reported cases and further spread of this new influenza virus into the community has not been documented. The last imported case had disease onset on 2 May, but the change in the case definition on 7 May including the US as an affected area may lead to notification of new imported cases.

The preliminary findings from the analysis of the first 98 laboratory-confirmed cases of the new influenza A(H1N1) virus infection in Spain indicate that symptoms in these cases appear to be similar to those of seasonal influenza. Cases observed are mainly distributed among young adults, reflecting the age structure of returning travellers from Mexico. This group has no risk factors for influenza complications and is difficult at this stage to assess the potential severity of this virus. For the time being, the impact of this outbreak on the healthcare services has been negligible.

Conclusion

The evolution of this outbreak of influenza A(H1N1) in Spain is difficult to predict. Though notification of new confirmed cases has decreased and the disease seems mild, we will continue monitoring changes in the epidemiology and/or clinical severity of new influenza A(H1N1) virus infections in Spain in order to implement appropriate prevention and control measures.

References

1. Centers for Disease Control and Prevention (CDC). Swine Influenza A(H1N1) infections— California and Texas, April 2009. MMWR Morb Mortal Wkly Rep. 2009;58:(16):435-7. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5816a7.htm
2. Centers for Disease Control and Prevention (CDC). Outbreak of swine-origin influenza A(H1N1) virus infection- Mexico, March-April 2009. MMWR Morb Mortal Wkly Rep. 2009;58(Dispatch):1-3. Available from: www.cdc.gov/mmwr/preview/mmwrhtml/mm58d0430a2.htm
3. World Health Organization. International health regulations (2005). 2nd ed. Geneva: World Health organization; 2008. Available from: http://www.who.int/ihr/9789241596664/en/index.html
4. Centers for Disease Control and Prevention (CDC). Swine Influenza A(H1N1) infection in two children—Southern California, March-April 2009. MMWR Morb Mortal Wkly Rep. 2009; 58(15):400-2. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5815a5.htm
5. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, D'eirdre Hollingsworth T, et al. Pandemic Potential of a Strain of Influenza A (H1N1): Early Findings Science Express 11 May 2009. DOI: 10.1126/science.1176062. Available from: http://www.sciencemag.org/cgi/content/abstract/1176062v1

Spotlight on measles 2010: An epidemiological overview of measles outbreaks in Poland in relation to the measles elimination goal

J Rogalska (jrogalska@pzh.gov.pl)[1], S Santibanez[2], A Mankertz[2], A Makowka[3], L Szenborn[4], P Stefanoff[1]
1. Department of Epidemiology, National Institute of Public Health – National Institute of Hygiene (NIZP-PZH), Warsaw, Poland
2. Regional Reference Laboratory WHO EURO, Robert Koch Institute, Berlin, Germany
3. Department of Virology, National Institute of Public Health – National Institute of Hygiene (NIZP-PZH), Warsaw, Poland
4. Department of Pediatric Infectious Diseases, Medical University, Wrocław, Poland

Citation style for this article:
Citation style for this article: Rogalska J, Santibanez S, Mankertz A, Makowka A, Szenborn L, Stefanoff P. Spotlight on measles 2010: An epidemiological overview of measles outbreaks in Poland in relation to the measles elimination goal. Euro Surveill. 2010;15(17):pii=19549. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19549

This article has been published on 29 April 2010

The objective of this study was to describe transmission chains of measles observed in Poland during 2008-2009. A decade ago, the incidence of measles in Poland declined and approached one case per million inhabitants one of the World Health Organization's criteria for measles elimination. Following a period of very few reported measles cases (2003 to 2005), an increase in incidence was observed in 2006. Since then, the incidence has constantly exceeded one case per million inhabitants. Of 214 measles cases reported in 2008 and 2009 in Poland, 164 (77%) were linked to 19 distinct outbreaks, with 79% of cases belonging to the Roma ethnic group. Outbreaks in the non-Roma Polish population had different dynamics compared to those in the Roma population. On average, measles outbreaks in Roma communities involved 10 individuals, seven of whom were unvaccinated, while outbreaks in the non-Roma Polish population involved five individuals, half of whom were incompletely vaccinated. The majority of outbreaks in Roma communities were related to importation of virus from the United Kingdom. In six outbreaks, the epidemiologic investigation was confirmed by identification of genotype D4 closely related to measles viruses detected in the United Kingdom and Germany. Our data indicate that Poland is approaching measles elimination, but measles virus circulation is still sustained in a vulnerable population. More efforts are needed to integrate the Roma ethnic group into the Polish healthcare system and innovative measures to reach vulnerable groups should be explored.

Background

In 1998 Poland implemented a measles elimination programme, coordinated by the World Health Organization (WHO) Regional Office for Europe. It requires monitoring consecutive stages of the elimination by tracking secondary outbreak cases, genotyping of detected measles viruses (MV) and serological testing of all suspected cases of measles [1].

Measles has been a notifiable disease in Poland since 1919. National case-based notification was initiated in 1996 and WHO case definitions [2] have been adopted. Since 2005, the case classification of the European Union [3] has been used. The first dose of the monovalent measles vaccine for children aged 13-15 months was introduced in Poland in 1975, and the second dose for seven year old children was implemented in 1991. In 2005 the monovalent measles vaccine was replaced by the combined measles-mumps-rubella (MMR) vaccine, administered at the age of 13-15 months and 10 years.

Poland belongs to the European countries with moderate incidence of measles [4,5]. Following the introduction of routine immunisation, the incidence of measles has decreased. From 2003 to 2005 the number of locally acquired cases in Poland was below the elimination threshold of one case per million inhabitants. Since 2006 the measles incidence has increased and remained continuously above this elimination indicator (Figure 1) [6]. In 2006, measles cases were mostly related to importation of MV-D4, whereas MV-D6 was detected in 2007. In 2008-2009 a substantial increase in the frequency of outbreak-related cases was observed, often related to importation.

The vaccine coverage in Poland with MMR vaccine remains well above the target of >95% for the first dose of measles vaccine (MCV1), another WHO marker for measles elimination [7]. Coverage with the first dose of MMR vaccine in three-year-olds in 2008 was 98.4%, and for two doses of MMR in eleven-year-olds it was 97.2%. Information on measles vaccine coverage in ethnic groups such as the Roma ethnic minority is not available in Poland.

The objective of this study was to describe the patterns of chains of transmission investigated in Poland between 1999 and 2009, with special focus on 2008-2009, in relation to the measles elimination goal.

Methods

In the present study, measles cases reported within the Polish enhanced measles surveillance between 1999 and 2009 were investigated. Physicians were required to report all suspected measles cases to the local health departments and to obtain samples for confirmatory IgM testing. The information collected during case investigation included demographic characteristics, vaccination status, and clinical and laboratory data. Although not routinely collected in the national surveillance system, the ethnic background

Figure 1

Secular trends of measles incidence in Poland, 1966-2009

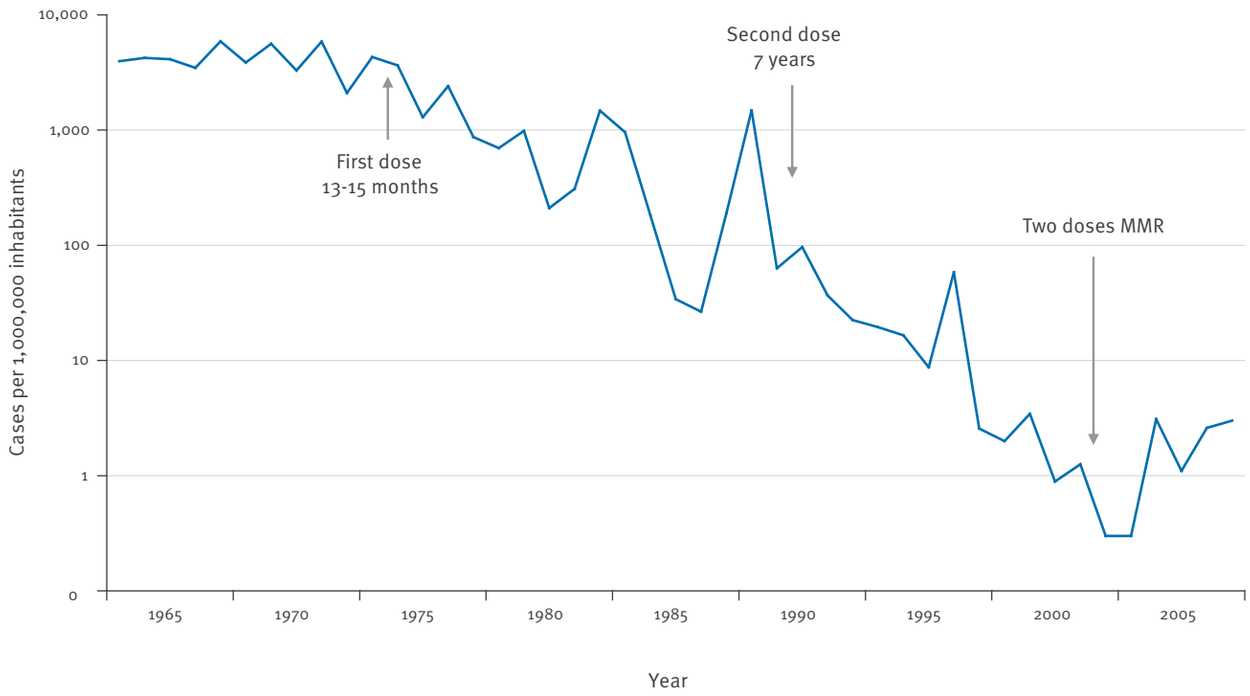
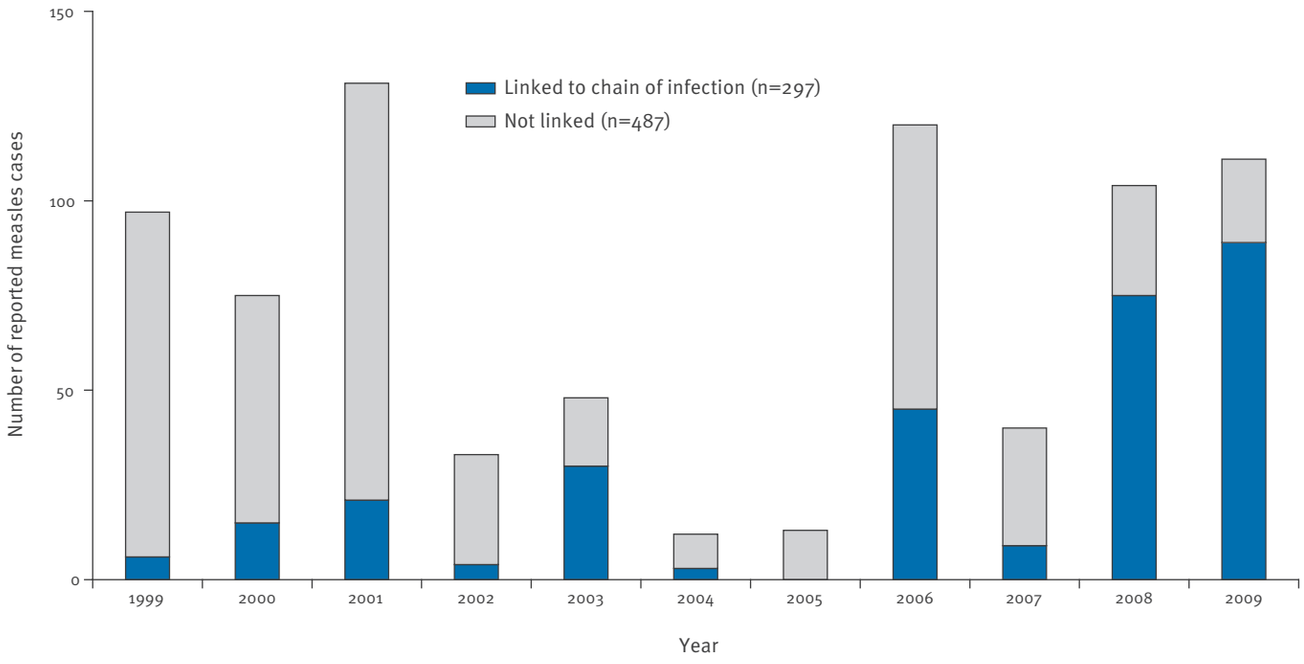


Figure 2

Number of reported measles cases, including those which could be linked to transmission chain, Poland, 1999-2009 (n=784)



of reported measles cases was recorded. Contact tracing is routinely undertaken, especially for unvaccinated and exposed individuals. Serological testing and detection of measles virus RNA are performed in the National Reference Laboratory at the National Institute of Public Health. Measles virus-containing samples are sent to the WHO Regional Reference Laboratory for Measles and Rubella (Robert Koch Institute, Berlin) for genotyping.

For the present study, we defined an imported outbreak as resulting from importation of measles virus by a person arriving from abroad who was exposed and developed symptoms outside Poland, and subsequently was the source of documented local transmission to other cases linked to the outbreak. If available, genotyping results were used for confirmation of importation-related transmission chains.

Measles case reports from 1999 to 2009 are described. Measles cases with an established link to the infection transmission chain (outbreak cases) in 2008-2009 are described in more detail to determine the role of disease importation and outbreak patterns.

Results

Over time, an increasing proportion of measles cases could be linked to identified chains of transmission in Poland (Figure 2), from 6% in 1999 to 80% in 2009. Of 569 cases of measles reported between 1999 and 2007, 133 (23%) were linked to outbreaks. In 2008 and 2009, this proportion was higher, with 77% reported measles cases linked to outbreaks.

During 2008 and 2009, 19 measles outbreaks with 164 cases were reported in Poland. Seven outbreaks were due to importation of the disease from the United Kingdom (UK), and 12 involved only indigenous transmission. Outbreaks in that period were reported from nine of the 16 provinces of Poland. One of the 164 outbreak cases, excluded from further analysis, occurred in a Ukrainian citizen who arrived in Poland in February 2009. He contracted measles while staying in a hospital where an outbreak occurred.

Fifty-three percent of cases in 2008 and 2009 were female and 90.2% of the patients were residents of urban areas. Cases were seen in all age groups, although adults aged over 19 years were predominantly affected (45 cases, 27.4%). One hundred and thirty patients (79.3%) were admitted to hospital. The proportion of hospitalised cases was highest in children aged five to nine years (90.9%). Seventy-nine percent of all outbreak-related cases during 2008 and 2009 occurred among the Roma ethnic group.

Important differences were observed between the outbreaks among the Roma community and those occurring in non-Roma Polish population (Table).

Table

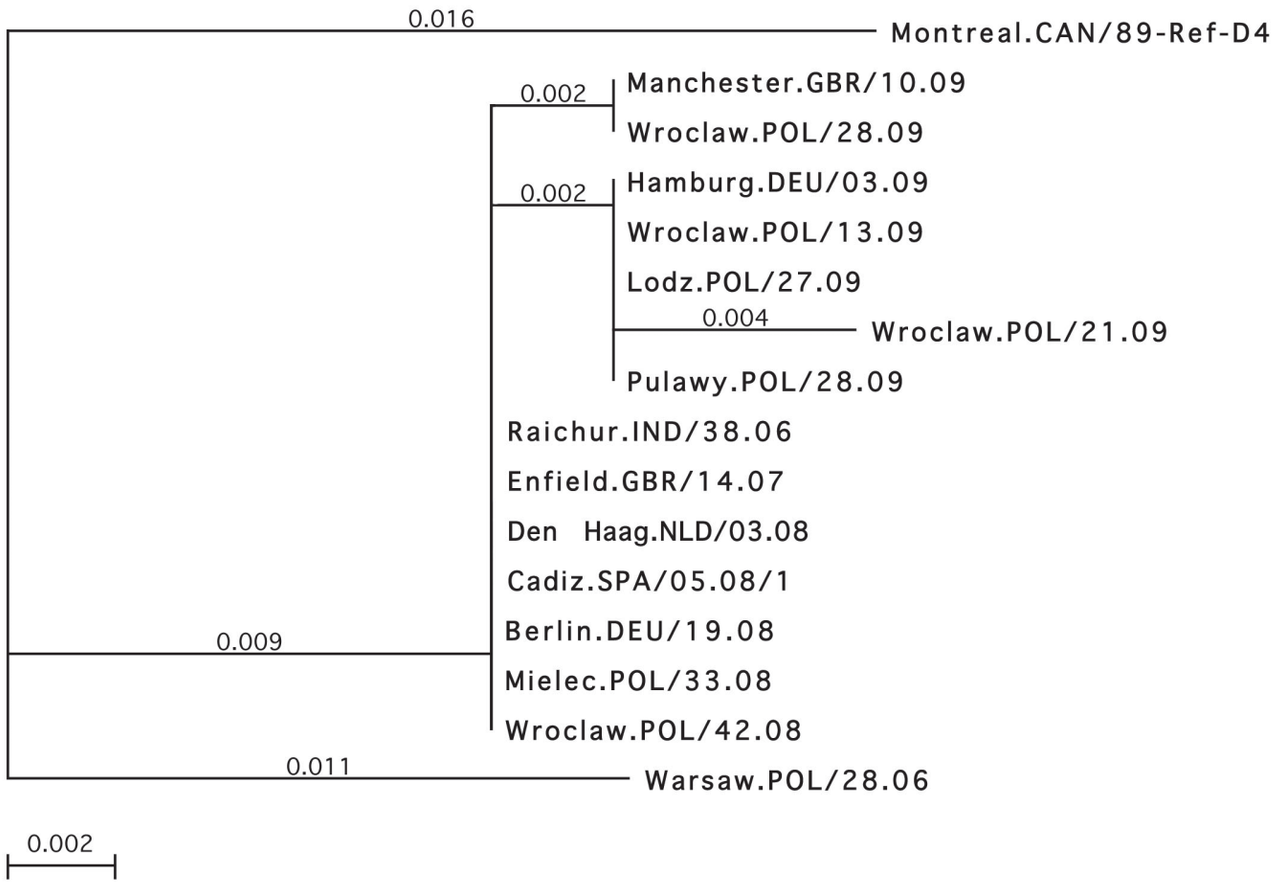
Characteristics of cases linked to chain of transmission, Poland, 2008-2009 (n=163)

Characteristic	Roma		Non-Roma Polish population		Total	
	N	%	N	%	N	%
Number of outbreaks	13	68.4	6	31.6	19	100.0
Number of cases	126	77.3	37	22.7	163	100.0
Sex						
Female	64	50.8	23	62.2	87	53.4
Male	62	49.2	14	37.8	76	46.6
Confirmation of cases						
Laboratory-confirmed	72	57.1	35	94.6	107	65.6
Epidemiologically linked	54	42.9	2	5.4	56	34.4
Vaccination status						
Vaccinated according to age	18	14.3	12	32.4	30	18.4
Incompletely vaccinated	91	72.2	18	48.6	109	66.9
Unknown vaccination status	17	13.5	7	19.0	24	14.7
Importation status (number of outbreaks)						
Import-related	7 (68 cases)	53.8 (54.0)	1 (3 cases)	16.7 (8.1)	8 (71 cases)	42.1 (43.6)
Local	6 (58 cases)	46.2 (46.0)	5 (34 cases)	83.3 (91.9)	11 (92 cases)	57.9 (56.4)
Generations of transmission identified (number of outbreaks)						
1-2	9	69.2	4	66.7	13	68.4
3 or more	4	30.8	2	33.3	6	31.6
D4 genotype identified	4 (19 cases)	30.8 (15.1%)	2 (2 cases)	33.3 (5.4)	6 (21 cases)	31.6 (12.9)

Outbreaks among Roma were considerably larger with an average of 10 cases, who were mostly unvaccinated (72% of outbreak cases), while outbreaks in the non-Roma Polish population involved an average of five cases, with 48% of outbreak cases incompletely vaccinated. The majority of outbreaks in Roma communities were related to importation of virus from the UK. In six outbreaks, measles virus genotyping identified a genotype D4 strain that was most closely related to viruses from the UK and Germany. Figure 3 presents the exact genetic relationship between viruses isolated from outbreak cases in 2008 and 2009 to closely related strains isolated in other countries. Laboratory testing was performed more often for cases from the non-Roma Polish

Figure 3

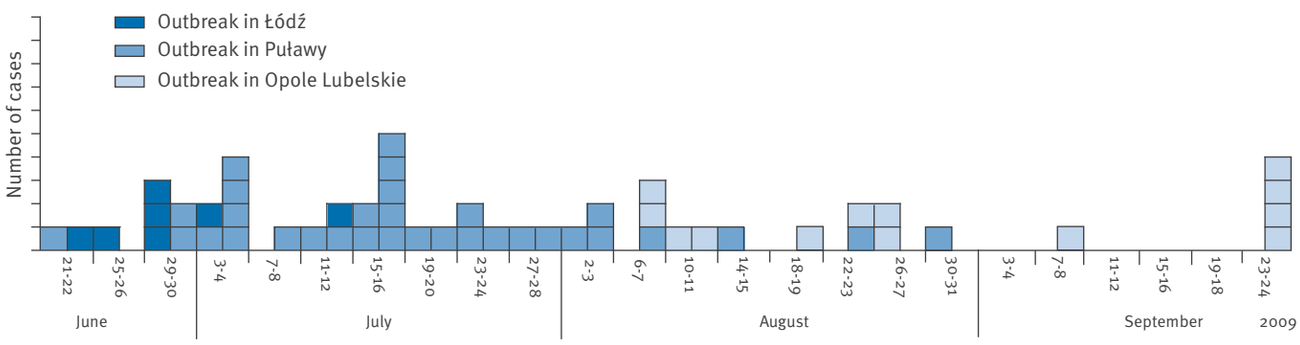
Phylogenetic analysis of measles viruses of genotype D4 detected from 2006 to 2009 in Poland and other European countries



The phylogenetic tree is based on a 456 nt sequence encoding the carboxyterminus of the nucleoprotein. It includes all measles strains identified in Poland in 2006-2009 and world strains most closely related to them.
Method: Neighbor Joining; Best Tree; tie breaking = Systematic.
Distance: Tamura-Nei; Gamma correction = Off; Gaps distributed proportionally.
Source: Robert Koch Institute, Berlin, Germany.

Figure 4

Number of reported measles cases by week of illness onset, Poland, 2009



population (94%) than for cases from the Roma community (57%). Based on the dates recorded for onset of disease, the same proportion of outbreaks recorded up to four generations of transmission among the Roma and non-Roma Polish population.

In some cases, separate outbreaks could be linked by detailed epidemiological and molecular investigation. From August to October 2008 two outbreaks occurred in Mielec and Wroclaw, which are approximately 400 km apart. A total of 32 cases were recorded from those two outbreaks in Roma communities, and both could be linked to the strain Enfield/GBR/14.07 (Accession No. EF600554) of measles virus genotype D4. The index cases were among families with young children returning from London, UK. In the same period numerous importations from England, confirmed by the detection of the Enfield strain, were notified in several other European countries (Figure 3), i.e. the Netherlands (Den Haag.NLD/03.08, GenBank Accession No. EU585844), Spain (Cadiz.SPA/05.08/1, GenBank Accession No. EU982301) and Germany (Berlin.DEU/19.08).

From June to October 2009, 54 cases were linked to three outbreaks in Roma communities living in different towns (Figure 4). The first outbreak with seven measles cases was reported in the city of Lodz. Subsequently, 47 measles cases were reported in the city of Pulawy and Opole Lubelskie in Lubelskie province. The outbreaks in Lodz and Pulawy were linked by epidemiological investigation and measles virus genotyping, since the measles virus detected in Lodz and Pulawy was identical to the strain Hamburg/DEU/03.09(D4) observed in northwest Germany in the first quarter of 2009. The outbreak in Opole Lubelskie was linked to the Pulawy outbreak by an epidemiological link, and no samples were collected for genotyping.

Discussion

Measles outbreaks have recently been described in many European countries. Large outbreaks were reported in 2008 and 2009 in France [8], Switzerland [9], and Bulgaria [10].

WHO defined measles elimination as a situation in a large geographical area in which endemic transmission of measles virus cannot occur and imported measles cases do not initiate sustained transmission [11]. Despite public health efforts and maintaining high levels of vaccination coverage, outbreaks due to measles virus importation continue to occur in Poland. Similarly as in other European countries, herd immunity has not been achieved despite a national measles vaccination coverage above 95%. This failure is possibly related to the existence of specific vulnerable populations, who are often not reached by the public health services regarding vaccination. Common causes of limited access to public health services may involve particular attitudes or beliefs of these populations [12-14].

There could be several reasons for the increased proportion of cases for which a chain of infection could be traced in 2008 and 2009, compared with the previous period. On the one hand, local public health officers may have been investigating the epidemiological links more efficiently during the recent years. When approaching the measles elimination phase, it becomes more important to monitor infection chains and, if necessary, to intervene. On the other hand, well defined outbreaks were identified in 2008 and 2009 with several cases occurring in the same households. This rather indicates an appearance of pockets of unvaccinated persons, who are sustaining measles transmission, possibly in relation to anti-immunisation beliefs, or poor access to healthcare.

Similar to other European countries, Poland has not succeeded in controlling measles enough to reach one case per million inhabitants, one of the WHO criteria for measles elimination. In recent years, most outbreaks in Poland were detected in ethnic minorities and were often related to measles importation from the United Kingdom or Germany. Currently, the emphasis of measles elimination activities should be directed to immunising all sections of the population that are not adequately protected. Considering that ethnic minorities are often marginalised and discriminated against, we need to better understand the health problems, attitudes and beliefs of these communities. An assessment performed during a large outbreak in August 2009, revealed limited access to healthcare and low life expectancy of a settled Roma community [15]. Both in Roma and in the non-Roma Polish population, a considerable proportion of unvaccinated cases in the under 19-year-olds indicates the need to address at least some high-risk groups in Poland. The best approach would be to focus on healthcare workers and persons working in crowded environments like schools, universities or airports.

Genetic characterisation of detected measles viruses has been done in Poland continuously since 2006 [16]. Molecular and epidemiological investigation of the recent outbreaks revealed five independent transmission chains with a duration of under three months. Genetic data demonstrated a close relationship of four of the five distinct subvariants of genotype D4 identified in Poland to viruses of western Europe (GenBank Accession No. EF600554, EU585844, EU982301, GQ370461) from where they were imported, and to a virus from India (GenBank Accession No. EU812270) considered to be the source of the recent European D4 viruses [Regional Reference Laboratory WHO EURO, Robert Koch Institute, personal communication]. The present analyses document that Poland has made progress on its way to reach the elimination goal for measles virus in the WHO European region. Considering increasing airline travel, and anti-vaccination beliefs, continuous efforts are necessary to maintain a high vaccination status of the Polish population, and implement innovative measures to reach vulnerable groups.

References

1. World Health Organization, Regional Office for Europe. Strategic plan for measles and congenital rubella infection in the WHO European Region. Copenhagen, WHO Regional Office for Europe, 2003. Available from: http://www.euro.who.int/document/e81567.pdf
2. World Health Organization, Regional Office for Europe. Surveillance guidelines for measles and congenital rubella infection in the WHO European Region. Copenhagen, WHO Regional Office for Europe, 2003. Available from: http://www.euro.who.int/document/E82183.pdf
3. Commission Decision 2003/534/EC amending Decision No 2119/98/EC of the European Parliament and of the Council and Decision 2000/96/EC as regards communicable diseases listed in those decisions and amending Decision 2002/253/EC as regards the case definitions for communicable diseases. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:184:0035:0039:EN:PDF
4. Muscat M, Bang H, Wohlfahrt J, Glismann S, Mølbak K; EUVAC.NET Group. Measles in Europe: an epidemiological assessment. Lancet. 2009;373(9661):383-9.
5. EUVAC.NET. Measles surveillance annual report 2008. EUVAC.NET, pages 1-8. Available from: http://www.euvac.net/graphics/euvac/pdf/annual_2008.pdf
6. Stefanoff P, Czarkowski MP. Unexpected rise in measles incidence in Poland in 2006 may be related to Ukrainian outbreak. Euro Surveill. 2006;11(26). pii=2990. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2990
7. Bulletin: Infectious diseases and poisonings in Poland in 2008. National Institute of Public Health – National Institute of Hygiene – Department of Epidemiology, Chief Sanitary Inspectorate – Department of Communicable Diseases Control. Warsaw 2009.
8. Thierry S, Alsibai S, Parent du Châtelet I, on behalf of the investigation team. An outbreak of measles in Reims, eastern France, January-March 2008 -- a preliminary report. Euro Surveill. 2008;13(13). pii=8078. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8078
9. Richard JL, Masserey-Spicher V, Santibanez S, Mankertz A. Measles outbreak in Switzerland - an update relevant for the European football championship (EURO 2008). Euro Surveill. 2008;13(8). pii=8043. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8043
10. Marinova L, Muscat M, Mihneva Z, Kojouharova M. An update on an ongoing measles outbreak in Bulgaria, April-November 2009. Euro Surveill. 2009;14(50). pii=19442. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19442
11. WHO/UNICEF. Measles mortality reduction and regional elimination strategic plan 2001-2005 (WHO/V&B/01.13). Geneva: World Health Organization and United Nations Children's Fund; September 2001. Available from: http://www.who.int/vaccines-documents/DocsPDF01/www573.pdf
12. Cohuet S, Bukasa A, Heathcock R, White J, Brown K, Ramsay M, Fraser G. A measles outbreak in the Irish traveller ethnic group after attending a funeral in England, March-June 2007. Epidemiol Infect. 2009;137(12):1759-65.
13. Noury U, Stoll J, Haeghebaert S, Antona D, Parent du Châtelet I, The investigation team. Outbreak of measles in two private religious schools in Bourgogne and Nord-Pas-de-Calais regions of France, May-July 2008 (preliminary results). Euro Surveill. 2008;13(35). pii=18961. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18961
14. Anis E, Grotto I, Moerman L, Warshavsky B, Slater PE, Lev B, Israeli A. Measles in a highly vaccinated society: the 2007-08 outbreak in Israel. J Infect. 2009;59(4):252-8.
15. Stefanoff P, Orlikova H, Rogalska J, Kazanowska-Zielinska E, Slodzinski J. Mass immunisation campaign in a Roma settled community created an opportunity to estimate its size and measles vaccination uptake, Poland, 2009. Euro Surveill. 2010;15(17). pii=19552. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19552
16. Makowka A, Gut W, Litwinska B, Santibanez S, Mankertz A. Genotyping of measles and rubella virus strains circulating in Poland in 2007. Euro Surveill. 2007;12(43). pii=3295. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3295

Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011

C Frank[1,2], M S Faber (FaberM@rki.de)[1,2], M Askar[1,3,4], H Bernard[1], A Fruth[5], A Gilsdorf[1], M Höhle[1], H Karch[6], G Krause[1], R Prager[5], A Spode[7], K Stark[1], D Werber[1], on behalf of the HUS investigation team[8]

1. Robert Koch Institute, Berlin, Germany
2. These authors contributed equally to the manuscript
3. Postgraduate Training for Applied Epidemiology (PAE, German Field Epidemiology Training Programme), Robert Koch Institute, Department for Infectious Disease Epidemiology, Berlin, Germany
4. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
5. National Reference Centre for Salmonella and other bacterial enteric pathogens at the RKI, Wernigerode, Germany
6. Consulting laboratory for haemolytic uraemic syndrome, Institute of Hygiene, University of Münster, Germany
7. Local health department Hamburg Nord, Hamburg, Germany
8. The members of the team are listed at the end of the article

Citation style for this article:
Frank C, Faber MS, Askar M, Bernard H, Fruth A, Gilsdorf A, Höhle M, Karch H, Krause G, Prager R, Spode A, Stark K, Werber D, on behalf of the HUS investigation team. Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011.
Euro Surveill. 2011;16(21):pii=19878. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19878

Article published on 26 May 2011

Since early May 2011, an increased incidence of haemolytic uraemic syndrome (HUS) and bloody diarrhoea related to infections with Shiga toxin-producing *Escherichia coli* (STEC) has been observed in Germany, with most cases in the north of the country. Cases reported from other European countries had travelled to this area. First results of a case–control study conducted in Hamburg suggest an association between the occurrence of disease and the consumption of raw tomatoes, cucumber and leaf salad.

An unusually high number of cases of haemolytic uraemic syndrome (HUS) has been observed in Germany since early May 2011. This report presents the preliminary results of the investigation as of 26 May 2011

Haemolytic uraemic syndrome (HUS) is a serious and sometimes deadly complication that can occur in bacterial intestinal infections with Shiga toxin (syn. verotoxin)-producing *Escherichia coli* (STEC/VTEC). The complete clinical picture of HUS is characterised by acute renal failure, haemolytic anaemia and thrombocytopenia. Typically it is preceded by diarrhoea, often bloody. Each year, on average 1,000 symptomatic STEC-infections and approximately 60 cases of HUS are notified in Germany, affecting mostly young children under five years of age [1]. In 2010 there were two fatal HUS cases [1].

STEC are of zoonotic origin and can be transmitted directly or indirectly from animals to humans. Ruminants are considered to be the reservoir, especially cattle, sheep and goats. Transmission occurs via the faecal-oral route through contact to animals (or their faeces), by consumption of contaminated food or water, but also by direct contact from person to person (smear infection). The incubation period of STEC is between two and 10 days, the latency period between the beginning of gastrointestinal symptoms and enteropathic HUS is approximately one week.

Outbreak description

The Table lists the number of cases of HUS or suspected HUS notified to local health departments and communicated by the federal states to the Robert Koch Institute (RKI). Suspected HUS are included as the syndrome is a process and suspected HUS typically develops over the course of a few days into the full clinical picture.

Disease onset (regarding diarrhoea) in the 214 patients detected so far was between 2 and 24 May 2011. A total of 119 (56%) of the cases were communicated from four northern federal states (Hamburg, Schleswig-Holstein, Lower Saxony and Bremen). The highest cumulative incidence has been recorded in the two northern city states of Hamburg and Bremen. An additional 31 cases occurred in Hesse. They were connected to a catering company supplying the cafeterias of a company and a residential institution. It is likely that these cases constitute a satellite outbreak.

Besides the geographic clustering, the age and sex distribution of the cases is conspicuous: Of the 214 cases, 186 (87%) are 18 years of age or older (mostly young to middle-aged adults) and 146 (68%) are female. In the notification data for HUS cases from 2006 to 2010, the proportion of adults lay between 1.5% and 10% annually, and the sexes were affected equally.

Cases linked to this outbreak were also communicated from other European countries: On 25 May 2011, Sweden reported through the European Warning and Response System (EWRS) nine cases of HUS, four of whom had

travelled in a party of 30 to northern Germany from 8 to 10 May. Denmark reported four cases of STEC infection, two of them with HUS. All cases had a recent travel history to northern Germany. Another two HUS cases with travel history to northern Germany in the relevant period were communicated, one each by the Netherlands and by the United Kingdom.

So far two German HUS cases have died of the disease (both female, one in her 80s, one in her 20s) .

Laboratory investigations

Investigations at the National Reference Centre for Salmonella and other bacterial enteric pathogens at the RKI (Wernigerode) of isolates from two patients from Hesse and Bremerhaven suggests that the outbreak strain is an *E. coli* strain of serotype O104 with the following characteristics: Shiga toxin 2 (*vtx2a*, EQA nomenclature 2011, WHO Centre *E. coli* SSI Copenhagen)- producing, intimin (*eae*)-negative and enterohaemolysin (*hly*)-negative. The strain shows a high resistance to third generation cephalosporins (through extended spectrum beta-lactamases, ESBL, CTX-M-type), and a broad antimicrobial resistance to, among others, trimethoprim/sulphonamide and tetracycline.

Table

Cases of HUS and suspected HUS with onset of diarrhoea since 2 May 2011, Germany (n=214)

Federal State	Number of HUS cases and suspected-HUS cases	Cumulative incidence (per 100,000 population)
Hamburg	59	3.33
Bremen	11	1.66
Schleswig-Holstein	21	0.74
Mecklenburg-Vorpommern	10	0.61
Hesse	31	0.51
Saarland	5	0.49
Lower Saxony	28	0.35
North Rhine-Westphalia	31	0.17
Berlin	3	0.09
Baden-Württemberg	8	0.07
Bavaria	5	0.04
Thuringia	1	0.04
Rhineland-Palatinate	1	0.02
Brandenburg	0	0.00
Saxony	0	0.00
Saxony-Anhalt	0	0.00
Total	**214**	**0.26**

HUS: haemolytic uraemic syndrome.

Data as of 26 May 2011, 8am, communicated to the Robert Koch Institute by the federal states.

A further 13 isolates from Muenster, Paderborn, Hamburg and Frankfurt were analysed in the consulting laboratory for haemolytic uraemic syndrome in the Institute of Hygiene at the University hospital in Muenster. All were sequence-typed as ST678 (*stx1-, stx2+, eae-*, flagellin-coding gene *flicH4*), group HUSEC 41, also indicating serotype O104 [2,3]. Whether these results reflect the entire situation in Germany needs to be confirmed by the analysis of a greater number of isolates. As in the past most outbreaks of HUS in Germany and elsewhere were found to be connected with STEC O157 strains, the identification of serotype O104 in this context is highly unusual, although, *E. coli* O104 has previously been described as the cause of an outbreak in the United States in 1994 [4].

Investigation into the source of infection

The large number of persons suddenly affected, the geographical and demographic distribution as well as first interviews of patients suggested STEC-contaminated food as the vehicle of infection. Foods like raw milk and raw meat, which were identified as vehicles in former STEC outbreaks, appear not to be related to the current event. Preliminary results of a case–control study conducted by the RKI and the Hamburg health authorities demonstrate a significant association between disease and the consumption of raw tomatoes, cucumbers and leafy salads. This study collected food histories for the week before symptom onset for 25 patients hospitalised with HUS (n=20) or bloody diarrhoea with laboratory-confirmed STEC infection (n=5), who all had onset of disease between 9 and 25 May 2011. In addition, 96 controls matched by age, sex and residence were asked about their food consumption during the week before the interview. The food items they were asked about were those frequently mentioned in previous in-depth interviews of HUS cases. Consumption of each of the named food items was reported by around 90% of the cases in comparison to around 60% of the controls, yielding odds ratios between around 4 and 7, all statistically significant. Nevertheless it is possible that another or an additional food item is the source of infection. The results cannot necessarily be transferred to the whole of Germany because the study was limited to Hamburg.

Regarding the source of the suspicious food items the study showed a heterogeneous picture. It can be excluded that the source is a single shop or restaurant. Based on these findings, food trace-back investigations are currently ongoing.

Evaluation of the situation

The current events represent one of the largest described outbreaks of HUS/STEC worldwide and the largest in Germany, with a very atypical age and sex distribution of the cases. Incident cases of HUS or suspected HUS are continuing to be reported at least in Northern Germany, where the emergency room consultations for bloody diarrhoea remain elevated. Thus it has to be assumed that the source of infection is still

active. Many patients with bloody diarrhoea need to be admitted to hospital, and HUS patients often need intensive care with dialysis and/or plasmapheresis, which puts a severe strain on hospital resources in some areas. The epidemiological studies that were conducted in cooperation with regional and local health departments rapidly delivered important clues as to certain food items that could be linked to the outbreak. Further epidemiological studies, laboratory investigations and trace back of food items is needed to confirm these results and to narrow down the source of infection.

Recommendations for consumers and patients

Considering the ongoing outbreak that included many cases with a severe course of disease, the RKI and the Federal Institute for Risk Assessment (BfR) recommend to abstain from consuming raw tomatoes, cucumbers and leafy salads, especially in northern Germany, until further notice. Regular food hygiene rules remain in effect [5].

For persons with diarrhoea the importance of strict hand hygiene is emphasised. Patients with bloody diarrhoea should seek medical aid immediately. Physicians are reminded to initiate STEC stool diagnostics for these patients and to closely monitor them for the development of HUS. Patients suspected of developing HUS should be referred to appropriate stationary care.

Diagnostic laboratories are requested to send STEC isolates to the National Reference Centre for Salmonella and other bacterial enteric pathogens. The Protection Against Infection Act of 2001 renders both the laboratory confirmation of an STEC infection and the clinical diagnosis of HUS or suspected HUS notifiable to the local health department.

Acknowledgments

We gratefully acknowledge the contribution of all local and state health departments, the Federal Institute for Risk Assessment (BfR) and all patients and healthy citizens of Hamburg who agreed to being interviewed. We would also like to thank all hospitals in Hamburg who supported the work of the field teams.

The members of the HUS investigation team were:

Karen Atzpodien, Susanne Behnke, Merle Böhmer, Udo Buchholz, Manuel Dehnert, Julia Hermes, Kerstin Krügermann, Marion Marx, Ingeborg Mücke, Mathias Nachtnebel, Janina Neifer, Stine Nielsen, Ines Noll, Kerstin Prahm, Ute Preuß, Cornelius Remschmidt, Thorsten Rieck, Bettina Rosner, Susanne Schink, Daniel Schmidt, Franziska Schwarz, Britta Schweickert, Petra Stöcker, Thorsten Süß, Bernhard Ultsch, Maria Wadl
(Robert Koch Institute, Department for Infectious Disease Epidemiology, Berlin, Germany)

Gerhard Fell (Institute for Hygiene and Environmental, Hamburg, Germany)
Johannes Dreesmann (State Health Department of Lower Saxony, Hannover, Germany)

Ulf Panzer, Sigrid Harendza (Department of Internal Medicine III, University Medical Center Hamburg-Eppendorf, Hamburg, Germany)
Jun Oh (Department of Pediatric Nephrology, Medical Center Hamburg-Eppendorf, Hamburg, Germany)

Stefan Sudmann (Department for emergency medicine, Marienkrankenhaus, Hamburg, Germany)
Ute Juers (Asklepios Klinik Barmbeck, Hamburg, Germany)

References

1. SurvStat. Berlin: Robert Koch Institute. German. Available from: http://www3.rki.de/SurvStat. [Accessed: 24 May 2011].
2. HUSEC Referenzstammsammlung. [HUSEC collection of reference strains]. Muenster: Konsiliarlabor für Hämolytisch–Urämisches Syndrom (HUS). German. Available from: http://www.ehec.org/index.php?lang=de&pid=HUSEC
3. Mellmann A, Bielaszewska M, Köck R, Friedrich AW, Fruth A, Middendorf B, et al. Analysis of collection of hemolytic uremic syndrome-associated enterohemorrhagic Escherichia coli. Emerg Infect Dis. 2008;14(8):1287-90.
4. Centers for Disease Control and Prevention (CDC). Outbreak of acute gastroenteritis attributable to Escherichia coli serotype O104:H21--Helena, Montana, 1994. MMWR Morb Mortal Wkly Rep. 1995;44(27):501-3.
5. Verbrauchertipps: Schutz vor Lebensmittelinfektionen im Privathaushalt. [Consumer advice: Protection from food-borne infections in the private household]. Berlin: Bundesinstitut für Risikobewertung; 2007. German. Available from: www.bfr.bund.de/cm/350/verbrauchertipps_schutz_vor_lebensmittelinfektionen_im_privathaushalt.pdf

Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013

R A Perera[1,2], P Wang[2,3,4], M R Gomaa[5], R El-Shesheny[5], A Kandeil[5], O Bagato[5], L Y Siu[3], M M Shehata[5], A S Kayed[5], Y Moatasim[5], M Li[3], L L Poon[1], Y Guan[1], R J Webby[6], M A Ali[5], J S Peiris (malik@hku.hk)[1], G Kayali (ghazi.kayali@stjude.org)[6]

1. Centre of Influenza Research, School of Public Health, The University of Hong Kong, Hong Kong, China
2. These authors contributed equally to the work and are joint first authors
3. Hong Kong University-Pasteur Research Pole, The University of Hong Kong, Hong Kong, China
4. Key Laboratory of Protein and Peptide Pharmaceuticals, Chinese Academy of Sciences - University of Tokyo Joint Laboratory of Structural Virology and Immunology, Beijing, China
5. Division of Environmental Research, National Research Centre, Giza, Egypt
6. Division of Virology, Department of Infectious Diseases, St Jude Children's Research Hospital, Memphis, United States

Citation style for this article:
Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, Siu LY, Shehata MM, Kayed AS, Moatasim Y, Li M, Poon LL, Guan Y, Webby RJ, Ali MA, Peiris JS, Kayali G. Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013.
Euro Surveill. 2013;18(36):pii=20574. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20574

Article submitted on 26 August 2013 / published on 5 September 2013

We describe a novel spike pseudoparticle neutralisation assay (ppNT) for seroepidemiological studies on Middle East respiratory syndrome coronavirus (MERS-CoV) and apply this assay together with conventional microneutralisation (MN) tests to investigate 1,343 human and 625 animal sera. The sera were collected in Egypt as a region adjacent to areas where MERS has been described, and in Hong Kong, China as a control region. Sera from dromedary camels had a high prevalence of antibody reactive to MERS-CoV by MERS NT (93.6%) and MERS ppNT (98.2%) assay. The antibody titres ranged up to 1,280 and higher in MN assays and 10,240 and higher in ppNT assays. No other investigated species had any antibody reactivity to MERS-CoV. While seropositivity does not exclude the possibility of infection with a closely related virus, our data highlight the need to attempt detection of MERS-CoV or related coronaviruses in dromedary camels. The data show excellent correlation between the conventional MN assay and the novel ppNT assay. The newly developed ppNT assay does not require Biosafety Level 3 containment and is thus a relatively high-throughput assay, well suited for large-scale seroepidemiology studies which are needed to better understand the ecology and epidemiology of MERS-CoV.

Introduction

A novel lineage C beta-coroanvirus was isolated from a patient with fatal viral pneumonia in Saudi Arabia in 2012 and termed Middle East respiratory syndrome coronavirus (MERS-CoV) [1]. As of 3 September 2013, 108 human cases have been confirmed, 50 of which were fatal [2]. Locally acquired cases have been reported from Jordan, Qatar, Saudi Arabia and the United Arab Emirates, and imported index cases, sometimes with secondary local transmission, have been reported in France, Germany, Italy, Tunisia and the United Kingdom [2-4]. Clusters of cases suggestive of limited human-to-human transmission have been reported; the largest cluster of cases to date occurred at a healthcare facility in Al-Hasa, Saudi Arabia [5]. The epidemiology of the disease so far is suggestive of multiple zoonotic transmissions from an animal reservoir leading to human infection, sometimes with secondary transmission events in humans.

Phylogenetically closely related, although not identical, viruses have been found in insectivorous bats in Africa and Europe [6,7]. More recently, a very short fragment (181 bp) of the RNA-dependent RNA polymerase gene that was genetically identical to MERS-CoV has been detected in a *Taphozous perforatus* bat captured in the vicinity of the residence of a human case with MERS [8]. These findings remain to be confirmed with more definitive sequence data. Even if MERS-CoV is found in bats, the possibility of an intermediate peridomestic host remains important to explore.

Since antibody responses following coronavirus infection remain detectable for many years [9], seroepidemiology of potential animal species for MERS-CoV-specific antibody is a logical approach to identify candidate species for further investigation. A recent report suggests that MERS-CoV antibody was detected in dromedary camels in the Arabian peninsula [10]. While a number of serological tests, including ELISA assays, immunoflourescence assays and immunoassays using recombinant viral proteins have been used for detecting serological responses in infected humans [11,12], virus neutralisation is the most specific serological

test and currently considered the gold-standard. However, virus neutralisation requires the handling of live virus and requires Biosafety Level 3 containment. We have therefore developed a pseudoparticle neutralisation (ppNT) assay where the spike protein of MERS-CoV is expressed by a replication-incompetent human immunodeficiency (HIV) virus that contains a luciferase reporter gene. Similar pseudotype viruses have been used successfully in serological tests for severe acute respiratory syndrome coronavirus (SARS-CoV) and influenza viruses such as the highly pathogenic avian influenza A(H5N1) virus [13]. Pseudotyped MERS-CoV has been used to study the mechanisms of virus entry, and it has been shown that cell transduction by such particles is blocked by neutralising antibodies specific for MERS-CoV [14].

The geographical distribution of MERS-CoV in its animal reservoir is not defined. Being a Middle Eastern country with an ecology and domestic livestock practices fairly similar to some countries where human MERS infections have occurred, we reasoned that Egypt would be a relevant geographical location for seroepidemiological studies. We have used both the ppNT assay and conventional microneutralisation (MN) tests to carry out seroepidemiological surveillance in humans and livestock in Egypt. Human and animal sera collected in Hong Kong were used as controls.

Methods

Sera from dromedary camels (n=110), water buffaloes (n=8) and cows (n=25) were collected from two abattoirs, one located in Cairo and the second located in the Qalyubia governorate in the Nile Delta region. The dromedary camels were mostly imported from Sudan for slaughter and were five to seven years-old. Upon import, they were held on Egyptian farms for four to five months before transport to the abattoirs in open trucks. Sera from sheep (n=5) and goats (n=13) were collected from backyard animals from a village in the Nile Delta. All sera were collected in June 2013.

Human sera (n=815) were collected in 2012–13 as part of an ongoing community-based seroepidemiological study on influenza virus among healthy subjects in Cairo and the Nile Delta region. The age range of the subjects was between two and 79 years-old (median: 29 years). Fifty-eight per cent of the study subjects were female.

Sera collected in Hong Kong served as un-exposed controls. These included archived age-stratified human sera (n=528) collected in Hong Kong in 2011 and 2012, with more than 50 sera from each decade of age (range: <10 to 80 years-old). Swine sera (n=260) were collected from an abattoir in Hong Kong in 2011 and 2012. Sera (n=204) from wild northern pintails (Anas acuta) and Eurasian widgeons (*Anas penelope*) were collected in December 2010 from the Mai Po wetlands nature reserve in Hong Kong.

As positive controls, we used a convalescent serum from a human patient with MERS, kindly provided by Dr C Drosten (Institute of Virology, University of Bonn Medical Centre, Bonn, Germany), and sera from two experimentally infected macaques and a non-infected control macaque kindly provided by Bart Haagmans (Erasmus University Medical Center, Rotterdam, the Netherlands).

An acute and convalescent serum from a patient with SARS was used as a further negative control. The MN antibody titre was <10 to SARS-CoV in the acute serum, and 160 in the convalescent serum.

The study was approved by the institutional review boards of the University of Hong Kong and St Jude Children's Research Hospital and the Ethics Committee of the National Research Centre, Egypt.

Viruses and virus titration

MERS-CoV (strain EMC) virus was obtained from Dr R Fouchier (Erasmus University Medical Center, Rotterdam, the Netherlands). SARS-CoV (strain HKU-39849) was taken from the virus repository at Hong Kong University. Virus stock for MERS-CoV was prepared in Vero cell culture (ATCC CCL-81) in minimal essential medium containing 2% fetal bovine serum, 100 units/mL penicillin and 100 µg/mL streptomycin. Virus aliquots were stored at -80 °C. Virus was titrated in serial half-$\log_{10}$ dilutions (from 0.5 log to 7 log) to obtain 50% tissue culture infectious dose ($TCID_{50}$) on 96-well tissue culture plates of Vero cells. The plates were observed in a phase contrast microscope for cytopathic effect (CPE) daily for three days. The endpoint of viral dilution leading to CPE in 50% of inoculated wells was estimated by using the Reed Muench method and designated as one $TCID_{50}$. SARS-CoV was grown and titrated in the same manner with the exception that Vero E6 cells (ATCC CRL-1586) were used.

Microneutralisation tests

Serial two-fold dilutions of heat-inactivated sera (56 °C for 30 minutes) were made, starting with a dilution of 1:10. The serum dilutions were mixed with equal volumes of 200 $TCID_{50}$ of MERS-CoV or SARS-CoV as indicated. After 1 h of incubation at 37 °C, 35 µL of the virus–serum mixture was added in quadruplicate to Vero or Vero-E6 cell monolayers for MERS-CoV and SARS-CoV, respectively, in 96-well microtiter plates. After 1 h of adsorption, an additional 150 µL of culture medium were added to each well and the plates incubated for three more days at 37 °C in 5% CO_2 in a humidified incubator. A virus back-titration was performed without immune serum to assess input virus dose. CPE was read at three days post infection. The highest serum dilution that completely protected the cells from CPE in half of the wells was taken as the neutralising antibody titre and was estimated using the Reed-Muench method. Positive and negative control sera were included to validate the assay.

MERS-CoV spike pseudoparticle neutralisation assay

A codon-optimised spike gene was designed according to published MERS-CoV genome sequence (GenBank accession number: JX869059.1), synthesised by GeneCust (Luxembourg) and subcloned into pcDNA3.1+ vector to generate pcDNA-S. To produce HIV/MERS spike pseudoparticles, 10 µg pNL Luc E^- R^- and 10 µg pcDNA-S were co-transfected into $4x10^6$ 293T cells [13]. Supernatants of transfected cells were harvested 48 h later and quantified for HIV p24 viral protein using a p24 ELISA Kit (Cell Biolabs, San Diego, United States).

For the ppNT assay, HIV/MERS pseudoparticles containing 5 ng p24 were used to infect Vero E6 cells (ATCC CRL-1586) in a single well (96-well plate format; $1x10^4$ cells/well). Infected cells were lysed in 20 µl lysis buffer and 100 µl of luciferase substrate at two days postinfection (Promega Corporation, Madison, United States). Luciferase activity was measured in a Microbeta luminometer (PerkinElmer, Waltham, United States).

For the ppNT, HIV/MERS pseudoparticles (5 ng of p24) were pre-incubated with serially diluted sera for 30 min at 4 °C and then added to cells in triplicate. Residual virus replication was assayed at two days post infection, as described above. The highest serum dilution giving a 90% reduction of luciferase activity was regarded as the ppNT antibody titre.

Results

Overall, 976 human and animal sera from Egypt and 992 human and animal sera from Hong Kong were tested by MN at a screening dilution of 1:10 and 1:20 (Table 1). None of the age-stratified human sera (n=528), swine sera (n=260) or wild bird sera (n=204) collected in Hong Kong had any neutralising activity for MERS-CoV in the MN tests. Similarly, none of the sera from humans (n=815), water buffaloes (n=8), cows (n=25), sheep (n=5) and goats (n=13) collected in Egypt were positive in the screening MN tests. In contrast, 103 of 110 sera collected in Egypt from dromedary camels neutralised MERS-CoV at the screening dilution of 1:20 or higher.

Entry of MERS pseudoparticles was shown to be inhibited by increasing concentrations of 0–20 mM NH_4Cl (data not shown), demonstrating pH dependent entry of the MERS pseudoparticles as previously reported [14]. The MERS ppNT assay was evaluated using two sera from experimentally infected macaques, one negative control serum from an uninfected macaque, a human convalescent serum from a MERS patient and five negative human control sera from Hong Kong (Figure 1).

The MERS ppNT assay was then used to screen 115 human sera from Hong Kong and 100 randomly selected human sera from Egypt which were all serologically negative for MERS-CoV. Sixteen dromedary camel sera that were positive in the MN screening assay were all found to have a high neutralising activity in the ppNT assay. In addition, five of six sera that were negative in the MN assay had a weak, but detectable, activity in the ppNT test (Table 1, Table 2, Figure 2). The camel sera that were found to be positive at a screening dilution of 1:20 in the MN test had antibody titres in the MERS NT screen ranging from 40 to 1,280 and higher, and MERS ppNT titres ranging from 640 to 10,240 and higher. One of the five MERS MN-negative sera was negative in the MERS ppNT assay, while the other four had low MERS ppNT titres ranging from 40 to 160.

Table 1

Screening results for MERS-CoV microneutralisation and MERS-CoV spike protein pseudoparticle neutralisation, human and animal samples from Egypt and Hong Kong, 2012–2013 (n=1,968)

Sera	Source of sera	MERS-CoV micro-neutralisation titre ≥1:20		MERS-CoV spike pseudotype antibody titre ≥1:20	
		Total tested	% Positive (n)	Total tested	% Positive (n)
Human[a]	Egypt	815	0 (0/815)	100	0 (0/100)
Goat[b]		13	0 (0/13)	ND	ND
Sheep[b]		5	0 (0/5)	ND	ND
Water buffalo[b]		8	0 (0/8)	ND	ND
Cow[b]		25	0 (0/25)	ND	ND
Camel[b]		110	93.6 (103/110)	110	98.2 (108/110)
Human	Hong Kong	528	0 (0/528)	115	0 (0/115)
Swine		260	0 (0/260)	ND	ND
Wild bird		204	0 (0/204)	ND	ND

MERS-CoV: Middle East respiratory syndrome coronavirus; ND: not done.

[a] Collected in 2012–13.

[b] Collected in June 2013.

Details of sera collected in Hong Kong as given in Methods.

The correlation of the MERS MN and MERS ppNT titres are shown in Figure 3 (Pearson's correlation coefficient: R=0.88). The MERS ppNT test appears to be more sensitive than the MERS MN test, and thus some of the apparently MN-negative camel sera give low titre-positive results in the MERS ppNT assay. However, none of the human sera from Egypt (n=100) or Hong Kong (n=115) had any detectable antibody in the MERS ppNT test. None of the camel sera with high antibody titres to MERS-CoV had any cross-neutralising activity to SARS-CoV (Table 2).

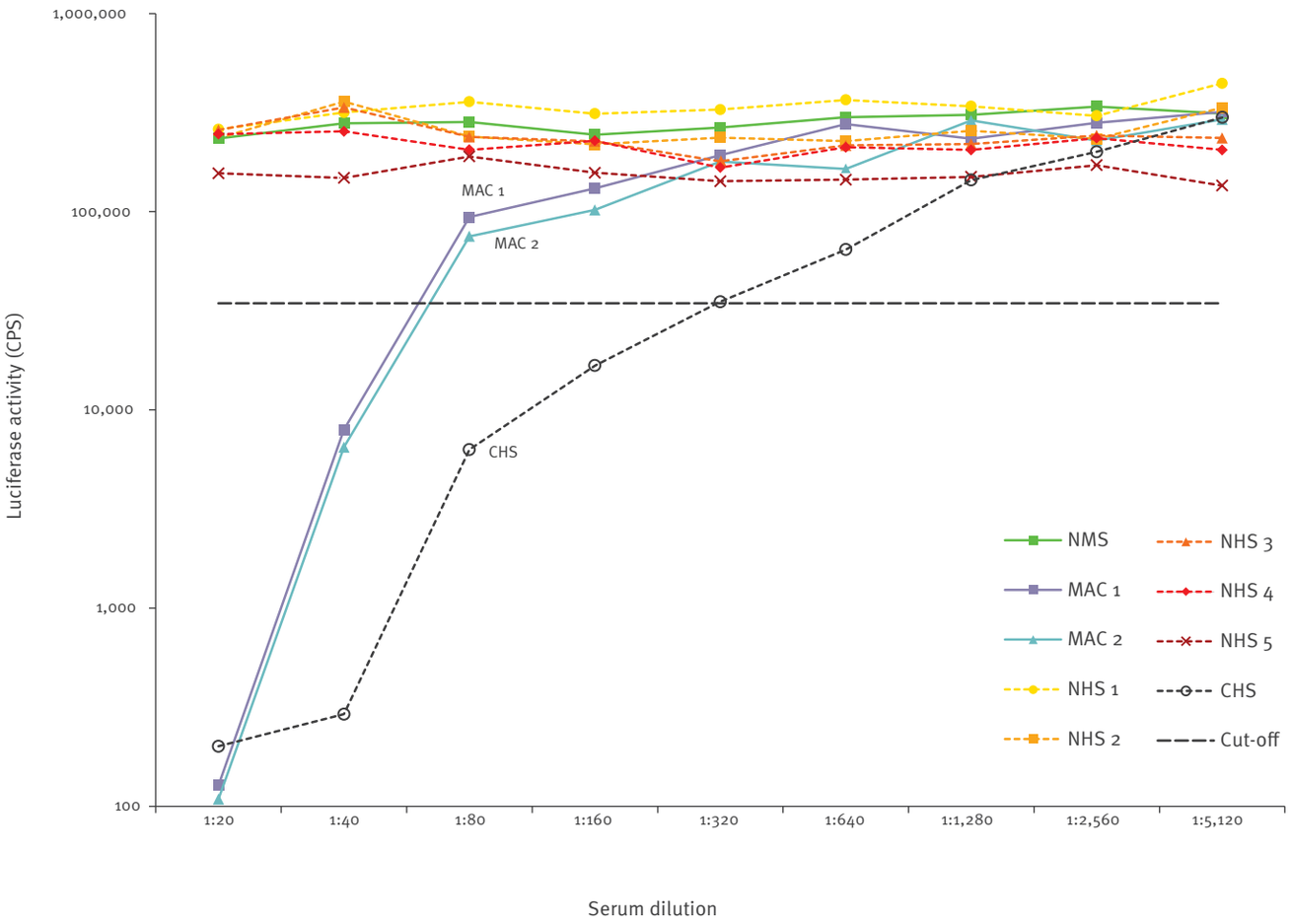
Discussion

Of 1,968 human and animal sera tested by MERS-CoV MN and 325 human and animal sera tested by MERS-CoV ppNT assays, only sera from dromedary camels had any neutralising antibody activity to the MERS-CoV. Of the 110 camel sera, 93.6% were seropositive by MERS-CoV MN test and 98.2% were seropositive by MERS-CoV ppNT test. The antibody titres were very high in MN as well as ppNT, suggesting that the virus infecting these camels was MERS-CoV virus itself or a very closely related virus.

It is known that dromedary camels host bovine coronaviruses (BCoV) which are lineage A beta-coronaviruses. However cross-neutralisation between MERS-CoV (lineage C beta-coronavirus) and BCoV was excluded by Reusken and colleagues in their study of sera from dromedary camels [10]. Furthermore, BCoV is antigenically closely related to the human coronavirus OC43. Human beta-coronavirus lineage A viruses OC43 and

Figure 1

MERS-CoV spike protein pseudoparticle neutralisation, human and animal samples from Egypt and Hong Kong, 2012–13 (n=9)



CPS: counts per second; MERS-CoV: Middle East respiratory syndrome coronavirus;

As positive controls, we used a convalescent human serum (CHS) from a patient with MERS, kindly provided by Dr C Drosten (Institute of Virology, University of Bonn Medical Centre, Bonn, Germany) and sera from two experimentally infected macaques (MAC1, MAC2), kindly provided by Bart Haagmans (Erasmus University Medical Center, Rotterdam, the Netherlands). As negative controls we used serum from a non-infected control macaque (NMS) and five human sera (NHS 1–5) from Hong Kong. The horizontal dotted line represents the 90% reduction in luciferase activity which represents the cut-off for positivity in the assay. Each batch of assays had the cut-off determined with reference to a serum-free negative control, and the data represented here are a compilation of two experiments. Thus the cut-off line is a representative indication based on an average of cut-offs used in seperate experiments.

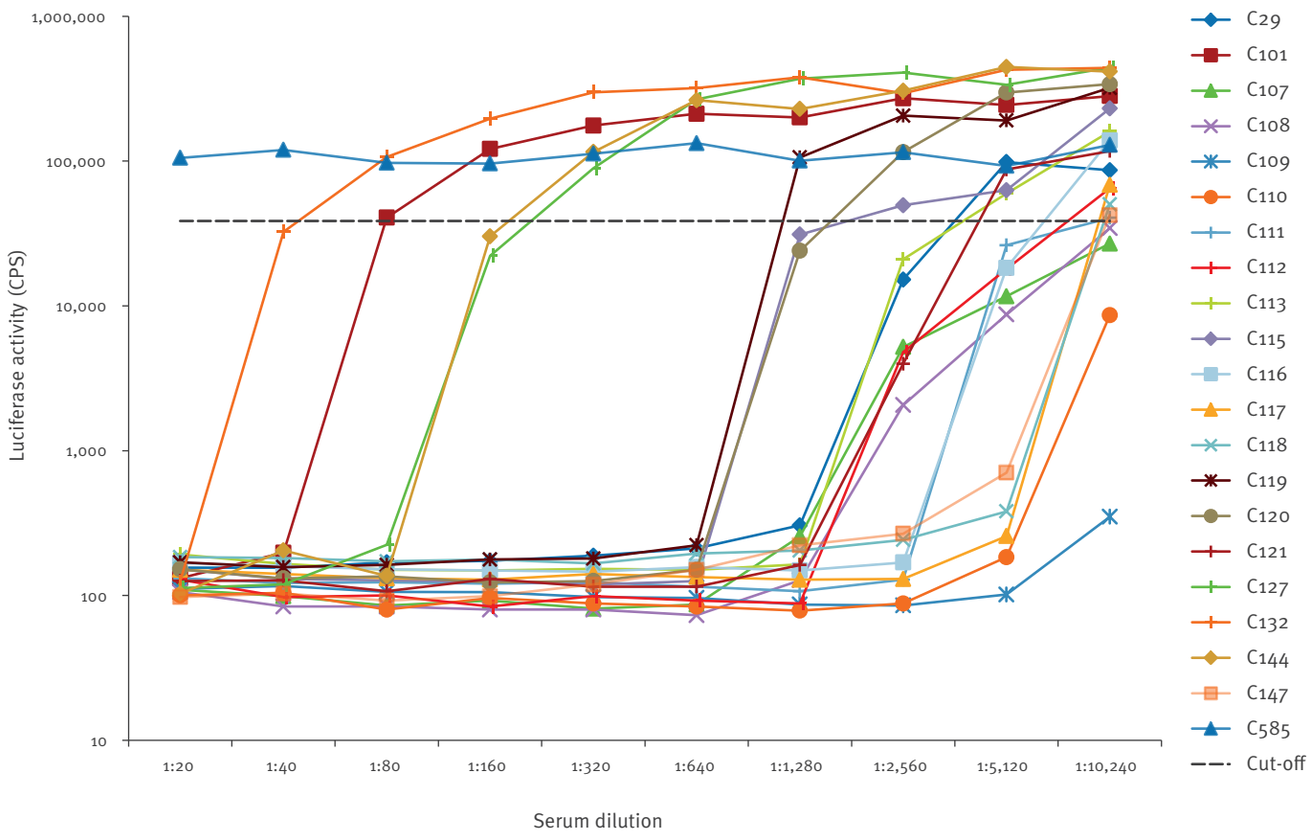
HKU1, and alpha-coronaviruses (229E and NL63) are ubiquitous respiratory viruses infecting humans and the panel of human sera of different ages tested can be expected to have varying levels of antibody to these viruses. The lack of any MERS-neutralising activity in the human sera we studied also indicates that the MN and ppNT assays are specific for MERS-CoV. The lack of cross-reactivity with convalescent serum from patients with SARS provides additional evidence of the lack of cross-reactivity in the MERS-CoV serology assays. Furthermore, it is notable that the camel sera with high antibody titres to MERS-CoV did not cross-react with SARS-CoV, a beta-coronavirus of lineage B. Taken together these data indicate that a MERS-CoV or a highly related virus is endemic in dromedary camels imported for slaughter in Egypt. These findings provide independent confirmation of the results recently reported by Reusken et al. who found very high antibody titres to MERS-CoV in dromedary camels [10].

The dromedary camels sampled in our study were those brought to abattoirs for slaughter in Cairo and in the Qalyubia governorate in the Nile Delta region. These animals were sourced from other East African countries such as Sudan and held in Egypt for some time prior to slaughter. Thus it is unclear where the animals originally acquired the infection. Considering the similar data from dromedary camels in Oman and the Canary Islands [10], it is likely that this coronavirus is widespread in North and East Africa and the Arabian peninsula.

There is substantial movement of people between Egypt and Saudi Arabia and other states on the Arabian peninsula, and thus it is possible that people may get infected, either as part of their travel to endemic areas or through zoonotic transmission within the country. There is also much movement of livestock across these Middle Eastern countries. The lack of antibody to MERS-CoV in sera of people resident in Egypt indicates

Figure 2

MERS-CoV spike protein pseudoparticle neutralisation on selected sera from dromedary camels, Egypt, June, 2013 (n=21)



CPS: counts per second; MERS-CoV: Middle East respiratory syndrome coronavirus; MN: microneutralisation; ppNT: pseudoparticle neutralisation.

Sixteen sera found to be positive and five sera found to be negative in the MERS-CoV MN screening assay were titrated in the MERS-CoV ppNT assay. The sera used are shown in Table 2. The horizontal dotted line represents the 90% reduction in luciferase activity which represents the cut-off for positivity in the assay.Each batch of assays had the cut-off determined with reference to a serum-free negative control and the data represented here are a compilation of two experiments. Thus the cut-off line is a representative indication based on an average of cut-offs used in seperate experiments.

that this infection is not common in Egypt, either as an infection acquired through travel or as an occasional zoonotic infection.

The MERS-CoV ppNT assay described here is a safe and specific assay for large scale seroepidemiological studies in a range of animal species, and such studies are urgently needed in regions where MERS-CoV cases have been detected as well as other regions. The HIV backbone used for pseudoparticle production is not replication-competent and the MERS-CoV pseudoparticles can therefore be produced and used in Biosafety Level 2 containment; in contrast, MN assays involve handling of the live MERS-CoV and require Biosafety Level 3 containment which is not always available in affected regions. Unlike immunoassays, there is no requirement for finding and optimising an enzyme-labelled anti-Ig conjugate for each species to be investigated. Furthermore, the MERS-CoV ppNT assay appears around 10 times more sensitive than the conventional MN assay (Figure 3, Table 2). The MN assay is a neutralisation assay based on $TCID_{50}$ rather than a plaque reduction assay, which perhaps makes it less sensitive than a plaque neutralisation assay. In any event, experience with influenza virus serology using pseudoparticle assays has shown that they are more sensitive than conventional MN assays for detecting neutralising antibodies. Thus MERS-CoV ppNT can be used as a screening assay, and positive sera can be retested for confirmation in a MERS MN tests.

Serological data does not provide proof that the virus infecting dromedary camels is the MERS CoV, and infection by a closely related coronavirus or a chimeric virus with a MERS-CoV-like spike protein cannot be ruled out until the dromedary camel virus is detected and genetically sequenced. However, it provides a strong impetus to attempt to seek the virus in specimens from these animals and to identify the MERS-related virus that appears to be infecting them. These serological studies also need to be extended to other domestic animals species to define the circulation of MERS-CoV or related viruses in animals in close contact with humans. Such studies should also include humans exposed to dromedary camels. It is important to note that waning antibody levels may result in false-negative serology results, and this is particularly relevant in mild or asymptomatic episodes of infection where the peak antibody titre may be lower and drop more quickly.

Table 2

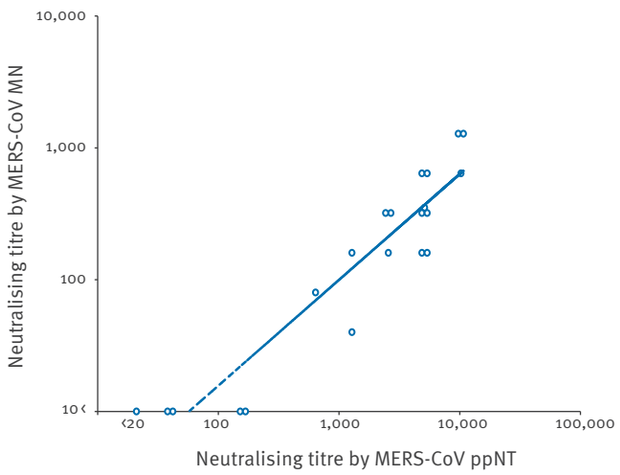
Antibody titres of selected sera from dromedary camels tested by microneutralisation for MERS-CoV and SARS-CoV and by MERS spike protein pseudoparticle neutralisation, Egypt, June, 2013 (n=21)

Camel sera	Antibody titres		
	MERS-CoV MN test	SARS-CoV MN test	MERS-CoV ppNT test
C101	<10 Negative	<10 Negative	40
C127	<10 Negative	<10 Negative	160
C132	<10 Negative	<10 Negative	40
C144	<10 Negative	<10 Negative	160
C585	<10 Negative	<10 Negative	<20 Negative
C29	320	<10 Negative	2,560
C107	160	<10 Negative	5,120
C108	160	<10 Negative	5,120
C109	640	<10 Negative	≥10,240
C110	≥1,280	<10 Negative	≥10,240
C111	320	<10 Negative	5,120
C112	320	<10 Negative	5,120
C113	320	<10 Negative	2,560
C115	160	<10 Negative	1,280
C116	320	<10 Negative	5,120
C117	640	<10 Negative	5,120
C118	640	<10 Negative	5,120
C119	80	<10 Negative	640
C120	40	<10 Negative	1,280
C121	160	<10 Negative	2,560
C147	≥1,280	<10 Negative	≥10,240

MERS-CoV: Middle East respiratory syndrome coronavirus; MN: microneutralisation; ppNT: pseudoparticle neutralisation; SARS-CoV: severe acute respiratory syndrome coronavirus.

Figure 3

Correlation of MERS-CoV antibody titres determined by MERS-CoV microneutralisation and MERS-CoV spike protein pseudoparticle neutralisation in selected sera from dromedary camels, Egypt, June, 2013 (n=21)



MERS-CoV: Middle East respiratory syndrome coronavirus; MN: microneutralisation; ppNT: pseudoparticle neutralisation.

The data used as those shown in Table 2. In the event of overlapping dots, their MN titre (X axis) was increased or decreased by 0.05% to slightly offset the overlap for ease of observation. The limit of detection in the MN and ppNT assays were titres of 10 and 20 respectively; and thus these values on the Y and X axis correspond to <10 and <20, respectively.

If the detection of MERS-CoV in insectivorous bats is confirmed [8] and if indeed the coronavirus we and others demonstrated to be common in dromedary camels is confirmed to be MERS-CoV, we will have a scenario of a virus reservoir in bats with a peridomestic animal such as the camel as intermediate host, which may in fact be the immediate source of human infection. It is notable that a number of index cases with MERS-CoV had a history of exposure to camels, although this is by no means universally the case. Given that the MERS-like coronavirus in camels appears to be ubiquitous, it remains to be explained why MERS in humans appears relatively rare. Coronaviruses are well known to mutate to markedly change virulence or host range. Examples are the emergence of the less pathogenic porcine respiratory coronavirus from virulent transmissible gastroenteritis virus of pigs, or virulent feline infectious peritonitis viruses emerging from low pathogenic feline coronaviruses [15]. Furthermore, the SARS-like virus detected in civets and other small mammals in live animal markets in southern China in 2002–03 initially appeared to infect humans, who appear to have seroconverted, but with minimal disease and onward transmission [16], while a few amino acid changes in the SARS-CoV spike protein allowed that virus to acquire efficient transmissibility and virulence in humans [17]. Thus, previous experience with animal and human coronaviruses highlights the public health urgency of investigations of MERS-CoV and MERS-CoV-like viruses in domestic and wild animals.

Acknowledgements

We thank Dr CYH Leung for providing wild bird sera from Hong Kong. This study was supported in part by research contract from the National Institute of Allergy and Infectious Diseases (NIAID) contract HHSN266200700005C and a grant from the European Community Seventh Framework Programme (FP7/2007-2013) under project European management Platform for Emerging and Re-emerging Disease entities (Grant agreement No. 223498) (EMPERIE).

Conflict of interest

None declared.

Authors' contributions

Pei-gang Wang developed the MERS-CoV pseudotype assay and carried out the tests. Ranawaka AMP Perera developed the MERS-CoV microneutralisation test and carried out the tests in BSL3 containment. Leo LLM Poon and Yi Guan provided advice on laboratory methods. Lewis YL Siu and Mingyuan Li carried out the MERS-CoV pseudoparticle assays. Mokhtar R. Gomaa, Rabeh El-Shesheny, Ahmed Kandeil, Ola Bagato, Mahmoud M. Shehata, Ahmed S. Kayed and Yassmin Moatasim collected human and animal sera in Egypt. Richard J. Webby and Mohamed A. Ali provided advice on field study design. Joseph SM Peiris and Ghazi Kayali designed and coordinated the study and wrote the manuscript. All authors reviewed and commented on the manuscript.

References

1. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-20. http://dx.doi.org/10.1056/NEJMoa1211721. PMid:23075143.
2. World Healh Organization (WHO). Middle East respiratory syndrome coronavirus (MERS-CoV) – update. Geneva: WHO; 29 Aug 2013. Available from: http://www.who.int/csr/don/2013_08_29/en/index.html
3. World Healh Organization (WHO). Middle East respiratory syndrome coronavirus (MERS-CoV) summary and literature update – as of 9 July 2013. Geneva: WHO. [Accessed 20 Aug 2013]. Available from: http://www.who.int/csr/disease/coronavirus_infections/update_20130709/en/index.html
4. Bermingham A, Chand MA, Brown CS, Aarons E, Tong C, Langrish C, et al. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. Euro Surveill. 2012;17(40):pii=20290. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20290
5. Assiri A, McGeer A, Perl TM, Price CS, Al Rabeeah AA, Cummings DA, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. N Engl J Med. 2013;369(5):407-16. http://dx.doi.org/10.1056/NEJMoa1306742. PMid:23782161.
6. Annan A, Baldwin HJ, Corman VM, Klose SM, Owusu M, Nkrumah EE, et al. Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. Emerg Infect Dis. 2013;19(3):456-9. http://dx.doi.org/10.3201/eid1903.121503. PMid:23622767. PMCid:PMC3647674.
7. Ithete NL, Stoffberg S, Corman VM, Cottontail VM, Richards LR, Schoeman MC, et al. Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. Emerg Infect Dis. 2013. EPub ahead of print. DOI: 10.3201/eid1910.130946. http://dx.doi.org/10.3201/eid1910.130946
8. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis. 2013. EPub ahead of print. DOI: 10.3201/eid1911.131172. http://dx.doi.org/10.3201/eid1911.131172
9. Cao WC, Liu W, Zhang PH, Zhang F, Richardus JH. Disappearance of antibodies to SARS-associated coronavirus after recovery. N Engl J Med. 2007;357(11):1162-3. http://dx.doi.org/10.1056/NEJMc070348. PMid:17855683.
10. Reusken CB, Haagmans BL, Müller MA, Gutierrez C, Godeke GJ, Meyer B, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. Lancet Infect Dis. 2013; pii: S1473-3099(13)70164-6. DOI: 10.1016/S1473-3099(13)70164-6. http://dx.doi.org/10.1016/S1473-3099(13)70164-6
11. Corman VM, Muller MA, Costabel U, Timm J, Binger T, Meyer B, et al. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Euro Surveill. 2012;17(49):pii=20334. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20334
12. Reusken C, Mou H, Godeke GJ, van der Hoek L, Meyer B, Muller MA, et al. Specific serology for emerging human coronaviruses by protein microarray. Euro Surveill. 2013;18(14):20441. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20441.PMid:23594517
13. Garcia JM, Lagarde N, Ma ES, de Jong MD, Peiris JS. Optimization and evaluation of an influenza A (H5) pseudotyped lentiviral particle-based serological assay. J Clin Virol. 2010;47(1):29-33. http://dx.doi.org/10.1016/j.jcv.2009.10.009. PMid:19897409.
14. Gierer S, Bertram S, Kaup F, Wrensch F, Heurich A, Krämer-Kühl A, et al. The spike protein of the emerging betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2, and is targeted by neutralizing antibodies. J Virol. 2013;87(10):5502-11. http://dx.doi.org/10.1128/JVI.00128-13. PMid:23468491.
15. Saif LJ. Animal coronaviruses: what can they teach us about the severe acute respiratory syndrome? Rev Sci Tech. 2004;23(2):643-60. PMid:15702725
16. Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003;302(5643):276-8. http://dx.doi.org/10.1126/science.1087139. PMid:12958366.
17. Li F. Structural analysis of major species barriers between humans and palm civets for severe acute respiratory syndrome coronavirus infections. J Virol. 2008;82(14):6984-91. http://dx.doi.org/10.1128/JVI.00442-08. PMid:18448527. PMCid:PMC2446986.

Genetic analysis of novel avian A(H7N9) influenza viruses isolated from patients in China, February to April 2013

T Kageyama[1,2], S Fujisaki[1,2], E Takashita[1], H Xu[1], S Yamada[3], Y Uchida[4], G Neumann[5], T Saito[4,6], Y Kawaoka[3,5,7,8], M Tashiro (mtashiro@nih.go.jp)[1]

1. Influenza Virus Research Center, National Institute of Infectious Diseases, Tokyo, Japan
2. These authors contributed equally to this work
3. Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, Japan
4. Influenza and Prion Disease Research Center, National Institute of Animal Health, National Agriculture and Food Research Organization, Ibaraki, Japan
5. Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, United States
6. The United Graduate School of Veterinary Sciences, Gifu University, Gifu, Japan
7. ERATO Infection-Induced Host Responses Project, Japan Science and Technology Agency, Saitama, Japan
8. Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Citation style for this article:
Kageyama T, Fujisaki S, Takashita E, Xu H, Yamada S, Uchida Y, Neumann G, Saito T, Kawaoka Y, Tashiro M. Genetic analysis of novel avian A(H7N9) influenza viruses isolated from patients in China, February to April 2013. Euro Surveill. 2013;18(15):pii=20453. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20453

Article submited on 08 April 2013 / published on 12 April 2013

Novel influenza viruses of the H7N9 subtype have infected 33 and killed nine people in China as of 10 April 2013. Their haemagglutinin (HA) and neuraminidase genes probably originated from Eurasian avian influenza viruses; the remaining genes are closely related to avian H9N2 influenza viruses. Several characteristic amino acid changes in HA and the PB2 RNA polymerase subunit probably facilitate binding to human-type receptors and efficient replication in mammals, respectively, highlighting the pandemic potential of the novel viruses.

Humans are rarely infected with avian influenza viruses, with the exception of highly pathogenic avian influenza A(H5N1) viruses, which have caused 634 infections and 371 deaths as of 12 March 2013 [1]. A few isolated cases of human infection with viruses of the H7N2, H7N3, and H7N5 subtypes have been reported, but none were fatal [2-11]. In 2003, in the Netherlands, 89 people were infected with an influenza virus of the H7N7 subtype that caused conjunctivitis and one fatality [5,7].

On 19 February 2013, an 87 year-old man in Shanghai developed a respiratory infection and died on 4 March, and on 27 February 2013, a 27 year-old pork seller in a Shanghai market became ill and died on 10 March. A 35 year-old woman in Chuzhou City in Anhui province (west of Shanghai), who had contact with poultry, became ill on 15 March 2013, and remains hospitalised in critical condition. There is no known epidemiological relationship among these three cases. A 38 year-old man in Hangzhou (Zhejiang province, south of Shanghai) became ill on 7 March 2013 and died on 27 March. All four cases presented with respiratory infections that progressed to severe pneumonia and breathing difficulties.

On 31 March 2013, the Chinese Centre for Disease Control and Prevention announced the isolation in embryonated eggs of avian influenza viruses of the H7N9 subtype (designated A/Shanghai/1/2013, A/Shanghai/2/2013, and A/Anhui/1/2013) from the first three cases. The sequences of the coding regions of all eight viral genes were deposited in the influenza sequence database of the Global Initiative on Sharing All Influenza Data (GISAID) on 31 March (Table 1). On 5 April 2013, the Hangzhou Center for Disease Control and Prevention deposited the haemagglutinin (HA), neuraminidase (NA), and matrix (M) gene sequences of A/Hongzhou/1/2013 virus (Table 1), which was isolated in cell culture from samples obtained from the 38 year-old man.

All four human influenza A(H7N9) viruses are similar at the nucleotide and amino acid levels, suggesting a common ancestor. The HA gene of the novel viruses belongs to the Eurasian lineage of avian influenza viruses and shares ca. 95% identity with the HA genes of low pathogenic avian influenza A(H7N3) viruses isolated in 2011 in Zhejiang province (south of Shanghai) (Figure 1, Table 2). The NA gene of the novel viruses is ca. 96% identical to the low pathogenic avian influenza A(H11N9) viruses isolated in 2010 in the Czech Republic (Figure 1, Table 2).

Table 1

Origin of influenza A(H7N9) isolates included in the phylogenetic analysis, China, February–April 2013 (n=7)

Segment ID	Segment	Isolate name	Collection date	Originating Laboratory	Submitting Laboratory	Submitter/ Authors
EPI439488	PB2	A/Shanghai/1/2013	2013	-	WHO Chinese National Influenza Center	Lei Yang
EPI439489	PB1					
EPI439490	PA					
EPI439486	HA					
EPI439491	NP					
EPI439487	NA					
EPI439493	M					
EPI439494	NS					
EPI439495	PB2	A/Shanghai/2/2013	2013	-		
EPI439501	PB1					
EPI439498	PA					
EPI439502	HA					
EPI439496	NP					
EPI439500	NA					
EPI439497	M					
EPI439499	NS					
EPI439504	PB2	A/Anhui/1/2013	2013	-		
EPI439508	PB1					
EPI439503	PA					
EPI439507	HA					
EPI439505	NP					
EPI439509	NA					
EPI439506	M					
EPI439510	NS					
EPI440095	HA	A/Hangzhou/1/2013	2013-03-24	Hangzhou Center for Disease Control and Prevention	Hangzhou Center for Disease Control and Prevention	Li,J; Pan,JC; Pu,XY; Yu,XF; Kou,Y; Zhou,YY
EPI440096	NA					
EPI440097	M					
EPI440682	PB2	A/Chicken/Shanghai /S1053/2013	2013-04-03	Harbin Veterinary Research Institute	Harbin Veterinary Research Institute	Huihui Kong
EPI440683	PB1					
EPI440681	PA					
EPI440685	HA					
EPI440678	NP					
EPI440684	NA					
EPI440680	M					
EPI440679	NS					
EPI440690	PB2	A/Environment/ Shanghai /S1088/2013	2013-04-03			
EPI440691	PB1					
EPI440689	PA					
EPI440693	HA					
EPI440686	NP					
EPI440692	NA					
EPI440688	M					
EPI440687	NS					
EPI440698	PB2	A/Pigeon/Shanghai /S1069/2013	2013-04-02			
EPI440699	PB1					
EPI440697	PA					
EPI440701	HA					
EPI440694	NP					
EPI440700	NA					
EPI440696	M					
EPI440695	NS					

We gratefully acknowledge the authors and laboratories for originating and submitting these sequences to the EpiFlu database of the Global Initiative on Sharing All Influenza Data (GISAID); these sequences were the basis for the research presented here.

All submitters of data may be contacted directly via the GISAID website www.gisaid.org

Figure 1

Phylogenetic analysis of the haemagglutinin (A) and neuraminidase (B) genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)

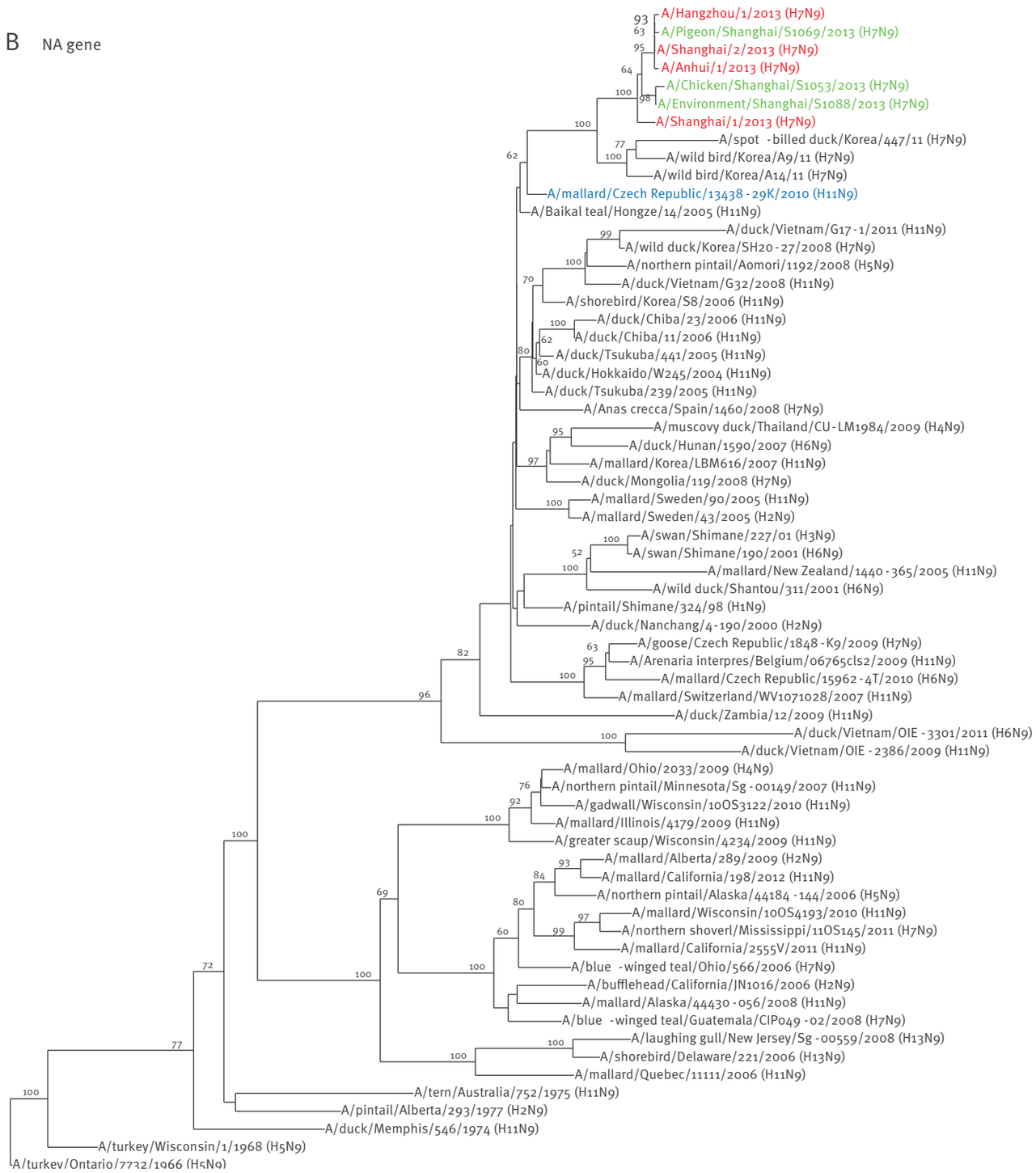
HA: haemagglutinin; NA: neuraminidase.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

Novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green; viruses with the highest similarities to the novel viruses are shown in blue. The HA clade names, North America, South America, and Eurasia, are based on epidemiological studies of H7 viruses [27,28].

Figure 1

Phylogenetic analysis of the haemagglutinin (A) and neuraminidase (B) genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



HA: haemagglutinin; NA: neuraminidase.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

Novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green; viruses with the highest similarities to the novel viruses are shown in blue. The HA clade names, North America, South America, and Eurasia, are based on epidemiological studies of H7 viruses [27,28].

Table 2

Nucleotide identity of novel influenza A(H7N9) virus genes and their closest relative, China, February–April 2013

Viral gene	Closest influenza virus relative	Nucleotide identity (%)
PB2	A/brambling/Beijing/16/2012(H9N2)	99
PB1	A/chicken/Jiangsu/Q3/2010(H9N2)	98
PA	A/brambling/Beijing/16/2012(H9N2)	99
HA	A/duck/Zhejiang/12/2011(H7N3)	95
NP	A/chicken/Zhejiang/611/2011(H9N2)	98
NA	A/mallard/Czech Republic/13438-29K/2010(H11N9)	96
M	A/chicken/Zhejiang/607/2011(H9N2)	98
NS	A/chicken/Dawang/1/2011(H9N2)	99

HA: haemagglutinin; M: matrix gene; NA: neuraminidase; NP: nucleoprotein; NS: non-structural gene; PA: RNA polymerase acidic subunit; PB1: RNA polymerase basic subunit 1; PB2: RNA polymerase basic subunit 2.

The sequences of the remaining viral genes are closely related (>97% identity) to avian influenza A(H9N2) viruses, which recently circulated in poultry in Shanghai, Zhejiang, Jiangsu, and neighbouring provinces of Shanghai (Table 2, Figure 2). These findings strongly suggest that the novel influenza A(H7N9) viruses are reassortants that acquired their H7 HA and N9 NA genes from avian influenza viruses, and their remaining genes from recent influenza A(H9N2) poultry viruses (Figure 1, Figure 3, Table 2).

At the nucleotide level, A/Shanghai/2/2013, A/Anhui/1/2013, and A/Hangzhou/1/2013 share more than 99% identity and differ by no more than three nucleotides per gene, even though they were isolated in different cities several hundred kilometres apart. On 7 April 2013, the Harbin Veterinary Research Institute deposited the full genome sequences of isolates from a pigeon (A/pigeon/Shanghai/S1069/2013), a chicken (A/chicken/Shanghai/S1053/2013), and an environmental sample (A/environment/Shanghai/S1088/2013) that were collected on 2 and 3 April from a Shanghai market (Table 1). All eight genes of these three isolates are similar to those of A/Shanghai/2/2013 and A/Anhui/1/2013 at the nucleotide level, except for the PB1 gene of A/pigeon/Shanghai/S1069/2013, which belongs to a different lineage than the PB1 of the other H7N9 isolates (Figures 1 and 2).

Interestingly, A/Shanghai/1/2013 and A/Shanghai/2/2013 differ by 52 nucleotides (for example, there are 13 nucleotide and nine amino acid differences in their HA sequences) even though these two cases were identified in the same city and at around the same time. These findings suggest that A/Shanghai/2/2013, A/Anhui/1/2013, A/Hangzhou/1/2013, as well as the viruses from the chicken and the environment, share a closely related source of infection, whereas A/Shanghai/1/2013 and A/pigeon/Shanghai/S1069/2013 are likely to have originated from other sources.

Highly pathogenic avian influenza viruses are characterised by a series of basic amino acids at the HA cleavage site that enable systemic virus spread. The HA cleavage sequence of the novel influenza A(H7N9) viruses possesses a single basic amino acid (EIPKGR*GL; *indicates the cleavage site), suggesting that these viruses are of low pathogenicity in avian species.

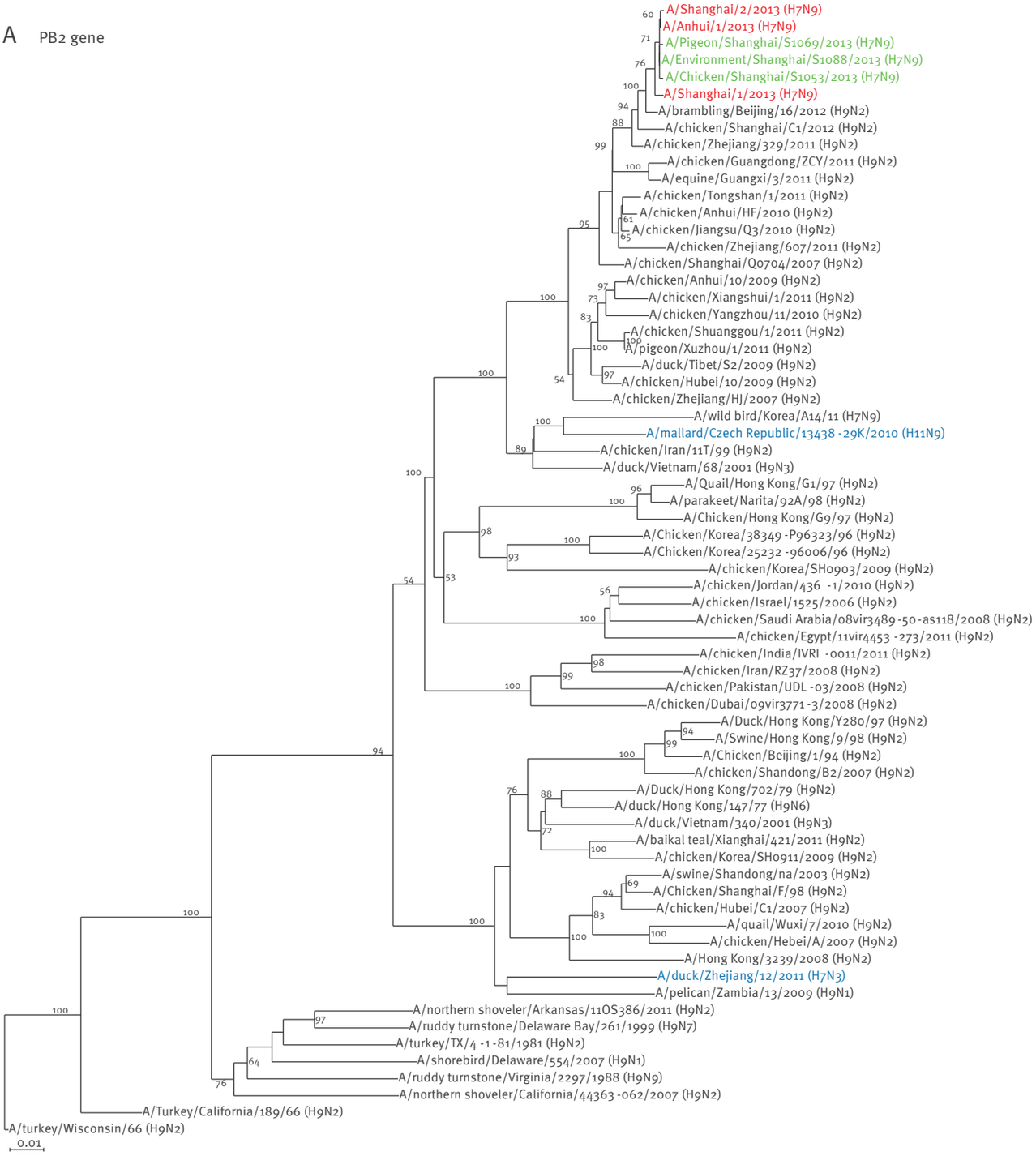
The amino acid sequence of the receptor-binding site (RBS) of HA determines preference for human- or avian-type receptors. At this site, A/Shanghai1/2013 encodes an A138S* mutation (H3 numbering; Figure 4, Table 3), whereas A/Shanghai/2/2013, A/Anhui/1/2013, the two avian isolates, and the virus from the environmental sample encode G186V and Q226L mutations; any of these three mutations could increase the binding of avian H5 and H7 viruses to human-type receptors [12-14]. The finding of mammalian-adapting mutations in the RBS of these novel viruses is cause for concern. The A/Hangzhou/1/2013 isolate encodes isoleucine at position 226, which is found in seasonal influenza A(H3N2) viruses.

In addition, all seven influenza A(H7N9) viruses possess a T160A substitution (H3 numbering; Table 3) in HA, which is found in recently circulating H7 viruses; this mutation leads to the loss of an *N*-glycosylation site at position 158 (H3 numbering; position 149 in H7 numbering), which results in increased virus binding to human-type receptors [15].

Lysine at position 627 of the polymerase PB2 protein is essential for the efficient replication of avian influenza viruses in mammals [16] and has been detected in highly pathogenic avian influenza A(H5N1) viruses and in the influenza A(H7N7) virus isolated from the fatal case in the Netherlands in 2003 [17]. PB2-627K is rare among avian H9N2 PB2 proteins (i.e. it has been found in only five of 827 isolates). In keeping with this finding, the avian and environmental influenza A(H7N9)

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



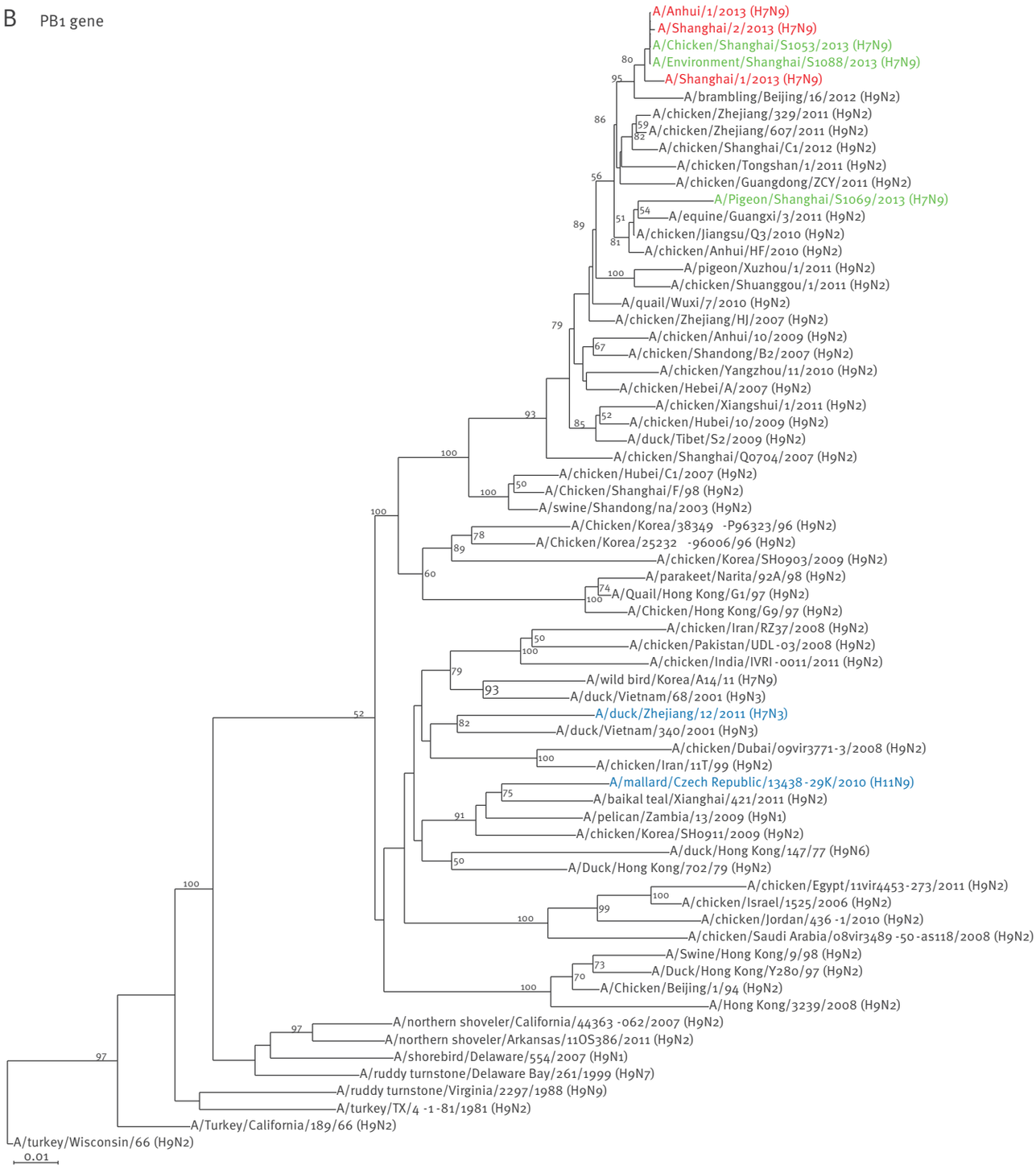
PB2: RNA polymerase basic subunit 2.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



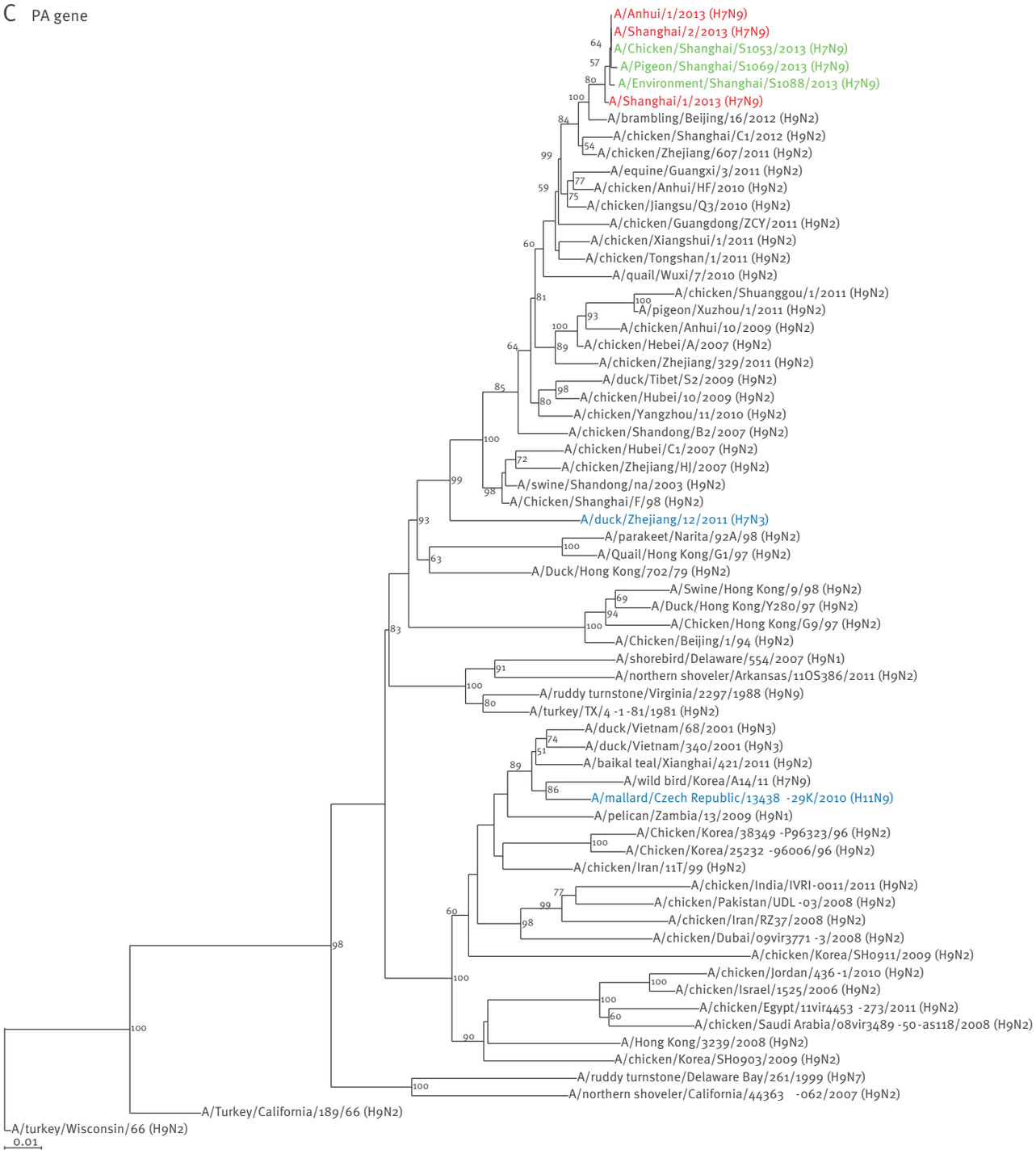
PB1: RNA polymerase basic subunit 1.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



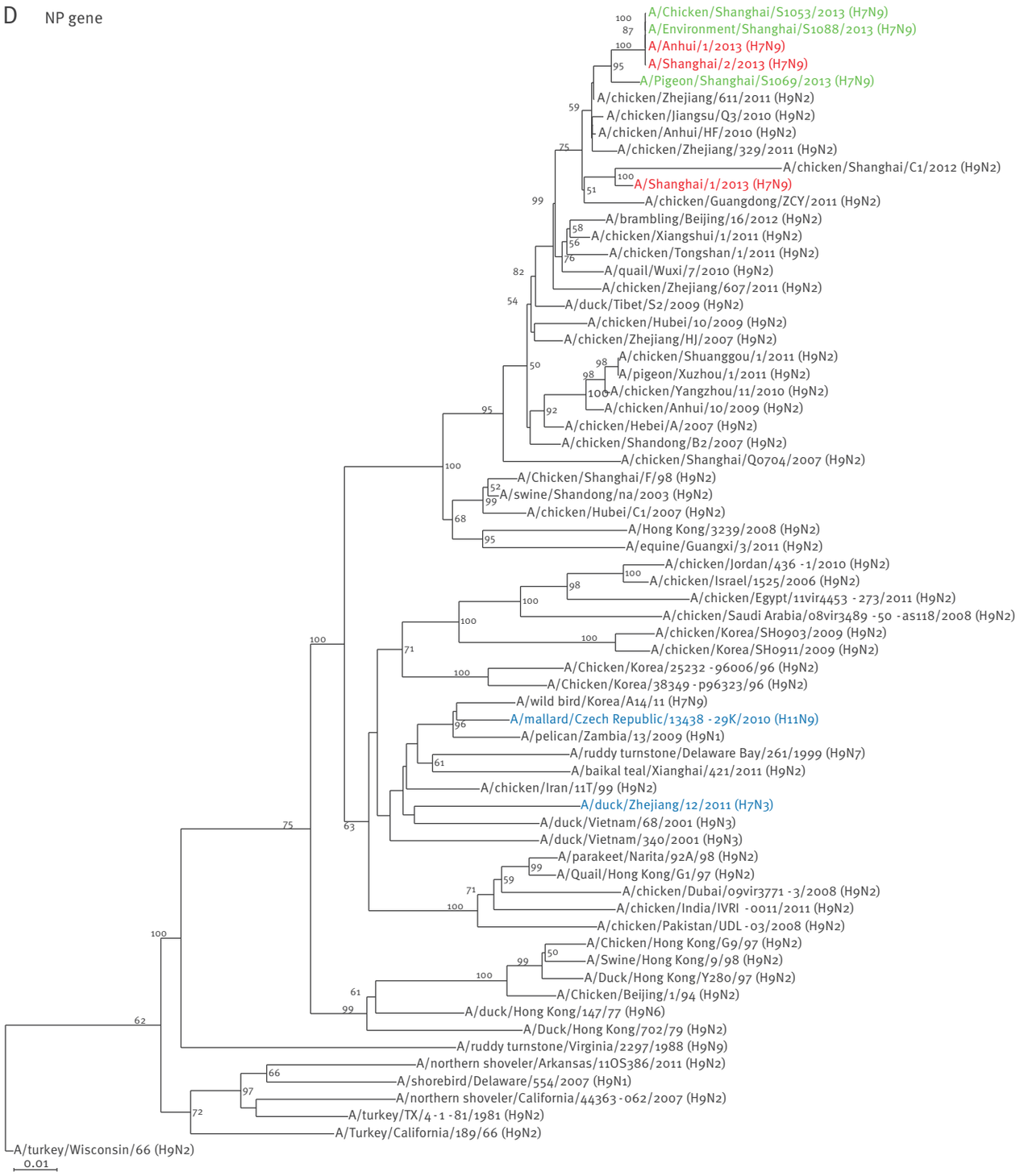
PA: RNA polymerase acidic subunit.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



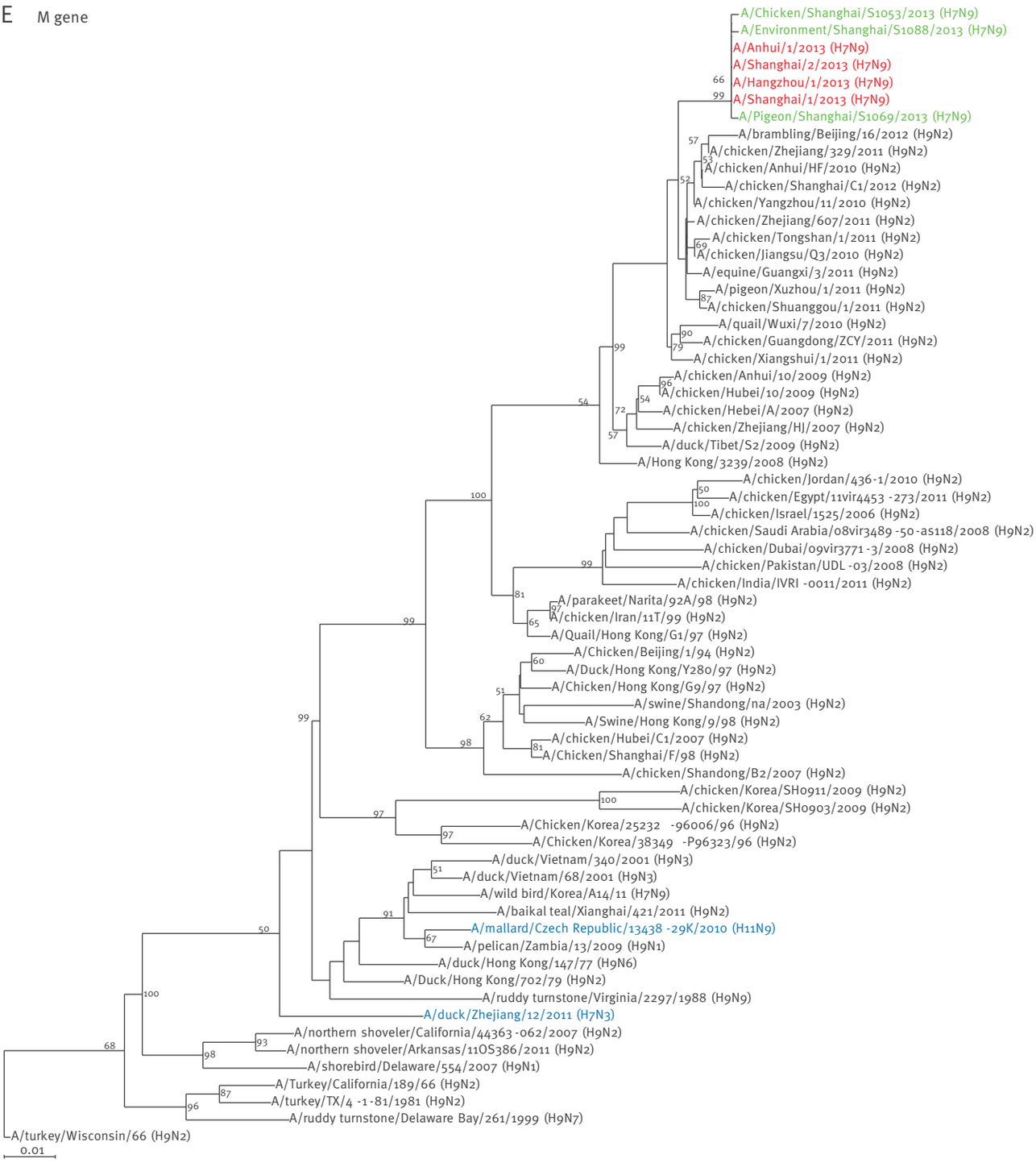
NP: nucleoprotein.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)



M: matrix gene.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 2

Phylogenetic analysis of the six remaining genes of the novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)

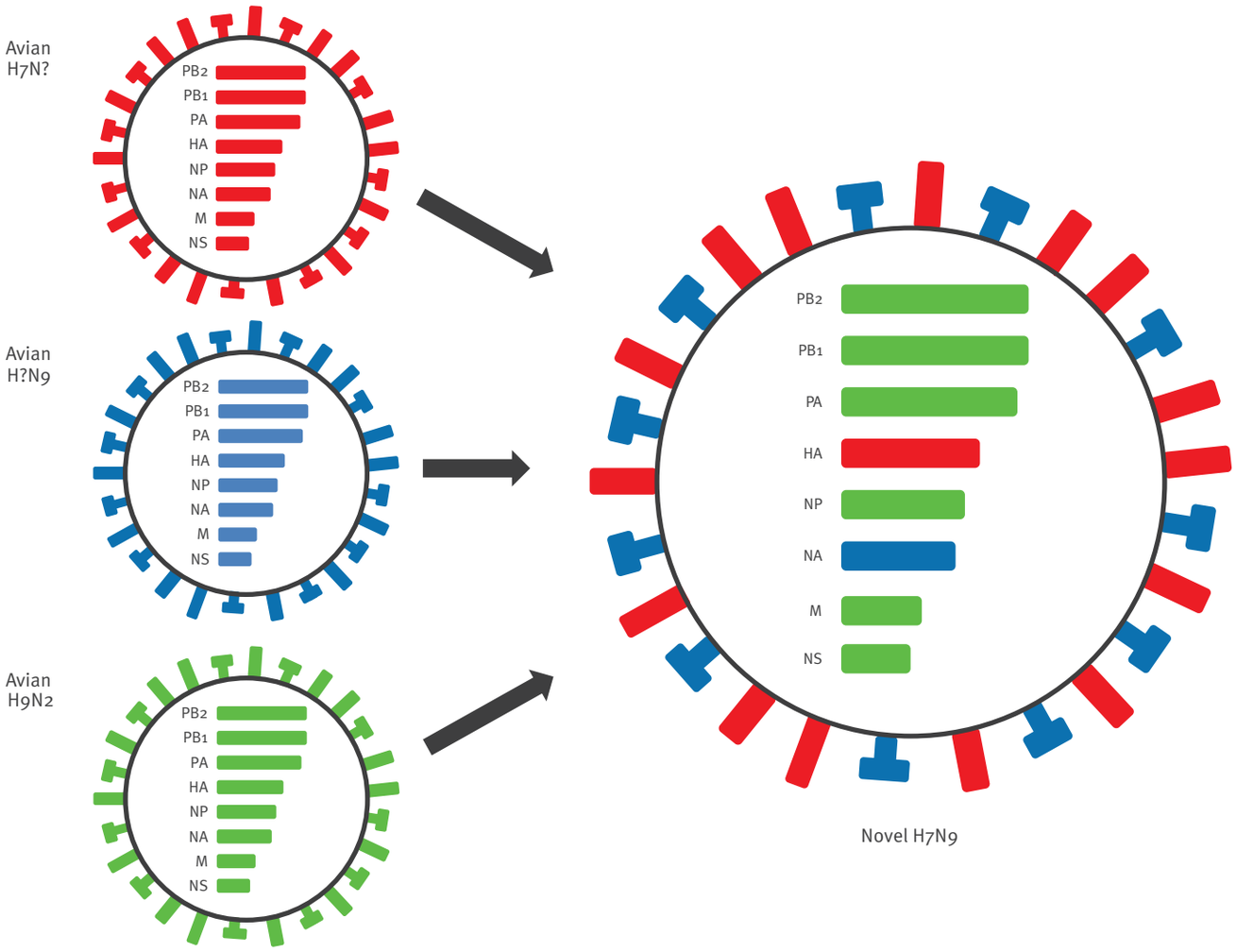
NS: non-structural gene.

Multiple alignments were constructed by using the CLUSTAL W algorithm. Genetic distances were calculated by using the Kimura's 2-parameter method [26], and phylogenetic trees were constructed by using the neighbour-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. Numbers next to nodes indicate bootstrap value percentages (>50%).

The novel human H7N9 viruses are shown in red; novel H7N9 viruses from birds and the environment are shown in green. Influenza viruses whose HA and NA genes are most closely related to the novel human H7N9 viruses are shown in blue.

Figure 3

Schematic diagram of novel influenza A(H7N9) virus generation



HA: haemagglutinin; NA: neuraminidase.

The novel influenza A(H7N9) viruses are likely to have acquired their HA gene from an avian H7 virus of unknown NA subtype, their NA gene from an avian N9 virus of unknown HA subtype, and their remaining six viral segments from avian H9N2 viruses circulating in poultry.

viruses analysed here encode PB2-627E. By contrast, all four human H7N9 viruses analysed here encode PB2-627K (Table 3).

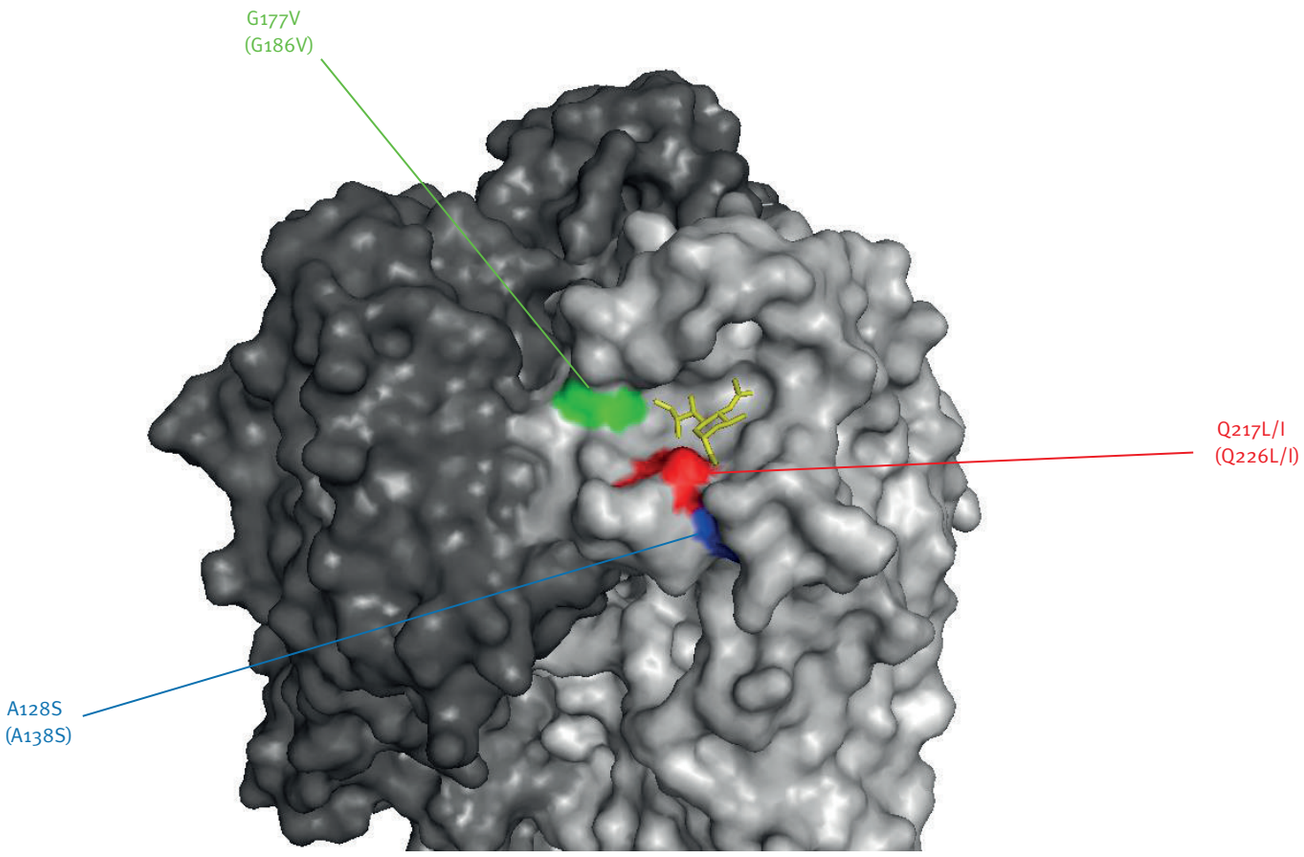
Antiviral compounds are the first line of defense against novel influenza viruses until vaccines become available. All seven novel influenza A(H7N9) viruses sequenced to date encode the S31N substitution in the viral ion channel M2 (encoded by the M segment) (Table 3), which confers resistance to ion channel inhibitors [18,19]. Based on the sequences of their NA proteins, all H7N9 viruses analysed here, with the exception of A/Shanghai/1/2013, should be sensitive to neuraminidase inhibitors (Table 3). However, the R294K mutation in the NA protein of A/Shanghai/1/2013 is known to confer resistance to NA inhibitors in N2 and N9 subtype viruses [20], and is therefore of great concern.

All H7N9 viruses encode a deletion at positions 69–73 of the NA stalk region (Table 3), which is reported to occur upon virus adaptation to terrestrial birds. This finding suggests that the novel H7N9 viruses (or their ancestor) may have circulated in terrestrial birds before infecting humans. Moreover, this deletion is associated with increased virulence in mammals [21].

The influenza A virus PB1-F2 protein (encoded by the PB1 segment) is also associated with virulence. The available sequences indicate that the H7N9 PB1 genes of all of the human viruses encode a full-length PB1-F2 of 90 amino acids, but lack the N66S mutation that is

Figure 4

Amino acid changes in the three novel influenza A(H7N9) viruses that may affect their receptor-binding properties, China, February–April 2013 (n=7)



Shown is the three-dimensional structure of three monomers (light and dark gray) of the influenza A(H7N7) virus (A/Netherlands/219/2003) haemagglutinin (accession code 4DJ8). Also shown is the part of 6'-sialyl-N-acetyllactosamine (a sialyloligosaccharide) to which human viruses bind preferentially (yellow). Indicated are amino acid changes in the H7N9 virus haemagglutinin protein at positions known to increase binding to human-type receptors.

associated with the increased pathogenicity of the 1918 pandemic virus and the highly pathogenic avian influenza A(H5N1) viruses [22]. Interestingly, the pigeon isolate encodes a truncated PB1-F2 of only 25 amino acids; the significance of this truncation is unknown.

The NS1 protein (encoded by the NS segment) is an interferon antagonist with several functions in the viral life cycle. All available H7N9 NS1 sequences lack the C-terminal PDZ domain-binding motif; the lack of the PDZ domain-binding motif may attenuate these viruses in mammals [23].

Other amino acids in the NS1 and matrix (M1; encoded by the M segment) proteins of the novel viruses are also associated with increased virulence (Table 3) [24.25]. However, these amino acids are found in many avian influenza viruses, and therefore, their significance for the biological properties of the novel influenza A(H7N9) viruses is currently unclear.

In conclusion, we here present a biological evaluation of the sequences of the avian influenza A(H7N9) viruses that caused fatal human infections in China. These viruses possess several characteristic features of mammalian influenza viruses, which are likely to contribute to their ability to infect humans and raise concerns regarding their pandemic potential.

***Authors' correction:**

The mutation A138S was erroneously written as S138A in the original publication. This mistake was corrected on 13 April 2013

Table 3

Selected characteristic amino acids of the three novel influenza A(H7N9) viruses, China, February–April 2013 (n=7)

Viral protein	Amino acid position	Shanghai/ 1/2013	Shanghai/ 2/2013	Anhui/ 1/2013	Hangzhou /1/2013	Chicken/ Shanghai/ S1053/2013	Environment/ Shanghai/ S1088/2013	Pigeon/ Shanghai/ S1069/2013	Human influenza viruses	Avian influenza viruses	Comments	Reference(s)
PB2	627	**K**	**K**	**K**	Nd	E	E	E	K	E	E627K: Mammalian host adaptation	16
HA	128/138[a]	**S**	A	A	A	A	A	A	A	A[b]	S138A: Increased virus binding to human-type receptors	13
	151/160[a]	**A**	**A**	**A**	**A**	**A**	**A**	**A**	K	**A**[b]	T160A: Loss of N-glycosylation and increased virus binding to human-type receptors	15
	177/186[a]	G	**V**	**V**	**V**	**V**	**V**	**V**	G	G[b]	G186V: Increased virus binding to human-type receptors	14
	217/226[a]	Q	**L**	**L**	**I**	**L**	**L**	**L**	I	Q[b]	Q226L: Increased virus binding to human-type receptors	12
NA	69–73[c]	**Deletion**	**Deletion**	**Deletion**	**Deletion**	**Deletion**	**Deletion**	**Deletion**	No deletion	No deletion	Deletion of amino acids 69–73: Increased virulence in mice	21
	289/294/292[d]	**K**	R	R	R	R	R	R	R	R	R294K: Reduced susceptibility to oseltamivir and zanamivir	20
M1	30	D	D	D	D	D	D	D	D/(S)	D	N30D: Increased virulence in mice (most influenza A viruses encode 30D)	24
	215	A	A	A	A	A	A	A	A	A	T215A: Increased virulence in mice (most avian influenza A viruses encode 215A)	24
M2	31	**N**	**N**	**N**	**N**	**N**	**N**	**N**	S/N	S/(N)	S31N: Reduced susceptibility to amantadine and rimantadine	18,19
NS1	42	S	S	S	Nd	S	S	S	S	S/A	P42S: Increased virulence in mice (most avian influenza A viruses encode 42S)	25
	218–230	**Deletion**	**Deletion**	**Deletion**	Nd	**Deletion**	**Deletion**	**Deletion**	No deletion[e]	No deletion/ Deletion	Lack of PDZ domain binding motif: Decreased virulence in mice	23

Substitutions of particular concern are shown in bold.

Nd: not determined.

[a] H7/H3 numbering.
[b] H7 virus.
[c] N9 numbering.
[d] H7N9/avian N9/N2 numbering.
[e] Influenza A(H1N1)pdm09 viruses from the 2009 influenza pandemic have the deletion.

Acknowledgements

We are grateful to Dr. Shu Yuelong, Chinese National Influenza Center, Chinese Center for Disease Control and Prevention, Beijing, China, for his rapid publication of the entire gene sequence data of A(H7N9) viruses isolated from human cases in China, and also for his information sharing and advice to this study. We also thank Susan Watson for scientific editing. This work was supported by Grants-in-Aid for Pandemic Influenza Research (TK, SF, HX, and MT) and Grant-in-Aid for Specially Promoted Research (MT) from the Ministry of Health, Labour and Welfare, Japan, by the NIAID-funded Center for Research on Influenza Pathogenesis (CRIP, HHSN266200700010C)(YK), by a Grant-in-Aid for Specially Promoted Research, by the Japan Initiative for Global Research Network on Infectious Diseases from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (YK), and by ERATO, Japan (YK).

Authors contributions

Designed the analyses: TK, SF, ET, SY, GN, YK, MT. Analysed and interpreted data: TK, SF, ET, HX, SY, YU, GN, YK, MT. Drafted the article: TK, SF. Revised the article: ET, GN, TS, YK, MT.

Conflict of interest

None declared.

This manuscript was accepted on 11 April 2013 and published online on 12 April 2013

References

1. World Health Organization (WHO)/Global Influenza Programme. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2013. Geneva: WHO: 12 Mar 2013. Available from: http://www.who.int/influenza/human_animal_interface/EN_GIP_20130312CumulativeNumberH5N1cases.pdf
2. Campbell CH, Webster RG, Breese SS Jr. Fowl plague virus from man. J Infect Dis. 1970;122(6):513-6. http://dx.doi.org/10.1093/infdis/122.6.513. PMid:5489075.
3. Centers for Disease Control and Prevention (CDC). Update: influenza activity-United States and worldwide, 2003-04 season, and composition of the 2004-05 influenza vaccine. MMWR Morb Mortal Wkly Rep. 2004;53(25):547-52. PMid:15229411.
4. Editorial team. Avian influenza A/(H7N2) outbreak in the United Kingdom. Euro Surveill. 2007;12(22): :pii=3206. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3206
5. Fouchier RA, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SA, Munster V, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. Proc Natl Acad Sci U S A. 2004;101(5):1356-61. http://dx.doi.org/10.1073/pnas.0308352100. PMid:14745020 PMCid:337057.
6. Hirst M, Astell CR, Griffith M, Coughlin SM, Moksa M, Zeng T, et al. Novel avian influenza H7N3 strain outbreak, British Columbia. Emerg Infect Dis. 2004;10(12):2192-5. http://dx.doi.org/10.3201/eid1012.040743. PMid:15663859. PMCid:3323367.
7. Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. Lancet. 2004;363(9409):587-93. http://dx.doi.org/10.1016/S0140-6736(04)15589-X.
8. Kurtz J, Manvell RJ, Banks J. Avian influenza virus isolated from a woman with conjunctivitis. Lancet. 1996;348(9031):901-2. http://dx.doi.org/10.1016/S0140-6736(05)64783-6.
9. Nguyen-Van-Tam JS, Nair P, Acheson P, Baker A, Barker M, Bracebridge S, et al. Outbreak of low pathogenicity H7N3 avian influenza in UK, including associated case of human conjunctivitis. Euro Surveill. 2006;11(18): pii=2952. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2952. PMid:16816456.
10. Taylor HR, Turner AJ. A case report of fowl plague keratoconjunctivitis. Br J Ophthalmol. 1977;61(2):86-8. http://dx.doi.org/10.1136/bjo.61.2.86.
11. Tweed SA, Skowronski DM, David ST, Larder A, Petric M, Lees W, et al. Human Illness from Avian Influenza H7N3, British Columbia. Emerg Infect Dis. 2004;10(12):2196-9. http://dx.doi.org/10.3201/eid1012.040961. PMid:15663860 PMCid:3323407.
12. Srinivasan K, Raman R, Jayaraman A, Viswanathan K, Sasisekharan R. Quantitative description of glycan-receptor binding of influenza A virus H7 hemagglutinin. PLoS One. 2013;8(2):e49597. http://dx.doi.org/10.1371/journal.pone.0049597. PMid:23437033 PMCid:3577880.
13. Nidom CA, Takano R, Yamada S, Sakai-Tagawa Y, Daulay S, Aswadi D, et al. Influenza A(H5N1) viruses from pigs, Indonesia. Emerg Infect Dis. 2010;16(10):1515-23. http://dx.doi.org/10.3201/eid1610.100508. PMid:20875275. PMCid:3294999.
14. Yang H, Chen LM, Carney PJ, Donis RO, Stevens J. Structures of receptor complexes of a North American H7N2 influenza hemagglutinin with a loop deletion in the receptor binding site. PLoS Pathog. 2010;6(9):e1001081. http://dx.doi.org/10.1371/journal.ppat.1001081 PMid:20824086 PMCid:2932715.
15. Wang W, Lu B, Zhou H, Suguitan AL Jr, Cheng X, Subbarao K, et al. Glycosylation at 158N of the hemagglutinin protein and receptor binding specificity synergistically affect the antigenicity and immunogenicity of a live attenuated H5N1 A/Vietnam/1203/2004 vaccine virus in ferrets. J Virol. 2010;84(13):6570-7. http://dx.doi.org/10.1128/JVI.00221-10. PMid:20427525 PMCid:2903256.
16. Hatta M, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. Science. 2001;293(5536):1840-2. http://dx.doi.org/10.1126/science.1062882. PMid:11546875.
17. Munster VJ, de Wit E, van Riel D, Beyer WE, Rimmelzwaan GF, Osterhaus AD, et al. The molecular basis of the pathogenicity of the Dutch highly pathogenic human influenza A H7N7 viruses. J Infect Dis. 2007;196(2):258-65. http://dx.doi.org/10.1086/518792. PMid:17570113.
18. Hay AJ, Wolstenholme AJ, Skehel JJ, Smith MH. The molecular basis of the specific anti-influenza action of amantadine. EMBO J. 1985;4(11):3021-4. PMid:4065098 PMCid:554613.
19. Pinto LH, Holsinger LJ, Lamb RA. Influenza virus M2 protein has ion channel activity. Cell. 1992;69(3):517-28. http://dx.doi.org/10.1016/0092-8674(92)90452-I.
20. McKimm-Breschkin JL, Sahasrabudhe A, Blick TJ, McDonald M, Colman PM, Hart GJ, et al. Mutations in a conserved residue in the influenza virus neuraminidase active site decreases sensitivity to Neu5Ac2en-derived inhibitors. J Virol. 1998;72(3):2456-62. PMid:9499107. PMCid:109546.
21. Matsuoka Y, Swayne DE, Thomas C, Rameix-Welti MA, Naffakh N, Warnes C, et al. Neuraminidase stalk length and additional glycosylation of the hemagglutinin influence the virulence of influenza H5N1 viruses for mice. J Virol. 2009;83(9):4704-8. http://dx.doi.org/10.1128/JVI.01987-08. PMid:19225004 PMCid:2668507.
22. Conenello GM, Zamarin D, Perrone LA, Tumpey T, Palese P. A single mutation in the PB1-F2 of H5N1 (HK/97) and 1918 influenza A viruses contributes to increased virulence. PLoS Pathog. 2007;3(10):1414-21. http://dx.doi.org/10.1371/journal.ppat.0030141. PMid:17922571. PMCid:2000966.
23. Jackson D, Hossain MJ, Hickman D, Perez DR, Lamb RA. A new influenza virus virulence determinant: the NS1 protein four C-terminal residues modulate pathogenicity. Proc Natl Acad Sci U S A. 2008;105(11):4381-6. http://dx.doi.org/10.1073/pnas.0800482105. PMid:18334632 PMCid:2393797.
24. Fan S, Deng G, Song J, Tian G, Suo Y, Jiang Y, et al. Two amino acid residues in the matrix protein M1 contribute to the virulence difference of H5N1 avian influenza viruses in mice. Virology. 2009;384(1):28-32. http://dx.doi.org/10.1016/j.virol.2008.11.044. PMid:19117585.
25. Jiao P, Tian G, Li Y, Deng G, Jiang Y, Liu C, et al. A single-amino-acid substitution in the NS1 protein changes the pathogenicity of H5N1 avian influenza viruses in mice. J Virol. 2008;82(3):1146–54. http://dx.doi.org/10.1128/JVI.01698-07. PMid:18032512 PMCid:2224464
26. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111-20. http://dx.doi.org/10.1007/BF01731581. PMid:7463489.
27. González-Reiche AS, Morales-Betoulle ME, Alvarez D, Betoulle JL, Müller ML, Sosa SM, et al. Influenza A viruses from wild birds in Guatemala belong to the North American lineage. PLoS One. 2012;7(3):e32873. http://dx.doi.org/10.1371/journal.pone.0032873. PMid:22427902 PMCid:3302778.
28. Kim HR, Park CK, Lee YJ, Oem JK, Kang HM, Choi JG, et al. Low pathogenic H7 subtype avian influenza viruses isolated from domestic ducks in South Korea and the close association with isolates of wild birds. J Gen Virol. 2012;93(Pt 6):1278-87. http://dx.doi.org/10.1099/vir.0.041269-0. PMid:22422062.

Concurrent outbreaks of dengue, chikungunya and Zika virus infections – an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012–2014

A Roth (adamr@spc.int)[1], A Mercier[1], C Lepers[1], D Hoy[1], S Duituturaga[1], E Benyon[1], L Guillaumot[2], Y Souarès[1]
1. Secretariat of the Pacific Community, New Caledonia
2. Institut Pasteur de Nouvelle-Caledonie, Noumea, New Caledonia

Citation style for this article:
Roth A, Mercier A, Lepers C, Hoy D, Duituturaga S, Benyon E, Guillaumot L, Souarès Y. Concurrent outbreaks of dengue, chikungunya and Zika virus infections – an unprecedented epidemic wave of mosquito-borne viruses in the Pacific 2012–2014. Euro Surveill. 2014;19(41):pii=20929. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20929

Article submitted on 26 September 2014 / published on 16 October 2014

Since January 2012, the Pacific Region has experienced 28 new documented outbreaks and circulation of dengue, chikungunya and Zika virus. These mosquito-borne disease epidemics seem to become more frequent and diverse, and it is likely that this is only the early stages of a wave that will continue for several years. Improved surveillance and response measures are needed to mitigate the already heavy burden on island health systems and limit further spread to other parts of the world.

Since January 2012, the Pacific is experiencing a high burden of mosquito-borne disease due to concurrent epidemics of dengue, chikungunya and Zika virus infections. So far over 120,000 people have been reported to be affected, a figure that is likely to substantially underestimate the real numbers due to underreporting. For as long as there has been data available from the Region (i.e. 40 years), this epidemic wave of mosquito-borne viruses with 28 new mosquito-borne viral outbreaks (n=25) and circulation (n=3) documented since January 2012 (18 Dengue virus (DENV) serotype 1–4, 7 chikungunya virus and 3 Zika virus infection outbreaks, respectively) is unprecedented (Table) [1-3]. We here present an overview of the surveillance and epidemiology of these mosquito-borne disease epidemics in the Pacific Region, to help facilitate response measures that are needed to mitigate the already heavy burden on island health systems and to limit further spread to other parts of the world.

Surveillance of mosquito-borne viruses in the Pacific Region

The Pacific Public Health Surveillance Network (PPHSN) is a voluntary network of countries, territories and organisations created in 1996. It is dedicated to the promotion of public health surveillance and response to health emergencies in the Pacific Region. It covers 22 Pacific Island countries and territories (hereafter referred to as the Pacific Region) with a population of 10.6 million inhabitants [4]. The network services include the timely exchange of information on outbreak-prone disease through PacNet, an email list with around 680 health professionals, and diagnostic support through a network of laboratories for identification and verification of pathogens.

In 2010, the Pacific Syndromic Surveillance System was introduced in the PPHSN. It monitors four syndromes and aims at improved early warning to complement routine notifiable disease notification systems that generally are not timely and seldom used for regional surveillance purposes in the Pacific Region. The Syndromic Surveillance system is under development and currently includes sentinel reporting from primary healthcare or hospital sites in all countries [5]. Manifest dengue, chikungunya and Zika virus infections have a similar initial clinical presentation and may be reported as any of the first three of the following four monitored syndromes: (i) acute fever and rash, (ii) prolonged fever, (iii) influenza-like illness and (iv) diarrhoea. Due to similar initial clinical features to the three mosquito-borne diseases, concurrent measles epidemics and leptospirosis pose diagnostic challenges in the Region.

There is a need for timely, reliable and detailed data on mosquito-borne virus outbreaks and circulation of the viruses in the Pacific Region. To obtain a comprehensible overview of the present epidemiological picture, several sources of information are used. Further to PacNet, syndromic and laboratory-based surveillance, event-based surveillance (mainly media and personal communications with health professionals) and surveillance by-proxy (reports of exported cases to neighbouring countries) [6] are also important. To facilitate better risk assessments and efficiency of data dissemination, this data is visualized in a recently launched interactive map available from: www.spc.int/phd/epidemics. The map, updated weekly, provides the region for the first time with a dynamic real-time picture of the current epidemic situation.

Table

Characteristics of new dengue, chikungunya and Zika virus infection outbreaks and circulation[a,b], Pacific Region, January 2012–17 September 2014[c] (n=28)

	Country	Month of onset	Latest information	Implicated mosquito borne virus	Summary	Sources
Dengue						
●	Tuvalu	Mar-14	10/07/2014	DENV-2	408 suspected cases with 195 cases positive in rapid tests (NS1/IgM).	[32]
●	Nauru	Mar-14	7/08/2014	DENV-3	251 suspected cases with 91 confirmed using IgM ELISA and/or rapid test. Samples sent for confirmatory serotyping.	[32, 33]
●	Tonga	Feb-14	21/08/2014	DENV-3	Outbreak ongoing; 2 cases of dengue imported into New Zealand since 12 July 2014.	[32–34]
●	New Caledonia	Feb-14	17/09/2014	DENV-3	In 2014, 338 cases of dengue recorded of which 55% were DENV-3. Virus circulation ongoing, with latest reported dengue case on 12 September 2014.	[35]
●	Fiji	15/01/2014[c]	23/06/2014	DENV-2	Circulation of virus, no outbreak declared; 6 confirmed cases of DENV-2 imported into Queensland Australia from January to June 2014.	[33]
●	Vanuatu	Dec-13	20/04/2014	DENV-3	1,561 dengue cases reported; 5 imported cases in Queensland Australia since January 2014 and 10 imported cases in New Caledonia from January to March 2014; 4 cases of DENV with serotype unknown imported into New Zealand since April 2014, of which 1 in July 2014.	[32–35]
●	Kiribati	Nov-13	24/01/2014	DENV-3	As of 16 Jan 2014, 198 suspected dengue cases of which 85 were laboratory-confirmed. Outbreak over, only sporadic cases of fever.	[32], media: Radio New Zealand International
●	Vanuatu	27/10/2012[c]	20/04/2014	DENV-1	Circulation of virus, no outbreak declared; 2 confirmed cases imported into Queensland Australia (1 case in 2013 and 1 case in 2014) and 6 cases into New Caledonia (5 cases in 2013 and 1 case in 2014); 4 cases of dengue serotype unknown imported into New Zealand since April 2014, of which 1 in July 2014.	[33, 35]
●	Fiji	Oct-13	5/06/2014	DENV-3	25,300 suspected cases, 15 deaths. Outbreak is ongoing; 15 confirmed cases imported into Queensland Australia from December 2013 to May 2014.	[33], media: Radio Australia, Fiji Broadcasting Corporation
● ●	French Polynesia	Feb-13	6/09/2014	DENV-1 DENV-3	As of 23 May 2014, 2188 positive cases since February 2013, and between 16 400 and 34 000 clinical visits estimated. 11 severe cases in March 2014 and 5 severe cases requiring hospitalisation in July 2014. DENV1 outbreak is still ongoing, but there are no cases of DENV3 reported since April 2014.	[36]
●	Wallis & Futuna	Jan-13	28/03/2013	DENV-1	88 suspected cases and 16 confirmed of which 11 imported cases from New Caledonia.	[37]
●	Solomon Islands	Dec-12	15/08/2014	DENV-3	7,697 reported cases as of 31 December 2013. As of June 2014, 1,762 suspected cases since January 2014, and 282 out of 1,500 samples tested positive in rapid tests. In March 2014 DENV-3 confirmed. Outbreak still ongoing.	[32, 33], media: Solomon Star
●	Kosrae, Federated States of Micronesia	Sep-12	19/07/2013	DENV-4	729 reported clinical cases; 206 cases laboratory confirmed by rapid diagnostic tests. No deaths reported.	[9, 37]
●	New Caledonia	Sep-12	17/09/2014	DENV-1	Largest ever recorded outbreak of dengue in New Caledonia with 10,978 cases and 5 deaths from September 2012 to September 2013; 338 cases of dengue recorded in 2014 of which 45% were DENV-1. Virus circulation ongoing, with latest reported dengue case on 128 September 2014.	[35]
●	Fiji	15/07/2012[c]	31/12/2012	DENV-2	Circulation of virus, no outbreak declared. 2 imported cases in Queensland Australia;	[33]
●	Kiribati	Mar-12	4/05/2012	DENV-1	243 clinical cases.	[32]
●	Niue	Feb-12	20/07/2012	DENV-1	More than 100 cases.	Media: Radio New Zealand International

	Country	Month of onset	Latest information	Implicated mosquito borne virus	Summary	Sources
Chikungunya						
●	Tokelau	Jul-14	11/09/2014	CHIKV	164 suspected cases reported. CHIKV confirmed.	[32]
●	Samoa	Jul-14	1/09/2014	CHIKV	433 cases reported over 4 weeks. 21 RT-PCR positives out of 59 samples (as of 28 Aug 2014).	[37], media: Samoa Observer
●	American Samoa	Jun-14	17/09/2014	CHIKV	823 probable cases reported, with 15 hospitalisations. CHIKV confirmed.	[37], media Radio New Zealand International
●	Tonga	Feb-14	11/09/2014	CHIKV	Over 10,000 suspected cases reported. Ongoing circulation of CHIKV confirmed.	[32–34]
●	Yap, Federated States of Micronesia	Aug-13	10/09/2014	CHIKV	A total of 1,711 suspected cases identified in Yap State. Circulation of CHIKV reconfirmed.	[37]
●	New Caledonia	Jan-13	2/06/2014	CHIKV	A total of 32 confirmed cases from January to May 2013.	[35]
●	Papua New Guinea	Jun-12	25/11/2013	CHIKV	A major outbreak spread over Papua New Guinea in 2013. Number of cases not reported, but estimated in media to be tens of thousands of cases.	[2], media: Australia Network News, Pacnews
Zika virus infections[d]						
●	Cook Islands	Feb-14	29/05/2014	ZIKV	Outbreak is over. 932 suspected and 50 confirmed cases.	[32], media: Radio New Zealand International
●	New Caledonia	Jan-14	17/09/2014	ZIKV	Imported cases reported in November 2013, first autochtonous case reported in January 2014; 1,400 confirmed cases of which 35 imported cases. Outbreak peaked in April 2014. Last case reported on 2nd August 2014.	[35]
●	French Polynesia	Oct-13	4/05/2014	ZIKV	8,723 suspected cases reported and more than 30,000 estimated clinical visits due to Zika. Outbreak declared over but virus circulation may be ongoing.	[36]

● Cases reported are increasing or peaking
● Cases reported are decreasing or viral circulation is ongoing
● Outbreak is reported to be over and/or no cases have been reported for one year.

CHIKV: chikungunya virus; DENV: dengue virus serotype 1-4; RT-PCR: reverse-transcriptase polymerase chain reaction; ZIKV: Zika virus.

[a] Only incident outbreaks and circulation notified during the reported period. Outbreaks first reported in 2011 (DENV-4 in Marshal Islands, DENV-2 in Yap and circulation of DENV in Papua New Guinea and Fiji) and still ongoing in 2012 are not presented.

[b] An outbreak is considered an outbreak when reported as such or when the first autochtonous cases are reported, and new circulation if there have been no events reported during one year.

[c] Month of start equals the month of first report, as this reflects circulation of virus.

[d] Easter Island experienced a Zika virus infection outbreak starting February 2014, but is not presented in the table as it is not part of the 22 countries and territories of the Pacific Public Health Surveillance Network .

The epidemiology of mosquito-borne viruses in the Pacific Region

Mosquito-borne virus diseases in the Pacific Region have a distinct epidemiology due to small populations scattered over thousands of tropical and sub-tropical islands on both sides of the equator in relative geographic isolation, together with (nowadays) significant people's mobility and thereby exposure to circulating arboviruses through the airline networks of the Asia-Pacific region (Figure 1).

Between January 2012 and 17 September 2014, a total of 28 new mosquito-borne viral outbreaks (n=25) and circulation (n=3) were documented: 18 DENV 1–4 outbreaks (2012: 7; 2013: 6; 2014: 5), 7 chikungunya virus (CHIKV) (2012: 1; 2013: 2; 2014: 4) and 3 Zika virus infection outbreaks (2012: 0; 2013: 1; 2014: 2), respectively.

Looking at the first semester of 2014, the number of outbreaks and circulating mosquito-borne viruses seem to be increasing (Figure 2). During the same period, DENV-3 became the dominating dengue virus, and since Zika virus started to spread in the end of 2013, there was concurrent circulation of DENV-1,-2 and -3, CHIKV and Zika virus (Table, Figure 2)

Dengue

The epidemic pattern of dengue in the Pacific Region has typically presented in form of sporadic or rare epidemics rather than a hyperepidemic/endemic pattern, with one dominating serotype sweeping across the islands every 3 to 5 years, and with varying duration of circulation in different islands largely depending on population size [1,7- 8]. During 2012, there were outbreaks of all four serotypes of DENV documented for the first time during one year (Figure 2) [1]. DENV-1 was the dominating serotype in 2012 and beginning 2013, causing the largest documented dengue outbreak ever in New Caledonia, with 10,978 confirmed cases and 5 deaths from September 2012 to September 2013. Since 2012 there have only been reports of one outbreak with DENV-2 and -4 respectively: DENV- 2 recently caused an outbreak in Tuvalu with 408 suspected cases (4% of the population) and DENV-4 caused a large outbreak in Kosrae in September 2012 to March 2013 with 729 clinical cases (11% of the population) (Table) [9]. Furthermore there have been reports of new circulation of DENV-2 in Fiji. (Table) After having been absent in the region for 18 years, DENV- 3 has after the reintroduction in 2012, become the dominating DENV in the region with five ongoing outbreaks, one of them in Fiji, with 25,300 suspected cases and 15 deaths (Table, Figure 1) [1,10].

Figure 1

Map of newly reported dengue, chikungunya and Zika virus infection outbreaks or new virus circulation[a], Pacific Region[b], January 2012–17 September 2014[c] (n=28)

CHIKV: chikungunya virus; DENV: dengue virus serotype 1-4; ZIKV: Zika virus.

[a] Only incident outbreaks and virus circulation reported during the period. Outbreaks first reported in 2011 (DENV-4 in Marshal Islands, DENV-2 in Yap and circulation of DENV in Papua New Guinea and Fiji) and still ongoing in 2012 are not presented.

[b] The 22 Pacific Island countries and territories that are core members of the Pacific Public Health Surveillance Network and referred to as the Pacific Region.

[c] Real-time interactive map with current epidemiological situation and alerts is available from: www.spc.int?phd/epidemics

Chikungunya

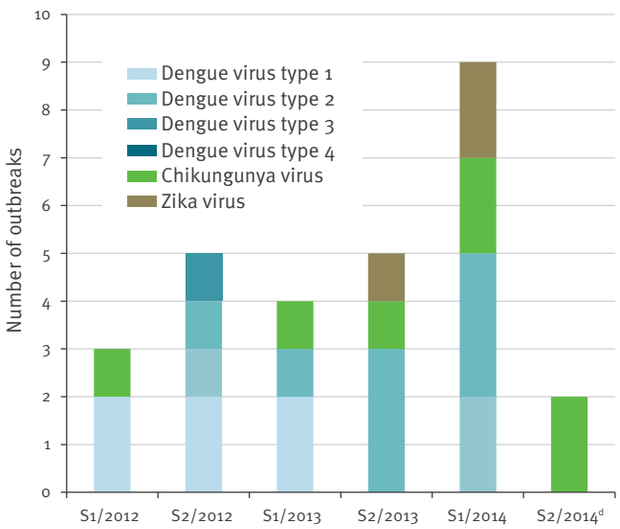
After being reported in the Pacific for the first time in a small tightly controlled outbreak in New Caledonia in 2011 [11], CHIKV is currently becoming established in the Region (Figure 1, Table) [2]. In Papua New-Guinea in 2012-13, the largest epidemic in the Region so far with estimated (though poorly documented) tens of thousands of cases, was caused by the East Central South African (ECSA) lineage of the virus [2]. The Asian lineage of the virus was responsible for the outbreak in Yap State (2013-14) [12] and also in New Caledonia (2013) where CHIKV re-emerged in the middle of a large DENV-1 epidemic and caused a small outbreak, similar to the 2011 outbreak (Table) [13]. Phylogenetic analyses of the CHIKV involved in the outbreaks in Tonga, Samoa and American Samoa are not yet available. Due to the on-going geographic expansion of *Aedes albopictus* in the Pacific region (Figure 3), virus genotype monitoring is a crucial aspect of surveillance.

Zika virus infections

After the first documented Pacific Zika outbreak in Yap in 2007 [14], the Asian lineage of the virus reappeared in French Polynesia in October 2013, and has since caused large outbreaks in New Caledonia (1,400 confirmed cases), Cook Islands (over 900 cases) and Easter Island that is not part of the PPHSN (Figure 1, Table) [3]. In French Polynesia, extrapolation of the 8,746 suspect cases reported by the sentinel surveillance network allows to infer that over 30,000 medical consultations were due to the spread of Zika virus throughout the archipelago. Between November 2013 and February 2014, increased incidence of neurological complications, including 42 cases of Guillain-Barré syndrome, was a unique and worrying feature of the French Polynesia outbreak that warrants further studies [3].

Figure 2

Incidence and aetiology of newly reported mosquito-borne virus outbreaks and circulation[a] by semester[b], Pacific Region, January 2012–17 September 2014[c] (n=28)



S: semester.

[a] An outbreak is considered an outbreak when reported as such, and new circulation of virus if there has been no event with the same virus reported during one year previously.

[b] S1 includes the months from January to June and S2 the months from July to December.

[c] Outbreaks or circulation that started before January 2012 or after 17 September 2014 are not presented in this graph to allow observation of a possible trend over time.

[d] Semester 2 in 2014 is not complete, and only includes reports from two full months out of six.

Discussion and Conclusions

Burden on the Pacific countries and territories

Mosquito-borne outbreaks are greatly exacerbating the pre-existing burden that Pacific Island primary healthcare systems face. If not managed well, the epidemic wave may threaten societies broadly, affecting trade, tourism and work force beyond the direct morbidity and mortality toll [2]. During the chikungunya outbreak in Reunion Island, one third of the around 800,000 inhabitants were infected, peaking at more than 47,000 estimated cases in one week, with estimated productivity loss of €17.4 million (range €6 to €28.9 million) and medical costs of €43.9 million that were met by the French state [15-17]. Much of the burden on the Pacific Region of the concurrent epidemics of all three diseases covered here is unknown and further studies are warranted, especially on co-infection and the effect of sequential infection with different viruses.

Zika virus disease, generally reported to have a mild clinical presentation, was associated with neurological complications during concurrent Zika virus disease and dengue epidemics in French Polynesia [3,18]. The Pacific Region may be particularly vulnerable to communicable diseases due to isolation and immunologically naive populations, but also due to rates of non-communicable disease, such as obesity, diabetes and cardiovascular disease, that are among the world's highest on some islands [19].

The risk for further spread

While there have been efforts to improve surveillance in the Pacific over the past two decades, it is not likely that the extent of the current increase in diversity and frequency of mosquito-borne virus outbreaks in the Pacific can be explained solely by improved surveillance systems. In the island setting of the Indian Ocean, the largest documented CHIKV outbreak lasted four years (2004–2007) [15]. Therefore, considering also the previous dengue outbreaks in the Pacific Region [1-2] and the diversity of the current outbreaks, it seems likely that the Pacific Region is in the early stages of an epidemic wave for the three mosquito-borne viruses that started in 2012 and is likely to continue for several years.

The risk for further spread in the Pacific Region is high for several reasons. Firstly, it is likely that there is little immunity to these diseases, as DENV-3 had not been

circulating in the Region since 1995 [1] and prior to the current wave, CHIKV and Zika virus occurrence in the Pacific was limited to two documented outbreaks [11, 14]. Secondly, competent vectors present in the Region, mainly *Ae. aegypti* and *Ae. albopictus*, but also other local mosquitoes such as *Ae. polynesiensis* or *Ae. hensilli* are known to transmit these viruses (Figure 3) [20]. These species have been incriminated in DENV transmission on epidemiological and/or experimental (laboratory infections) grounds. Several of them are confirmed or strongly suspected vectors of CHIKV and Zika viruses [21]. Thirdly, large population mobility and airline travel facilitate the spread [22].

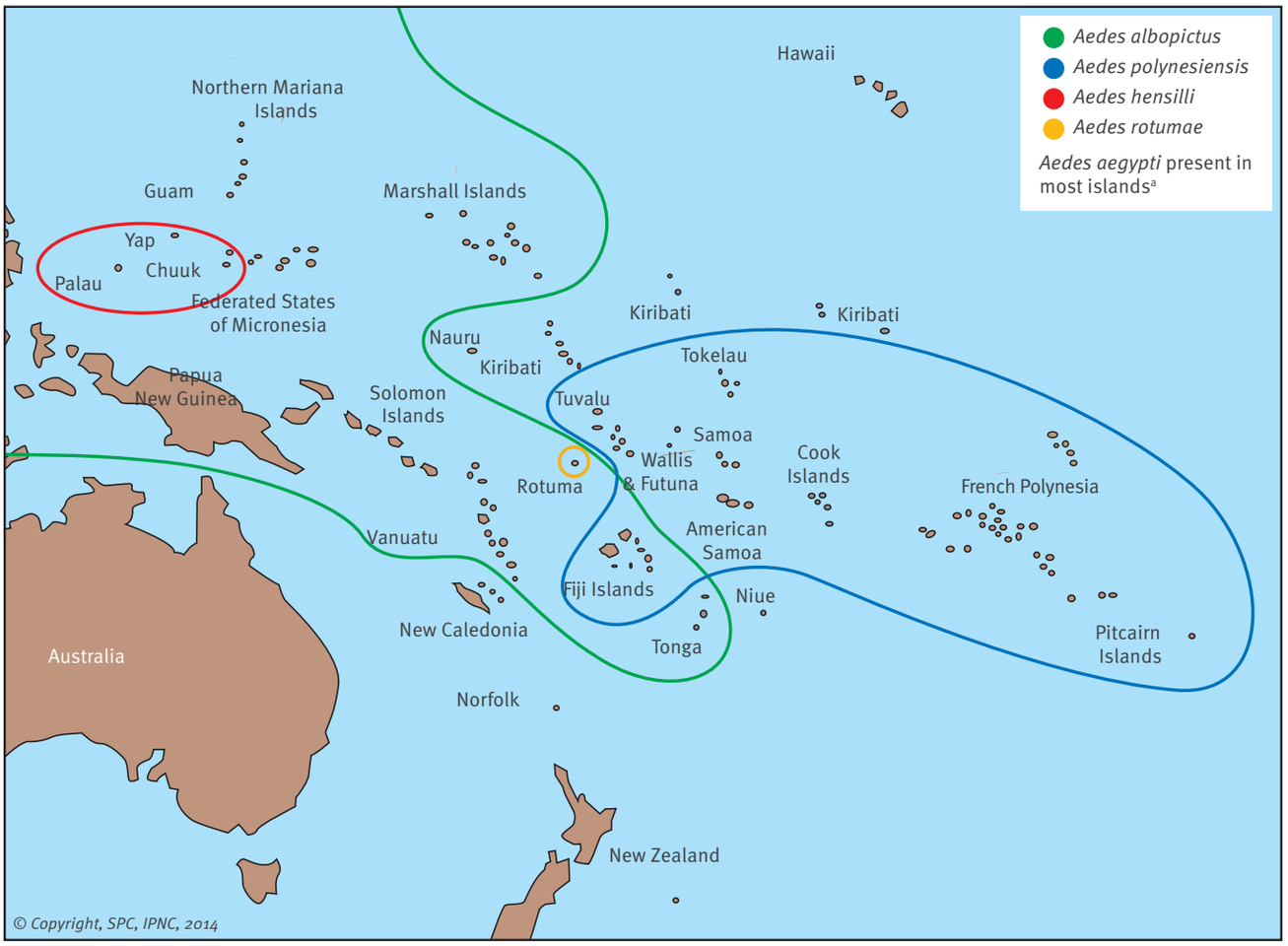
Vector control capacity in the Pacific Region is often limited or insufficient [11]. At present, there is no ongoing entomological surveillance system targeting vectors of dengue and other arboviruses established in the Region except in New Caledonia, Fiji and French Polynesia. The current knowledge about mosquito distribution in the other countries and territories is based on data collected during entomological investigations in surveys from the second half of the 20th century and from some more recent surveys [20]. Interestingly, the three viruses involved in this epidemic wave are not broadly mosquito-borne, but specifically *Aedes (Stegomyia)*-borne.

The cause of the recent increase in mosquito-borne disease in the Pacific Region is largely unknown, but is in line with a global increase of emerging diseases, and likely driven by a combination of socio-economic, environmental and ecological factors [23].

The continuous challenges of dengue and chikungunya [24] and more recently Zika virus infections [25]

Figure 3

Map of the known distribution of *Aedes (Stegomyia)* mosquitoes, vectors of dengue and possible vectors of chikungunya and Zika viruses, Pacific Region as of beginning October 2014



[a] *Aedes aegypti* (not represented on the map) is present throughout most of the region including North Queensland. It is absent from the rest of Australia, New Zealand, Hawaii, Futuna and some other remote islands, and it seems to be currently displaced by *Ae. albopictus* in many locations (e.g. Papua New Guinea and Solomon Islands).
The known or strongly suspected distributions of other vectors are as follows (not exhaustive): *Ae. scutellaris* (Indonesia; Northern Australia, Papua New Guinea); *Ae. marshallensis* (Marshall Islands; Western Kiribati; Kosrae; Pohnpei); *Ae. hebrideus* (Papua New Guinea; Solomon Islands; Vanuatu); *Ae. cooki* (Niue, Vava'u Group, Tonga); *Ae. tongae* (Ha'apai Group, Tonga); *Ae. tabu* (Tongatapu group, Tonga); *Ae. kesseli* (Niua group, Tonga); *Ae. pseudoscutellaris* (Fiji). [25]

for Europe, the re-emergence of dengue in Japan [26], and the first-time chikungunya transmission in the Americas [27], show that these viruses pose a threat to any country with competent vectors. The epidemiology of mosquito-borne viruses in the Pacific may be changing. There are close links between the several European overseas countries and territories in the Pacific Region and Europe and the United States [28]. Considering the extensive airline travel between the Pacific Region and other parts of the world where the viruses have not yet been established e.g. Europe and the United States, it should be of international interest to stay informed of the spread of the current Pacific Region wave of mosquito-borne viruses and to support surveillance and control efforts [2,23,29].

Examples of response from PPHSN partners to the epidemic situation include the provision of support and capacity building to Pacific Islands in surveillance, outbreak investigation and response, and mass-gathering surveillance. The Pacific Outbreak Manual is also being updated to include specific response guidelines for the three viruses [30].

To further enhance surveillance and response measures, Pacific Directors and Ministers of Health have shared the current risk assessment, and the upcoming Pacific International Health Regulations meeting will focus on mosquito-borne diseases. Island primary healthcare-based systems have difficulties to cope with high caseloads and there is a need for early multidisciplinary preparedness and response to face larger outbreaks adequately [2].

Acknowledgements

All PPHSN partners should be acknowledged for the hard work that has contributed to this overview. In particular we wish to acknowledge: the Pacific Island countries and territories and their health professionals for country reports and updates on PacNet and to the Pacific Syndromic Surveillance reports; the WHO Suva for managing the Pacific Syndromic Surveillance reports; all international and regional partner organisations that assist PPHSN member countries in surveillance and outbreak investigations and capacity building thereof; national laboratories for primary diagnosis and sampling and laboratories serving as regional references or assisting in first diagnostic confirmation of mosquito-borne disease – Fiji Centre for Communicable Disease Control (FCDCC) Mataika House, Guam Public Health Laboratory (GPHL), Institut Pasteur New Caledonia (IPNC), Institut Louis Malardé (ILM) Tahiti French Polynesia, CDC Atlanta US, CDC Puerto Rico, Hawaii State Laboratory (HSL), Fort Collins Laboratory USA, Queensland University of Technology (QUT) Australia, Queensland Health & Forensic Scientific Services (QHFSS) Australia; and the surveillance units reporting imported cases of mosquito-borne disease – La Direction des Affaires Sanitaires et Sociales de la Nouvelle-Calédonie New Caledonia, Queensland Health Australia notifiable conditions data and Institute of Environmental Science and Research (ESR) New Zealand dengue report.

Conflict of interest

None declared.

Authors' contributions

AR, YS, CL, DH conceived the idea of the paper. AR, AM, CL, SD and EB contributed to data gathering and cleaning. AR, CL and AM conducted the analysis. LG gathered and compiled all vector data and analysis. AR and AM drafted the first draft, and all
authors commented and agreed upon the final manuscript.

References

1. Singh N, Kiedrzynski T, Lepers C, Benyon EK. Dengue in the Pacific--an update of the current situation. Pacific Health Dialog. 2005;12(2):111-9.
2. Roth A, Hoy D, Horwood PF, Ropa B, Hancock T, Guillaumot L, et al. Preparedness for threat of chikungunya in the pacific. Emerging Infect Dis. 2014;20(8). http://dx.doi.org/10.3201/eid2008.130696
3. Musso D, Nilles EJ, Cao-Lormeau VM. Rapid spread of emerging Zika virus in the Pacific area. Clin Microbiol Infect. 2014. http://dx.doi.org/10.1111/1469-0691.12707
4. Les populations du Pacifique 2013. [Pacific Island Populations 2013]. French. Nouméa, 2013. Statistics for Development Division, Secretariat of the Pacific Community. Available from: http://www.spc.int/sdd/index.php/en/downloads/doc_download/738-2013-pacific-population-poster
5. Kool JL, Paterson B, Pavlin BI, Durrheim D, Musto J, Kolbe A. Pacific-wide simplified syndromic surveillance for early warning of outbreaks. Global Public Health. 2012;7(7):670-81. http://dx.doi.org/10.1080/17441692.2012.699536
6. Lau CL, Weinstein P, Slaney D. Dengue surveillance by proxy: travellers as sentinels for outbreaks in the Pacific Islands. Epidemiol Infect. 2013;141(11):2328-34. http://dx.doi.org/10.1017/S0950268813000058
7. Dupont-Rouzeyrol M, Aubry M, O'Connor O, Roche C, Gourinat AC, Guigon A, et al. Epidemiological and molecular features of dengue virus type-1 in New Caledonia, South Pacific, 2001-2013. Virol J. 2014;11:61. http://dx.doi.org/10.1186/1743-422X-11-61
8. Morens DM. Dengue fever: a prevention summary for Pacific health workers. Pacific Health Dialog. 1996;3(1):240-52.
9. Centers for Disease Control and Prevention (CDC). Dengue outbreak--Federated States of Micronesia, 2012-2013. MMWR. Morbidity and mortality weekly report. 2013;62(28):570-3.
10. Cao-Lormeau VM, Roche C, Musso D, Mallet HP, Dalipanda T, Dofai A, et al. Dengue virus type 3, South Pacific Islands, 2013. Emerg Infect Dis. 2014;20(6):1034-6. http://dx.doi.org/10.3201/eid2006.131413
11. Dupont-Rouzeyrol M, Caro V, Guillaumot L, Vazeille M, D'Ortenzio E, Thiberge JM, et al. Chikungunya virus and the mosquito vector Aedes aegypti in New Caledonia (South Pacific Region). Vector Borne Zoonotic Dis. 2012;12(12):1036-41. http://dx.doi.org/10.1089/vbz.2011.0937
12. Nhan TX, Claverie A, Roche C, Teissier A, Colleuil M, Baudet JM, et al. Chikungunya virus imported into French polynesia, 2014. Emerg Infect Dis. 2014;20(10):1773-4. http://dx.doi.org/10.3201/eid2010.141060
13. Dupont-Rouzeyrol M, O'Connor O. Phylogénie moléculaire des Arbobirus en Nouvelle-Calédonie. Institut Pasteur de Nouvelle-Calédonie: La Recherche: Rapport d'activité 2013. [Molecular Phylogeny of Arboviruses in New Caledonia. Pasteur Institute of New Caledonia; Annual report 2013. p 24. Doc. n° 156/2014-IPNC/DG of 25 June 2014. Noumea, 2013.] p 24. Doc.n° 156/2014-IPNC/DG du 25 juin 2014. Nouméa, 2013. Available from: http://www.institutpasteur.nc/wp-content/uploads/2013/07/Rapport-2013-IPNC-RECHERCHE-n%C2%Bo-156-26-juin-2014.pdf
14. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL, Lanciotti RS, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med. 2009;360(24):2536-43. http://dx.doi.org/10.1056/NEJMoa0805715
15. Renault P, Balleydier E, D'Ortenzio E, Baville M, Filleul L. Epidemiology of Chikungunya infection on Reunion Island, Mayotte, and neighboring countries. Med Mal Infect. 2012;42(3):93-101. http://dx.doi.org/10.1016/j.medmal.2011.12.002
16. Lagacherie P. [Coverage of the chikungunya epidemic on Reunion Island in 2006 by the French healthcare system]. Med Trop (Mars). 2012;72 Spec No:97-8.
17. Soumahoro MK, Boelle PY, Gauzere BA, Atsou K, Pelat C, Lambert B, et al. The Chikungunya epidemic on La Reunion Island in 2005-2006: a cost-of-illness study. PLoS Negl

Trop Dis. 2011;5(6):e1197. http://dx.doi.org/10.1371/journal.pntd.0001197

18. Oehler E, Watrin L, Larre P, Leparc-Goffart I, Lastere S, Valour F, et al. Zika virus infection complicated by Guillain-Barre syndrome - case report, French Polynesia, December 2013. Eurosurveill. 2014;19(9). pii: 20720.
19. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384(9945):766-81. http://dx.doi.org/10.1016/S0140-6736(14)60460-8
20. Guillaumot L, Ofanoa R, Swillen L, Singh N, Bossin HC, Schaffner F. Distribution of Aedes albopictus (Diptera, Culicidae) in southwestern Pacific countries, with a first report from the Kingdom of Tonga. Parasit Vectors. 2012;5:247. http://dx.doi.org/10.1186/1756-3305-5-247
21. Ledermann JP, Guillaumot L, Yug L, Saweyog SC, Tided M, Machieng P, et al. Aedes hensilli as a Potential Vector of Chikungunya and Zika Viruses. PLoS Negl Trop Dis 2014 8(10): e3188. http://dx.doi.org/10.1371/journal.pntd.0003188
22. Tatem AJ, Huang Z, Das A, Qi Q, Roth J, Qiu Y. Air travel and vector-borne disease movement. Parasitology. 2012;139(14):1816-30. http://dx.doi.org/10.1017/S0031182012000352
23. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008;451(7181):990-3. http://dx.doi.org/10.1038/nature06536
24. Chretien JP, Linthicum KJ. Chikungunya in Europe: what's next? Lancet. 2007;370(9602):1805-6. http://dx.doi.org/10.1016/S0140-6736(07)61752-8
25. European Centre for Disease Prevention and Control (ECDC). Rapid risk assessment: Zika virus infection outbreak, French Polynesia. 14 Feb 2014. Stockholm:,ECDC; 2014.
26. DENGUE - JAPAN: (SAITAMA) [Internet]. International Society for Infectious Diseases. 2014 [cited 2014-09-08]. Available with archive number: 20140828.2731079 from: http://www.promedmail.org/.
27. Staples JE, Fischer M. Chikungunya virus in the Americas--what a vectorborne pathogen can do. N Engl J Med. 2014;371(10):887-9. http://dx.doi.org/10.1056/NEJMp1407698
28. Jones J, Gastellu-Etchegorry M, Stenz FK, Baudon C, Bloem SJ, Bondonneau M, et al. Epidemiology, surveillance and control of infectious diseases in the European overseas countries and territories, 2011. Eurosurveill. 2011;16(29). pii: 19923.
29. Kuehn BM. World leaders push to prepare for global threats. JAMA. 2014;311(12):1189-90. http://dx.doi.org/10.1001/jama.2014.2272
30. The Pacific Outbreak Manual. Pacific Public Health Surveillance Network (PPHSN). February 2014. Available from: http://www.spc.int/phs/PPHSN/Publications/Pacific_Outbreak_Manual_wersion1-2.pdf
31. Guillaumot L. Arboviruses and their vectors in the Pacific--status report. Pac Health Dialog. 2005;12(2):45-52.
32. World Health Organization, West Pacific Region (WPRO). Emerging disease surveillance and response. Division of Pacific Technical Support. Weekly Pacific Syndromic Surveillance Report. Fiji: WPRO; 2014. Available from: http://www.wpro.who.int/southpacific/programmes/communicable_diseases/disease_surveillance_response/page/en/index2.html
33. Queensland Government. Queensland Health. Queensland notifiable conditions data. Last updated: 14 Oct 2014. Brisbane: Queensland Health; 2014.Available from: http://www.health.qld.gov.au/ph/cdb/sru_data.asp
34. Institute of Environmental Science and Research Ltd (ESR). Dengue weekly updates. Porirua, New Zealand. For details, contact ESR (Institute of Environmental Science and Research Ltd) (ange.bissielo@esr.cri.nz)
35. La direction des affaires sanitaires et sociales de Nouvelle-Calédonie (DASS). [Department of Health and Social Affairs] Veille sanitaire. French. Nouméa, 2014. [Accessed 17 Sep 2014]. Available from : http://www.dass.gouv.nc/portal/page/portal/dass/observatoire_sante/veille_sanitaire/
36. Bulletin de surveillance et veille sanitaire en Polynésie français - Bureau de veille sanitaire. [Public Health Surveillance and Monitoring in French Polynesia – Public Health Surveillance Office]. French. Accessed 17 Sep 2014. Available from: http://www.spc.int/phs/PPHSN/Surveillance/Routine_reports.htm
37. PacNet country reports. For details in PacNet report archives, contact the Focal Point of the Pacific Public Health Surveillance Coordinating Body (FocalPointPPHSNCB@spc.int‹mailto:FocalPointPPHSNCB@spc.int›)

First secondary case of Ebola outside Africa: epidemiological characteristics and contact monitoring, Spain, September to November 2014

M A López[1], C Amela[2], M Ordobas[1], M F Domínguez-Berjón[1], C Álvarez3, M Martínez[3], M J Sierra[2], F Simon[2], J M Jansá[4], D Plachouras[4], J Astray (jenaro.astray@salud.madrid.org)[1], Working group of Ebola outbreak investigation team of Madrid[5]

1. Epidemiology Area of the Autonomous Community of Madrid, General Directorate of Primary Care, Health Authority of the Autonomous Community of Madrid, Madrid, Spain
2. Coordinating Centre for Health Alerts and Emergencies (CCAES),General Directorate of Public Health, Quality and Innovation, Madrid, Spain
3. Subdirectorate of Health Prevention and Promotion, General Directorate of Primary Care, Health Authority of the Autonomous Community of Madrid, Madrid, Spain
4. European Centre for Disease Prevention and Control (ECDC),Stockholm, Sweden
5. The members of the group are listed at the end of the article

Citation style for this article:
López MA, Amela C, Ordobas M, Domínguez-Berjón MF, Álvarez C, Martínez M, Sierra MJ, Simon F, Jansá JM, Plachouras D, Astray J, Working group of Ebola outbreak investigation team of Madrid. First secondary case of Ebola outside Africa: epidemiological characteristics and contact monitoring, Spain, September to November 2014. Euro Surveill. 2015;20(1):pii=21003. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21003

Article submitted on 30 November 2014 / published on 08 January 2015

On 6 October 2014, a case of Ebola virus disease (EVD) acquired outside Africa was detected in Madrid in a healthcare worker who had attended to a repatriated Spanish missionary and used proper personal protective equipment. The patient presented with fever <38.6 °C without other EVD-compatible symptoms in the days before diagnosis. No case of EVD was identified in the 232 contacts investigated. The experience has led to the modification of national protocols.

Introduction

The current Ebola virus disease (EVD) epidemic affecting countries in West Africa is the largest ever registered outbreak of this disease [1]. Ongoing intensive transmission in the community and in healthcare facilities associated with weak health systems including limited human and material resources hinder adequate outbreak control and case management. Healthcare workers (HCW) in these areas have been significantly affected during this epidemic [2-5].

On 7 August 2014, the Spanish government decided to repatriate a Spanish missionary healthcare worker at the St. Joseph's hospital in Monrovia (Liberia) who had tested positive for Ebola virus. On arrival, the person was admitted to the infectious diseases isolation unit at the reference hospital (La Paz-Carlos III Hospital Complex in Madrid). The patient remained hospitalised until his death on 12 August. On 22 September, a second Spanish missionary healthcare worker who had worked at a hospital in Lunsar (Sierra Leone) and who was also suffering from Ebola virus infection was repatriated under the same procedure. This patient was admitted to the same reference hospital where he died on 25 September. One of the HCW who was caring for the second repatriated Ebola case was diagnosed with EVD on 6 October. This was the first secondary case of this disease outside Africa.

In this paper we describe the epidemiological characteristics and public health control measures adopted after the identification of this first transmission outside the epidemic area. The information and lessons learnt in Spain may contribute to improving preparedness and response guidelines and protocols in non-affected countries. The risk of transmission of Ebola virus to healthcare professionals associated with repatriated patients needs to be reassessed and considered for future surveillance and control measures in these settings [5-7].

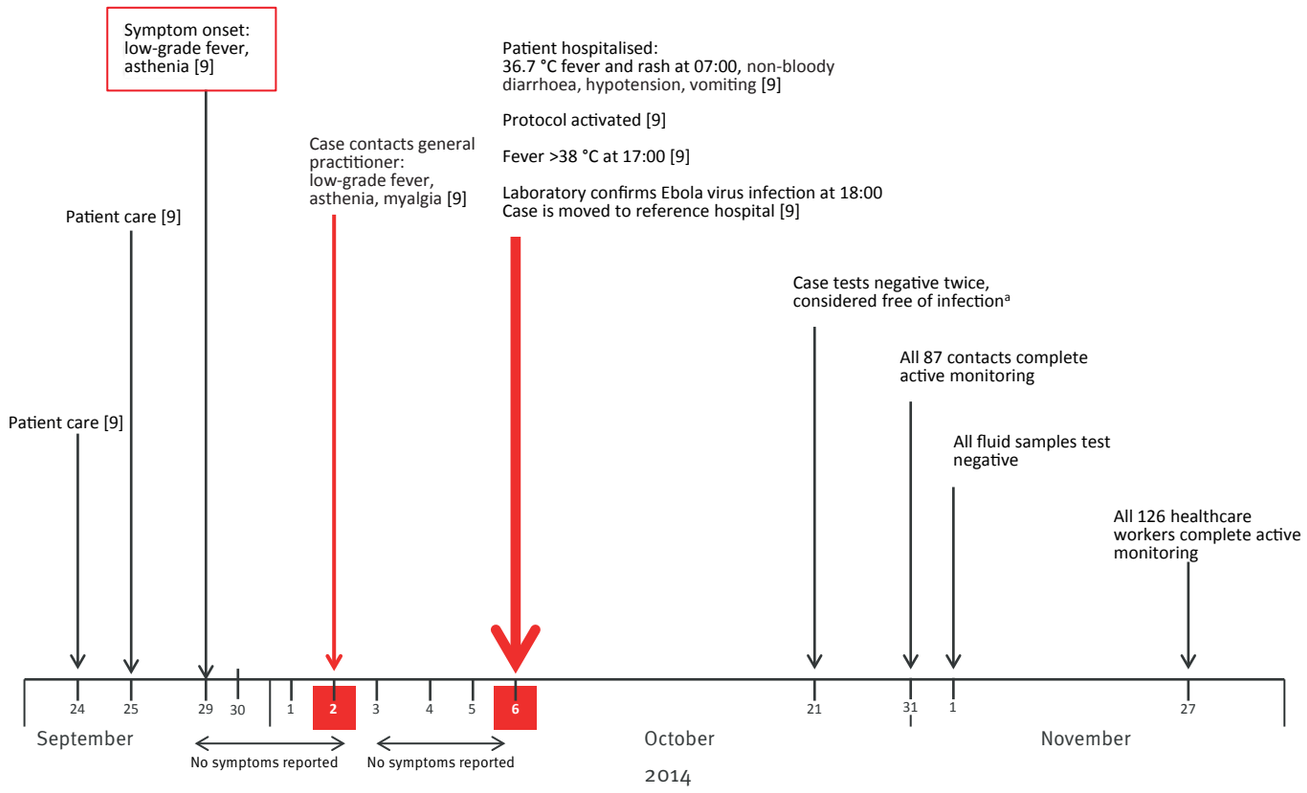
Epidemiological investigation and contact monitoring

Case description

The secondary case of EVD diagnosed in Spain on 6 October was one of the 117 HCW who had participated in the care of the two repatriated EVD cases. The HCW completed the 21-day monitoring period after caring for the first case on 30 August. On 21 and 25 September, she was exposed to the second patient and presumably contaminated fomites. She was classified as a low-risk contact and was therefore self-monitoring for symptoms, in accordance with the protocol [8]. The HCW had used appropriate personal protective equipment (PPE), i.e. waterproof long-sleeved clothing covering the feet, waterproof footwear, hood, face mask or goggles, double layer of gloves, and FP3 respirator [8], and she did not recall any incident during its use.

Figure 1

Timeline of events for secondary Ebola case, Madrid, 24 September–27 November 2014



[a] Culture results for all body fluids taken on 21 October were negative

Following the established procedures for HCW caring for EVD patients [8], the hospital recommended self-monitoring for 21 days from 25 September onwards. According to these procedures, the HCW was supposed to inform the monitoring official at the hospital in case of fever >38.6 °C and any of the symptoms of the disease: severe headache, vomiting, diarrhoea, abdominal pain or bleeding. On the following day, 26 September, she was off duty. She contacted the monitoring official for the first time on 2 October.

Symptoms started on 29 September. She presented malaise and low-grade fever <38 °C. The grade fever remained at this level for three days and increased to 38 °C in the three following days [9]. Figure 1 shows the evolution and timeline of events.

On 6 October at 04:00, she called the public health officials to report a temperature of 37.3°C, general malaise, nausea and cough. These symptoms led the public health officer to request medical evaluation at home and to refer her to the closest hospital. On admission at 07:00, she had a temperature of 36.7 °C, blood pressure of 90/60 mm Hg, 95% oxygen saturation measured by means of pulse oximetry, and a maculopapular rash. She reported that she had not received antipyretic agents [9]. At 08:00 on 6 October, the hospital contacted the public health services and they decided to classify the case as under investigation for EVD and send blood samples to the national reference laboratory. The patient's condition worsened in the following hours [9] and at 18:00, the reference laboratory confirmed the diagnosis of EVD. The patient was transferred to the reference hospital under strict isolation measures. The patient received antiviral treatment and convalescent serum from a recovered Ebola patient. On 21 October, the case tested EVD-negative in two samples taken 48 hours apart and, according to protocols, was considered free of Ebola virus infection on 1 November when a PCR test of all body fluid samples yielded negative results. The isolation measures were suspended on the same day, and the patient was finally discharged on 5 November 2014.

Contact monitoring

The epidemiological investigation began at the time of diagnosis. Information on the patient's possible exposure was requested and contact identification, risk classification and monitoring began at the same time. A committee of experts was established for the classification of contacts. High- and low-risk classification criteria and the action taken for each group are

Table 1

Classification of contacts and public health measures adopted for the secondary Ebola case, Madrid, 6 October–27 November 2014

CLASSIFICATION OF CONTACTS	PUBLIC HEALTH MESURES FOR CONTACTS
Low-risk contact	
A person who, with appropriate PPE and without incidences in the use of PPE, had direct contact with a confirmed case, with his/her body fluids or any material that has potentially been contaminated in the course of healthcare;	Active monitoring: professionals responsible for monitoring contacts have daily contact with the monitored individual, measure his/her axillary temperature twice a day and record the presence of any symptom;
A person who has stayed in a closed physical space in which there could have been fomites with biological remains from the case and who does not comply with high-risk contact criteria (e.g. seats in the waiting room, the same surgery, the same ambulance, etc	The identity of contacts for monitoring is sent to health centres and hospitals (alerts in electronic clinical records) for early detection in case they consult for Ebola-related symptoms. The Blood Donors Centres of the Madrid Region also receive electronic alerts in the clinical records to avoid any incident related to possible blood donations by these individuals.
High-risk contact	
Close contact (distance <1 m), without appropriate PPE or with incidences in the use of PPE, with a confirmed case who was coughing, vomiting, bleeding or had diarrhoea;	Quarantine is indicated. In order to facilitate the compliance with the quarantine, hospital quarantine is offered to these contacts. All contacts included in this group (15 people) agreed to be admitted voluntarily.
Unprotected sexual relation with a confirmed case three months after the onset of symptoms;	
Direct contact with clothing, bedclothes or fomites contaminated with the blood, urine or body fluids of a confirmed case, without appropriate PPE or with incidences in the use of PPE;	
Percutaneous wound (e.g. needle-stick injury) or mucosal exposure to body fluids, tissues or laboratory samples of a confirmed case;	
Healthcare given to a case or handling of his/her samples, without the appropriate PPE or with incidences in the use of PPE.	

Table 2

Number of contacts of the secondary Ebola case by exposure place, relationship with case and risk category (high risk contacts in brackets), Madrid, Spain, 29 September–27 November 2014 (n=232)

Relation with case/ place of exposure	Cleaner	Patient/ patient's aid	Spouse	HCW	Dog sacrifice	Ambulance technicians	Other	**Total**
Transport by ambulance[a]	4	12	0	3	0	10	0	**29**
Primary care	2 (1)	22	0	4 (1)	0	0	0	**28 (2)**
Home	8[b]	0	1 (1)	1 (1)	6	0	1	**17 (2)**
Hospital	2	0	0	7 (7)	0	0	3 (1)	**12 (8)**
Other activities	0	0	0	2	0	0	7 (3)	**9 (3)**
Subtotal	16 (1)	34	1 (1)	17 (9)	6	10	11 (4)	**95 (15)**
HCW at reference hospital	11	0	0	113	0	0	2	**126**
Reference laboratory	0	0	0	0	0	0	11	**11**
Total contacts	27 (1)	34	1 (1)	130 (9)	6	10	24 (4)	**232 (15)**

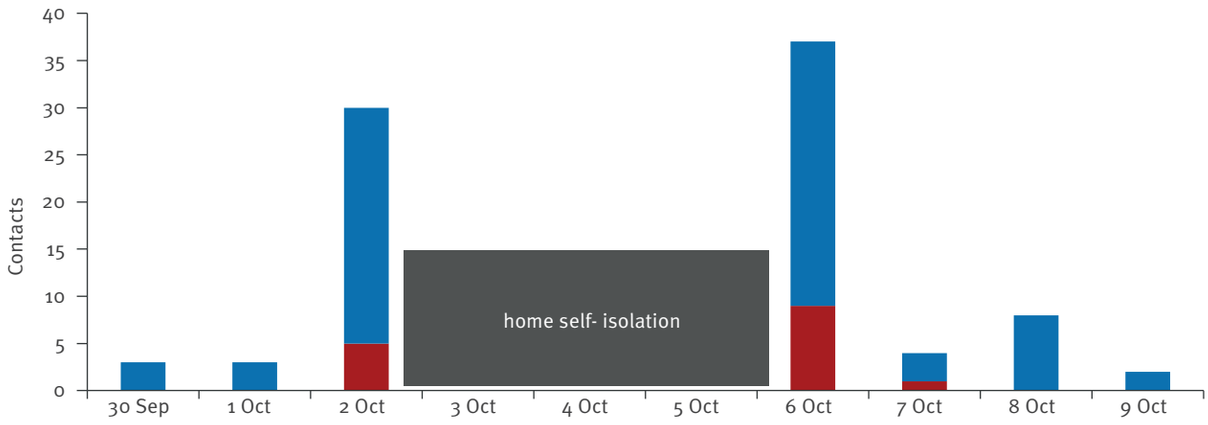
HCW: healthcare worker who attended to the secondary case.

[a] Two ambulances: from home to first hospital and from first hospital to reference hospital.

[b] The home cleaning was performed on the day after the patient was discharged from hospital.

Figure 2

Number of contacts of the secondary Ebola case, by exposure date and risk category[a], Madrid, Spain, 29 September–9 October 2014 (n=87)



	30 Sep	1 Oct	2 Oct	3 Oct	4 Oct	5 Oct	6 Oct	7 Oct	8 Oct	9 Oct
Low risk	3	3	25	0	0	0	28	3	8	2
High risk	0	0	5	0	0	0	9	1	0	0

[a] Excluded healthcare workers at the reference hospital, laboratory workers and home cleaners.

presented in Table 1. These actions were adapted from those established in the current protocol [8]. The first epidemiological information was provided by a family member of the patient at the hospital and was completed with available health and administrative records and the locations the patient reported to have visited from onset of symptoms until hospitalisation.

A total of 232 contacts were identified, of whom 15 were classified as high-risk and 217 as low-risk (Table 2). Most contacts, excluding HCW at reference hospital, occurred on the day of diagnosis at the hospital where the diagnosis was established (Figure 2). The 15 contacts classified as high-risk were informed of the risks associated with their contact with the case and were recommended a quarantine, at a hospital facility if possible. All of them voluntarily agreed to undergo hospital quarantine for 21 days after the last exposure day.

One of the low-risk contacts presented fever during the monitoring, but EVD was ruled out.

A total of 126 hospital employees were in contact with the patient during her stay at the hospital. Follow-up ended on 27 November, 21 days after the final exposure of the hospital cleaning staff. By that time, none of the contacts monitored had presented EVD.

Discussion

Action protocols are based on the evidence obtained in the outbreak in Africa [9-11]. Early detection of cases for minimising the probability of transmission is the key aim of contact monitoring. However, when the first secondary case was diagnosed in Spain, the case definition provided in the existing national protocol and in most international protocols (European Centre for Disease Prevention and Control [12], United States (US) Centers for Disease Control and Prevention [13,14]) required a fever of >38.6 °C and symptoms compatible with the disease. This definition was not sensitive enough to detect this case in the first stages of disease. The non-specific clinical presentation of Ebola also makes early case detection difficult. This situation was also observed in the two secondary cases diagnosed a few days later in the US [15-17].

We would like to draw attention to the 'paucisymptomatic' presentation of EVD in infected contacts closely monitored after exposure to confirmed cases outside of the epidemic area in Africa not described up to now.

The public health measures applied immediately to the contacts of the secondary case in Madrid included active monitoring of low-risk contacts and quarantine for high-risk contacts. All contacts accepted these measures. However, in the future it may be necessary to apply the quarantine to more people or to contacts who refuse to be quarantined. In our opinion, it is necessary to develop procedures and laws which would establish and help apply the quarantine.

The experience with the repatriated cases in several non-epidemic countries and the secondary transmissions identified in Spain and in the US have resulted in proposals to modify existing protocols. These proposals [18] include increased sensitivity of the case

definitions for persons under investigation in order to detect possible cases in the initial phases of the disease, particularly for contacts of confirmed cases, and a revision of contact classification and monitoring measures.

The Spanish experience highlights that the generation of secondary cases among HCW caring for repatriated EVD patients represents the currently main risk for Europe as has happened also in US [8,13-15]. The risk is very low, however it can not be excluded [19].

Despite the existence of preparedness and response plans, trained professional teams, 24/7 alert systems and contingency plans for control and response of communicable diseases in both hospitals, the number of exposed contacts among HCW was high. After the secondary case was diagnosed, training and assessment was reinforced for all healthcare professionals involved in the treatment and care of EVD and a committee was set up to classify incidents. This alert shows the need for constant updating and training of professionals in the use of PPE and strict application of donning and doffing procedures in order to minimise the risks. Hence it is necessary to provide adequate risk communication and create awareness in HCW who care for these patients.

Despite the rapid activation of the protocols and control measures, this first case of secondary transmission of EVD outside Africa has represented an unprecedented challenge for the health services and public health authorities in Spain [9,12-14] and has highlighted the need to strengthen continuous preparation and training in order to respond properly to this type of emergency.

Acknowledgements

We would like to thank all the clinicians, informatics and laboratory workers involved in the management of the outbreak.

Conflict of interest

None declared.

Authors' contributions

Jenaro Astray and Mª Ángeles Lópaz wrote the first draft of the manuscript. Mª Ángeles Lópaz managed the Ebola outbreak alert system, Jenaro Astray coordinated the Ebola response team of the Community of Madrid and acted as a liaison to the reference hospital, Maria Ordobás was responsible for contact monitoring, Felicitas Dominguez managed the alert information system, Carmen Álvarez and Manuel Martínez led the Ebola Crisis Committee. Carmen Amela, Mª José Sierra and Fernando Simón coordinated the Ebola response at the national level, and Carmen Amela also participated in the regional Ebola response team. Josep Jansa and Diamantis Plachouras participated in the contact classification and assessment. The working group participated in the fieldwork, conducting epidemiological survey, classifying cases and contact monitoring. All authors critically read and revised the drafts of the manuscripts.

Members of the working group of the Ebola outbreak investigation team of Madrid

Aguirre R, Aragón A, Arce A ,Camarero M, Córdoba E, Cuevas I, Domínguez MJ, García J, García-Comas L, Garcia N, Gascón MJ, Hernando M, Ibáñez C, Insua E, Jiménez S, Lasheras MD, Méndez I, Moratilla L, Navea A, Noguerales R, Nováková V, Ortiz H, Rodero I, Rodríguez E, San Miguel L, Taveira J A, Sanchez A, Santos S, Suarez B, Torijano MJ, Valcárcel MA, Velasco M, Zorrilla B.

References

1. WHO Ebola Response Team. Ebola virus disease in West Africa—the first 9 months of the epidemic and forward projections. N Engl J Med. 2014;371(16):1481-95. http://dx.doi.org/10.1056/NEJMoa1411100 PMID:25244186
2. Poletto C, Gomes M, Pastore Y, Piontti A, Rossi L, Bioglio L, Chao D, et al. Assessing the impact of travel restrictions on international spread of the 2014 West African Ebola epidemic. Euro Surveill.2014;19(42):20936. http://dx.doi.org/10.2807/1560-7917.ES2014.19.42.20936 PMID:25358040
3. Fitzpatrick G, Vogt F, MoiGbabai O, Black B, Santantonio M, Folkesson E, et al. Describing readmissions to an Ebola case management centre (CMC), Sierra Leone, 2014. Euro Surveill.2014;19(40):20924. http://dx.doi.org/10.2807/1560-7917.ES2014.19.40.20924 PMID:25323075
4. Fasina FO, Shittu A, Lazarus D, Tomori O, Simonsen L, Viboud C, et al. Transmission dynamics and control of Ebola virus disease outbreak in Nigeria, July to September 2014. Euro Surveill.2014;19(40):20920. http://dx.doi.org/10.2807/1560-7917.ES2014.19.40.20920 PMID:25323076
5. Sprenger M, Coulombier D. Preparedness is crucial for safe care of Ebola patients and to prevent onward transmission in Europe - outbreak control measures are needed at its roots in West Africa. Euro Surveill.2014;19(40):20925. http://dx.doi.org/10.2807/1560-7917.ES2014.19.40.20925 PMID:25323074
6. Infection prevention and control recommendations for hospitalized patients with known or suspected Ebola virus disease in U.S. hospitals. Atlanta: Centers for Disease Control and Prevention; Nov 2014. Available from: http://www.cdc.gov/vhf/ebola/hcp/infection-prevention-and-control-recommendations.html
7. Wiwanitkit V. Ebola, fear and preparedness. Epidemiol Health. 2014;36:e2014015. doi: 10.4178/epih/e2014015. PMID: 25220146.
8. Protocolo de actuación frente a casos sospechosos de enfermedad por virus ébola (eve). [Protocol for actionagainst suspected cases of Ebola virus disease]. Madrid: Ministry of Health,Social Services and Equality; 15 Sep 2014. Spanish. Available from: http://www.osakidetza.euskadi.net/contenidos/informacion/protocolos_epidem/es_4328/adjuntos/Protocolo%20de%20actuaci%C3%B3n%20EVE_15%2009%202014.pdf
9. Parra JM, Salmerón OJ, Velasco M. The First case of Ebola virus disease acquired outside Africa. N Engl J Med. 2014. PMID: 25409262.
10. Forrester JD, Pillai SK, Beer KD, Neatherlin J, Massaquoi M, Nyenswah TG, et al. Assessment of ebola virus disease, health care infrastructure, and preparedness - four counties, Southeastern Liberia, august 2014. MMWR Morb Mortal Wkly Rep. 2014;63(40):891-3. PMID:25299605
11. Benowitz I, Ackelsberg J, Balter SE, Baumgartner JC, Dentinger C, Fine AD, et al. Surveillance and preparedness for Ebola virus disease—New York City, 2014. MMWR Morb Mortal Wkly Rep. 2014;63(41):934-6. PMID:25321072
12. McCarty CL, Basler C, Karwowski M, Erme M, Nixon G, Kippes C, et al. Response to importation of a case of ebola virus disease - ohio, october 2014. MMWR Morb Mortal Wkly Rep. 2014;63(46):1089-91. PMID:25412070
13. European Centre for Disease Prevention and Control (ECDC). Ebola virus disease case definition for reporting in EU. Stockholm: ECDC. [Accessed: 21 Nov 2014]. Available from: http://ecdc.europa.eu/en/healthtopics/ebola_marburg_fevers/EVDcasedefinition/Pages/default.aspx
14. Centers for Disease Control and Prevention (CDC).Ebola virus disease information for clinicians in U.S. healthcare settings. Atlanta: CDC. [Accessed 21 Nov 2014]. Available from: http://

www.cdc.gov/vhf/ebola/hcp/clinician-information-us-healthcare-settings.html

15. Centers for Disease Control and Prevention (CDC). Risk of Exposure. Atlanta: CDC. [Accessed: 21 Nov 2014]. Available from: http://www.cdc.gov/vhf/ebola/exposure/index.html
16. Chevalier MS, Chung W, Smith J, Weil LM, Hughes SM, Joyner SN, et al. Ebola virus disease cluster in the United States - dallas county, Texas, 2014. MMWR Morb Mortal Wkly Rep. 2014;63(46):1087-8. PMID:25412069
17. Centers for Disease Control and Prevention (CDC). Cases of Ebola diagnosed in the United States. Atlanta: CDC. [Accessed: 21 Nov 2014]. Available from: http://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/united-states-imported-case.html
18. Protocolo de actuación frente a casos sospechosos de Enfermedad por virus ébola (EVE). [Protocol for action against suspected cases of Ebola virus disease]. Madrid: Ministry of Health, Social Services and Equality; 13 Oct 2014. Spanish. Available from: http://www.madrid.org/cs/Satellite?blobcol=urldata&blobheader=application%2Fpdf&blobheadername1=Content-disposition&blobheadername2=cadena&blobheadervalue1=filename%3DProtocolo-de-actuacion-EVE+aprobado+CIT+SNS+y+CC_CEGE_13_10_14_Adaptado+CM.pdf&blobheadervalue2=language%3Des%26site%3DPortalSalud&blobkey=id&blobtable=MungoBlobs&blobwhere=1352862624089&ssbinary=true
19. Evaluación del riesgo para España de la epidemia de fiebre hemorrágica por el virus de Ébola en África Occidental. [Risk assessment Spain epidemic hemorrhagic fever virus Ebola in West Africa]. Madrid: Ministry of Health, Social Services and Equality; 9 Dec 2014. Spanish. Available from: https://www.msssi.gob.es/profesionales/saludPublica/ccayes/alertasActual/ebola/docs/E_Riesgo_para_Espana_Fiebre_hemorragica

Research article

Migration-related tuberculosis: epidemiology and characteristics of tuberculosis cases originating outside the European Union and European Economic Area, 2007 to 2013

C Ködmön [1], P Zucs [1], MJ van der Werf [1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Correspondence: Csaba Ködmön (csaba.kodmon@ecdc.europa.eu)

Citation style for this article:
Ködmön C, Zucs P, van der Werf MJ. Migration-related tuberculosis: epidemiology and characteristics of tuberculosis cases originating outside the European Union and European Economic Area, 2007 to 2013. Euro Surveill. 2016;21(12):pii=30164. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.12.30164

Article submitted on 03 February 2015 / accepted on 05 November 2015 / published on 24 March 2016

Migrants arriving from high tuberculosis (TB)-incidence countries may pose a significant challenge to TB control programmes in the host country. TB surveillance data for 2007–2013 submitted to the European Surveillance System were analysed. Notified TB cases were stratified by origin and reporting country. The contribution of migrant TB cases to the TB epidemiology in EU/EEA countries was analysed. Migrant TB cases accounted for 17.4% (n = 92,039) of all TB cases reported in the EU/EEA in 2007–2013, continuously increasing from 13.6% in 2007 to 21.8% in 2013. Of 91,925 migrant cases with known country of origin, 29.3% were from the Eastern Mediterranean, 23.0% from south-east Asia, 21.4% from Africa, 13.4% from the World Health Organization European Region (excluding EU/EEA), and 12.9% from other regions. Of 46,499 migrant cases with known drug-susceptibility test results, 2.9% had multidrug-resistant TB, mainly (51.7%) originating from the European Region. The increasing contribution of TB in migrants from outside the EU/EEA to the TB burden in the EU/EEA is mainly due to a decrease in native TB cases. Especially in countries with a high proportion of TB cases in non-EU/EEA migrants, targeted prevention and control initiatives may be needed to progress towards TB elimination.

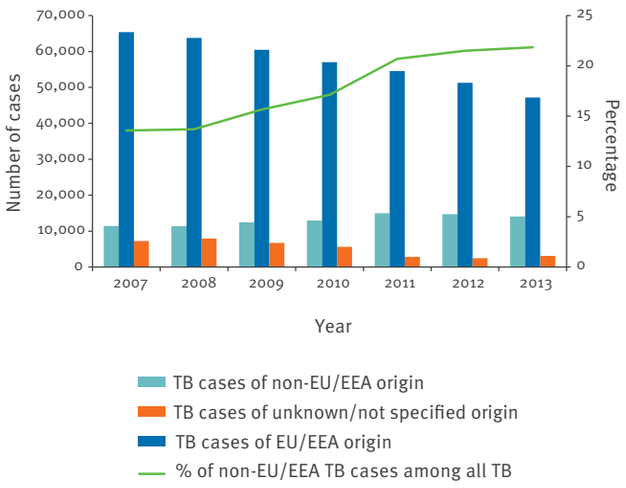
Introduction

The tuberculosis (TB) notification rate in the European Union and European Economic Area (EU/EEA) declined from 16.8 per 100,000 population in 2007 to 12.7 per 100,000 in 2013 [1]. However, in some low-incidence countries, the decline in TB notification rate has slowed down, especially in countries reporting a high proportion of TB cases in individuals of foreign origin, i.e. migrants. In general, migration is influenced by socioeconomic and political factors [2]. Economic, social and political stability is relatively high in the EU/EEA which thus attracts immigrants from many low-income countries around the world [3]. On average (years 2007–2012), 1.5 million migrants from outside the EU/EEA were registered annually in EU and EEA countries [4]. A considerable proportion of these migrants are coming from countries with a high TB burden such as Bangladesh, Brazil, China, India, Morocco, Pakistan, Russian Federation, Somalia and Ukraine [5]. They may arrive in the EU/EEA with active TB disease, or with latent TB infection (LTBI). To detect TB disease in migrants, several EU/EEA countries have introduced (pre-)entry screening programmes [6-8]. Screening of migrants for LTBI is also being explored by some countries, such as the Netherlands [9]. However, screening programmes will not identify all TB or LTBI cases among migrants, due to the limited sensitivity of the current screening tests (mainly chest x-ray and tuberculin skin test or interferon gamma release assay). Also, not all migrant groups are covered by the screening programme, e.g. undocumented migrants are often not included. In addition, migrants frequently travel back to their country of origin where they may be (re-) infected with TB [10].

Migrants developing TB may pose a challenge to TB programmes in the EU/EEA due to language and cultural differences [11]. Also, undocumented migrants may not access the healthcare system due to fear of deportation, and migrants whose stay is legal may be unfamiliar with the healthcare system and therefore encounter problems in seeking healthcare [12]. Since countries with low TB notification rates report high numbers of TB cases in migrants in particular, it is important to study this phenomenon because addressing TB in migrants will be essential to achieving the goal of TB programmes, i.e. TB elimination [13]. Therefore, the aim of this study is to quantify and to geographically

Figure 1

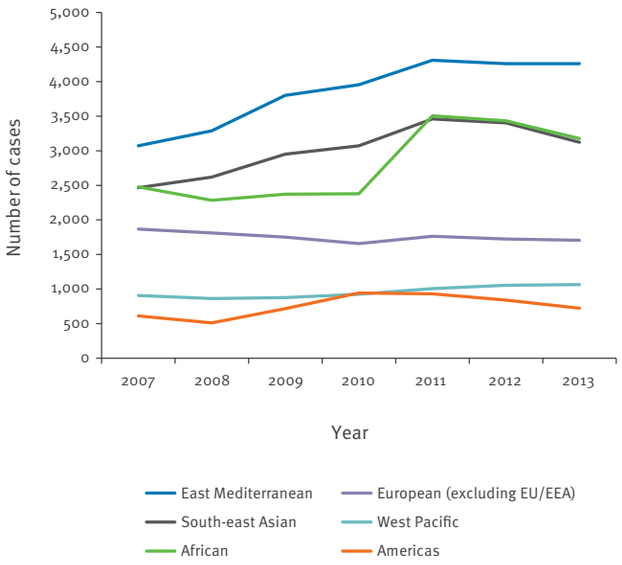
Number of tuberculosis cases by year and origin, and percentage of non-European Union/European Economic Area cases among all tuberculosis cases, European Union/European Economic Area, 2007–2013



EEA: European Economic Area; EU: European Union; TB: tuberculosis.

Figure 2

Number of tuberculosis cases of non- European Union/European Economic Area origin by year and World Health Organization Region, 2007–2013 (n=91,925)



EEA: European Economic Area; EU: European Union.

and epidemiologically characterise migration-related importation of TB to EU/EEA countries.

Methods

The European Centre for Disease Prevention and Control (ECDC) has collected case-based TB surveillance data from EU and EEA countries since 2007 and stored them in a common database (The European Surveillance System, TESSy) hosted by ECDC. Designated national surveillance institutions are responsible for data reporting to TESSy and for data validation.

The detailed data collection methods and definitions are described elsewhere [1]. TB cases were defined according to agreed case definitions published by the European Commission [14] and confirmed, probable and possible cases were included in the analysis. Surveillance data reported by 29 EU/EEA countries and covering the period from 2007 to 2013 were extracted from the database on 3 October 2014. Place of birth was used as a proxy indicator for the geographic origin of a TB case in most countries; except for Austria, Belgium, Greece, Poland, Hungary (from 2010 onwards) and for Malta (only in 2010) where citizenship was used. Place of birth outside EU/EEA borders was used as proxy for migrant TB in most countries. Non-EU/EEA citizenship was used for Austria, Belgium, Greece, Poland, Hungary (from 2010 onwards) and for Malta (only in 2010).

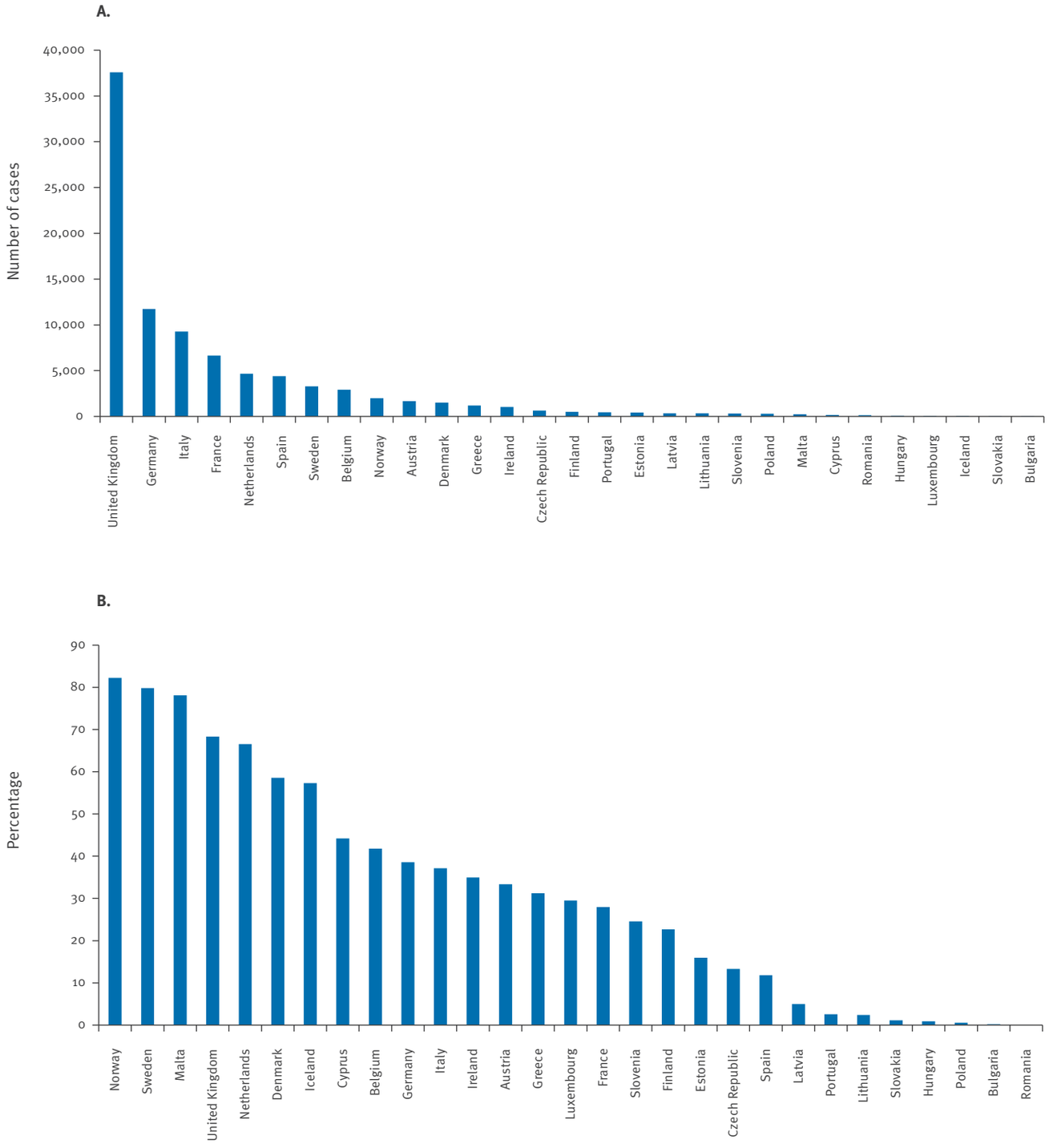
The analysis was restricted to TB cases with known origin. The areas of origin were defined according to the World Health Organization (WHO) regions described in the *Global Tuberculosis Report, 2013* [15].

The European Region refers to the WHO European Region excluding the EU and EEA (Iceland, Liechtenstein and Norway) countries. To assign country of origin (based on place of birth), we used the ISO 3166–1 codes for countries, dependent territories, and special areas of geographical interest which are published by the International Organization for Standardization [16]. The origin of cases reported by or from populated Overseas Countries and Territories of EU countries was assigned according to their geographic location and such cases counted as cases in individuals of non-EU/EEA origin. Cases reported/coded in the system as originating (based on place of birth) either from 'Soviet Union' (Former Soviet Union (FSU) countries: Armenia, Azerbaijan, Belarus, Estonia (EU), Georgia, Kazakhstan, Kyrgyzstan, Latvia (EU), Lithuania (EU), Moldova, Russian Federation, Tajikistan, Turkmenistan, Ukraine and Uzbekistan) or 'Yugoslavia' (Bosnia and Herzegovina, Croatia (EU), Kosovo*, Montenegro, Serbia, Slovenia (EU) and the former Yugoslav Republic of Macedonia) were classified as cases of unspecified origin (n = 114), because some parts of those two historical countries belong to the EU today as indicated in brackets.

Liechtenstein reported TB surveillance data to TESSy only for 2007 and was therefore excluded from the analysis. Croatia joined the EU in July 2013 and was considered a non-EU/EEA country in the analysis. France, Italy, and Spain are not reporting drug resistance data to TESSy and were excluded from the analysis of laboratory data and drug resistance. Treatment outcome data were not reported by France, Greece,

Figure 3

Number of tuberculosis cases (A) and percentage of tuberculosis cases (B) of non- European Union/European Economic Area origin among tuberculosis cases with known country of origin (B), by reporting country, European Union/European Economic Area, 2007–2013 (n=91,925)

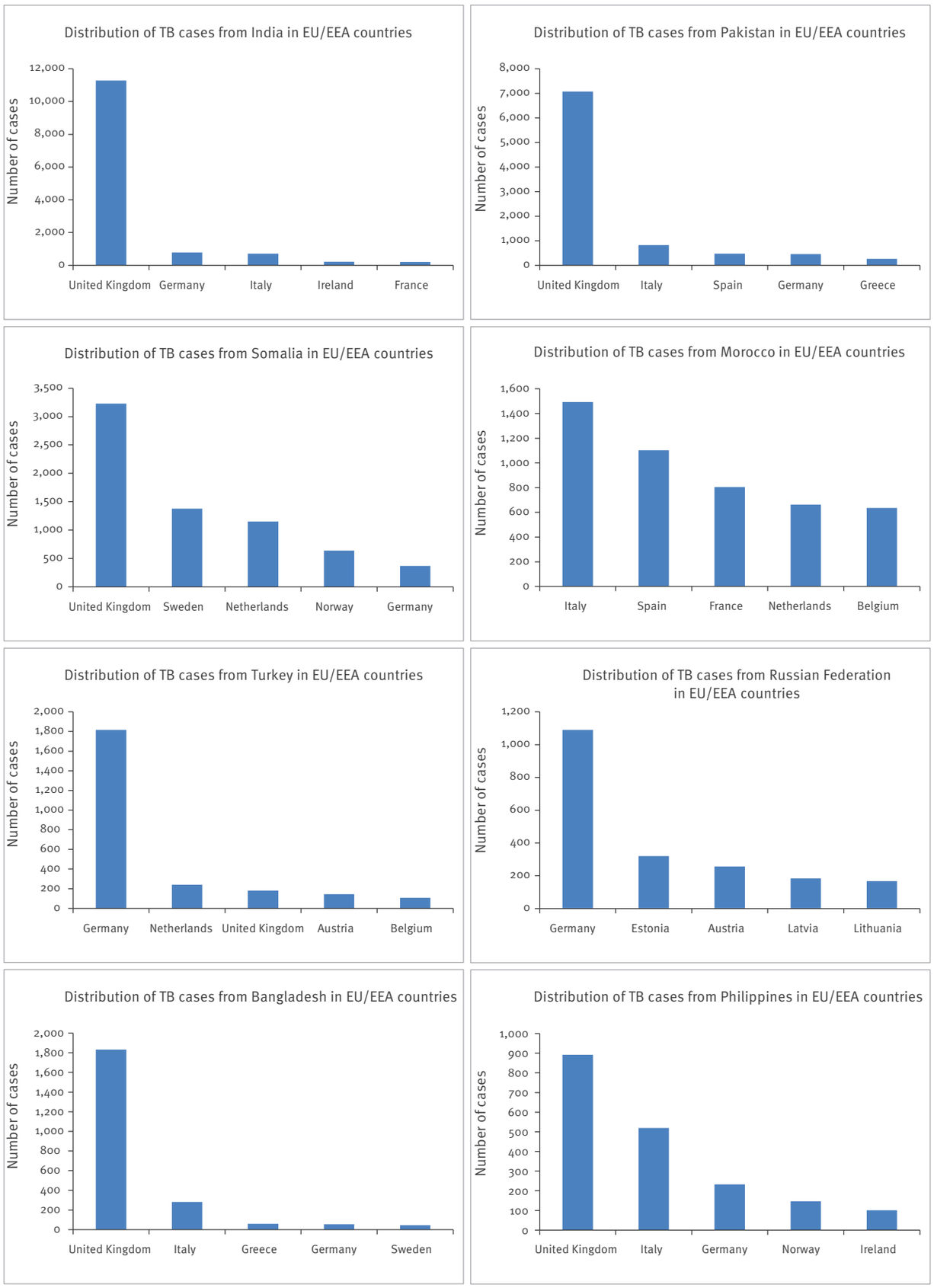


and Italy in 2007–2012, and by Spain in 2007–2009. Therefore, these countries were excluded from the treatment outcome analysis. TB treatment was considered successful if a case was cured or their treatment completed 12 months after start of treatment.

TB cases were described by year of reporting, origin and country of reporting. Native cases (EU/EEA origin) and cases from outside the EU/EEA were compared by sex, age, previous treatment history, TB site, laboratory confirmation status, drug resistance, HIV status and treatment outcome. Differences were considered statistically significant, if $p<0.01$ as determined by

Figure 4

Distribution of tuberculosis cases originating from India, Pakistan, Somalia, Morocco, Turkey, Russian Federation, Bangladesh and the Philippines across the five European Union/European Economic Area countries with the highest reported numbers, 2007–2013 (n=47,440)



EEA: European Economic Area; EU: European Union; TB: tuberculosis.

Table a

Characteristics of tuberculosis cases with reported country of origin by region of origin, European Union/European Economic Area, 2007–2013 (n=491,538)

	WHO Region																Total	
	EU/EEA		Total non-EU/EEA		Eastern Mediterranean		South-East Asian		African		European (excluding EU/EEA)		Western Pacific		Americas			
	N	%	N	%	N	%[a]	N	%[a]	N	%[a]	N	%[a]	N	%[a]	N	%[a]	N	%
Total	**399,613**	**81.3**	**91,925**	**18.7**	**26,945**	**29.3**	**21,097**	**23.0**	**19,629**	**21.4**	**12,280**	**13.4**	**6,697**	**7.3**	**5,277**	**5.7**	**491,538**	**100**
Sex																		
Male	264,068	66.1	53,122	57.8	16,348	60.7	12,022	57.0	11,667	59.4	7,381	60.1	3,112	46.5	2,592	49.1	317,190	64.5
Female	135,220	33.8	38,580	42.0	10,545	39.1	9,016	42.7	7,918	40.3	4,868	39.6	3,561	53.2	2,672	50.6	173,800	35.4
Unknown	325	0.1	223	0.2	52	0.2	59	0.3	44	0.2	31	0.3	24	0.4	13	0.2	548	0.1
Age groups (years)																		
0–14	18,034	4.5	2,601	2.8	1,052	3.9	276	1.3	612	3.1	368	3.0	138	2.1	155	2.9	20,635	4.2
15–24	39,266	9.8	14,741	16.0	5,538	20.6	3,007	14.3	3,338	17.0	1,071	8.7	1,049	15.7	738	14.0	54,007	11.0
25–44	122,780	30.7	48,683	53.0	13,012	48.3	12,439	59.0	11,584	59.0	4,910	40.0	3,740	55.8	2,998	56.8	171,463	34.9
45–64	135,147	33.8	17,611	19.2	4,786	17.8	3,499	16.6	3,210	16.4	3,680	30.0	1,379	20.6	1,057	20.0	152,758	31.1
65+	83,946	21.0	8,157	8.9	2,504	9.3	1,864	8.8	856	4.4	2,236	18.2	379	5.7	318	6.0	92,103	18.7
Unknown	440	0.1	132	0.1	53	0.2	12	0.1	29	0.1	15	0.1	12	0.2	11	0.2	572	0.1
Previous TB history																		
No	317,268	79.4	70,386	76.6	21,080	78.2	17,409	82.5	14,728	75.0	7,838	63.8	5,105	76.2	4,226	80.1	387,654	78.9
Yes	58,781	14.7	5,721	6.2	1,627	6.0	1,137	5.4	996	5.1	1,411	11.5	337	5.0	213	4.0	64,502	13.1
Unknown	23,564	5.9	15,818	17.2	4,238	15.7	2,551	12.1	3,905	19.9	3,031	24.7	1,255	18.7	838	15.9	39,382	8.0
Site of disease																		
Pulmonary	333,989	83.6	53,111	57.8	13,737	51.0	9,215	43.7	11,961	60.9	10,168	82.8	4,287	64.0	3,743	70.9	387,100	78.8
Extra-pulmonary	64,968	16.3	38,463	41.8	13,109	48.7	11,818	56.0	7,592	38.7	2,032	16.5	2,384	35.6	1,528	29.0	103,431	21.0
Unknown	656	0.2	351	0.4	99	0.4	64	0.3	76	0.4	80	0.7	26	0.4	6	0.1	1,007	0.2
Laboratory confirmation																		
Confirmed	214,612	53.7	47,925	52.1	13,920	51.7	12,278	58.2	9,202	46.9	7,748	63.1	3,577	53.4	1,200	22.7	262,537	53.4
Not confirmed	119,397	29.9	23,693	25.8	7,457	27.7	7,013	33.2	4,103	20.9	3,105	25.3	1,484	22.2	531	10.1	143,090	29.1
Laboratory data not reported	65,604	16.4	20,307	22.1	5,568	20.7	1,806	8.6	6,324	32.2	1,427	11.6	1,636	24.4	3,546	67.2	85,911	17.5
Drug resistance among DST done																		
DST done among laboratory confirmed	147,090	68.5	46,499	97.0	13,580	97.6	12,030	98.0	8,945	97.2	7,322	94.5	3,443	96.3	1,179	98.3	193,589	73.7
Susceptible	126,945	86.3	40,538	87.2	12,044	88.7	10,794	89.7	8,046	89.9	5,679	77.6	2,912	84.6	1,063	90.2	167,483	86.5
Mono-resistant	8,664	5.9	3,492	7.5	1,069	7.9	813	6.8	614	6.9	552	7.5	358	10.4	86	7.3	12,156	6.3
Poly-resistant	2,821	1.9	1,107	2.4	270	2.0	199	1.7	145	1.6	387	5.3	92	2.7	14	1.2	3,928	2.0
MDR among DST done	8,660	5.9	1,362	2.9	197	1.5	224	1.9	140	1.6	704	9.6	81	2.4	16	1.4	10,022	5.2
XDR among MDR	691	8.0	80	5.9	6	3.0	2	0.9	2	1.4	68	9.7	2	2.5	0	0.0	771	7.7
HIV status																		
Tested for HIV	83,062	20.8	5,876	6.4	1,626	6.0	372	1.8	1,189	6.1	1,206	9.8	422	6.3	1,061	20.1	88,938	18.1
HIV-positive among tested	3,999	4.8	567	9.6	32	2.0	18	4.8	289	24.3	114	9.5	14	3.3	100	9.4	4,566	5.1

DST: drug susceptibility testing; MDR: multidrug resistant; EEA: European Economic Area; EU: European Union; N: number; WHO: World Health Organization; XDR: extensively drug resistant.

[a] Percentage among TB cases in individuals of non-EU/EEA origin.

Table b

Characteristics of tuberculosis cases with reported country of origin by region of origin, European Union/European Economic Area, 2007–2013 (n=491,538)

	WHO Region																Total	
	EU/EEA		Total non-EU/EEA		Eastern Mediterranean		South-East Asian		African		European (excluding EU/EEA)		Western Pacific		Americas			
	N	%	N	%	N	%[a]	N	%[a]	N	%[a]	N	%[a]	N	%[a]	N	%[a]	N	%
Treatment outcome[b]																		
Number of reported cases 2007–2012	352,428		77,875		22,687		17,975		16,452		10,574		5,632		4,555		430,303	
Treatment outcome reported	305,945	86.8	63,600	81.7	18,841	83.0	16,492	91.7	11,994	72.9	9,148	86.5	4,443	78.9	2,656	58.3	369,545	85.9
Success	228,351	74.6	49,256	77.4	15,141	80.4	12,839	77.8	9,328	77.8	6,444	70.4	3,349	75.4	2,155	81.1	277,607	75.1
Failed	6,900	2.3	109	0.2	29	0.2	9	0.1	10	0.1	48	0.5	10	0.2	3	0.1	7,009	1.9
Defaulted	20,176	6.6	3,436	5.4	848	4.5	1,083	6.6	615	5.1	538	5.9	271	6.1	81	3.0	23,612	6.4
Died	25,123	8.2	2,052	3.2	503	2.7	475	2.9	303	2.5	595	6.5	101	2.3	75	2.8	27,175	7.4
Still on treatment	9,427	3.1	4,312	6.8	1,202	6.4	1,309	7.9	829	6.9	606	6.6	275	6.2	91	3.4	13,739	3.7
Not evaluated	15,968	5.2	4,435	7.0	1,118	5.9	777	4.7	909	7.6	917	10.0	437	9.8	251	9.5	20,403	5.5

DST: drug susceptibility testing; MDR: multidrug resistant; EEA: European Economic Area; EU: European Union; N: number; WHO: World Health Organization; XDR: extensively drug resistant.

[a] Percentage among TB cases in individuals of non-EU/EEA origin.

[b] Treatment outcome 12 months after starting treatment for cases notified in 2007–2012.

chi-squared test. Statistical analysis was performed using Stata 13 software (StataCorp, Texas, US).

Results

Of 527,467 TB cases notified in the EU/EEA from 2007 to 2013, 399,613 (75.8%) were reported as originating from EU/EEA countries, 92,039 (17.4%) as originating from non-EU/EEA countries, and for 35,815 (6.8%), country of origin was not reported. Among 491,652 TB cases with reported country of origin, 122,627 (24.9%) originated from outside the reporting country. Of these, 91,925 (75%) originated from outside the EU/EEA, 30,588 (24.9%) were of EU/EEA origin, and 114 (0.1%) originated from 'Soviet Union' or 'Yugoslavia'. The proportion of TB cases with reported non-EU/EEA origin increased from 13.6% (n=11,403) in 2007 to 21.8% (n=14,050) in 2013, the proportion of TB cases with reported EU/EEA origin decreased from 77.8% (n=65,390) in 2007 to 73.4% (n=47,185) in 2013, while the proportion of TB cases with unknown or unspecified origin decreased from 8.6% (n=7,221) to 4.8% (n=3,092) in the same period (p<0.001) (Figure 1).

Of 92,039 cases with non-EU/EEA origin, the country of origin was reported for 91,925 (99.9%) cases, with the majority coming from the Eastern Mediterranean Region (29.3%, n=26,945), the South-East Asian Region (23.0 %, n=21.097) and the African Region (21.4%, n=19,629) (Table).

Compared with native TB cases, TB cases in individuals of non-EU/EEA origin were more frequently female (42.0% vs 33.8%, p<0.001) and under 45 years of age (71.8% vs 45.1%, p<0.001) (Table). Cases of non-EU/EEA origin had a previous TB history less frequently (6.2% vs 14.7%, p<0.001), but a proportion of cases with unknown previous history three times higher than native cases. Extrapulmonary TB was much more commonly diagnosed in cases of non-EU/EEA origin (41.8% vs 16.3%, p<0.001). Very similar proportions, just over 50% of cases were laboratory-confirmed in both native and migrant cases, but the latter were much more extensively tested for drug susceptibility (97.0% vs 68.5%, p<0.001), and were found to be mono-resistant and poly-resistant slightly more frequently, but not multidrug-resistant (9.9% vs 2.9%, p<0.001). The majority of multidrug-resistant (MDR) TB and extensively drug-resistant (XDR) TB cases in individuals of non-EU/EEA origin were from the European Region, where the highest percentage of MDR-TB cases among the cases with available drug susceptibility testing (DST) results (9.6%, n=704) was observed, as well as the highest percentage of XDR-TB cases among MDR-TB (9.7%, n=68). Of 704 MDR-TB cases originating from the European Region, 678 (96.3%) were notified in cases coming from 13 non-EU/EEA 'Soviet Union' countries (data not shown). The highest percentage of mono-resistance to a first-line anti-TB drug was observed in cases originating from the Western Pacific Region (10.4%, n=358). Most cases with mono-resistance originated from the Philippines, Vietnam and China (145, 117 and 48 respectively). Among the mono-resistant TB cases from the Philippines, 83.4% (n=121) were resistant to isoniazid, while in cases originating from Vietnam and China, 55.6% (n=65) and 60.4% (n=29) were resistant to isoniazid (data not shown). In the period 2007–2013 the trend in MDR-TB prevalence among cases of non-EU/EEA origin did

not change significantly (p=0.94, data not shown). Cases of non-EU/EEA origin were tested for HIV much less frequently than native cases (6.4% vs 20.8%, p<0.001), but tested HIV-positive twice as often (9.6% vs 4.8%, p<0.001). Among cases of non-EU/EEA origin, the majority and highest prevalence of HIV co-infection was found in cases originating from the African Region. A higher proportion of treatment success was reported in migrant cases (77.4% vs 74.6%, p<0.001), while the proportion that died during treatment was lower (3.2% vs 8.2%, p<0.001). The percentage of TB cases where the treatment outcome was 'lost to follow-up' was lower in the cases of non-EU/EEA origin (5.4% vs 6.6%), but the percentage of non-evaluated cases was higher (7.0% vs 5.2%). The lowest treatment success rate, 70.4%, was observed among cases from the European Region.

From 2007 to 2011, the number of notified TB cases in individuals of non-EU/EEA origin increased for all WHO Regions except for the European region (Figure 2). Thereafter, the number remained the same or decreased slightly. In the same period, the number of TB cases with unknown country of origin decreased from 8.6% in 2007 to 4.8% in 2013. The mean annual increase in the period 2007–2011 was highest for cases originating from Americas (13.5%; standard deviation (SD): 18.4), followed by the African Region (10.9%; SD: 20.4), the South-East Asian Region (8.9%; SD: 8.1), the Eastern Mediterranean Region (8.9%; SD: 5.3) and the Western Pacific Region (2.8%; SD: 4.2), while for cases originating from the European Region a mean annual decrease of 1.3% (SD: 3.7) was observed. The mean increase in the number of notified cases was the highest for cases originating from the Eastern Mediterranean Region (n=309; SD: 183.1), followed by the African Region (n=256; SD: 411.3), the South-East Asian Region (n=248; SD: 238.2), the Americas (n=75; SD: 145.1) and the Western Pacific Region (n=25; SD: 52.7). The notification of cases originating from the European Region showed the mean decrease of 26 cases annually (SD: 63.2).

Of all TB cases in individuals of non-EU/EEA origin, 40.9% (n=37,573) were reported by the United Kingdom (UK), 12.8% (n=11,728) by Germany and 10.1% (n=9,264) by Italy (Figure 3A. The highest contribution of TB cases in individuals of non-EU/EEA origin to the national TB burden was observed in Norway with 82.4% (n = 1,997), Sweden with 79.9% (n = 3,274) and Malta with 78.1% (n = 228) (Figure 3B).

The reported non-EU/EEA TB cases originated from 186 countries, dependent territories, and special areas of geographical interest with 51.6% coming from India (15.3%), Pakistan (10.9%), Somalia (8.5%), Morocco (5.7%), Turkey (3.0%), Russian Federation (2.9%), Bangladesh (2.7%), and the Philippines (2.6%). Their distribution mirrors the typical migration flows and destination country preferences (Figure 4). Between 2007 and 2013, increasing numbers of TB cases from India, Pakistan and Morocco were notified (p<0.001, data not shown).

Most cases from India (80.3%, n=11,293) were reported by the UK (Figure 4). The UK also reported a large percentage of the cases originating from Pakistan (70.5%, n=7,073), from Somalia (41.2%, n=3,228), from Bangladesh (74.7%, n=1,833), and from Philippines (36.7%, n=892). Germany reported 66.8% (n=1,818) of all reported cases from Turkey and 40.6% (n=1,091) of all reported cases from Russian Federation. While, Italy reported the largest percentage of cases from Morocco (28.7%, n=1,493).

Discussion

Almost one in five TB cases notified in the EU/EEA between 2007 and 2013 originated from a country outside the EU/EEA, but this varied from<1% to>80% between the 29 countries included in this study. The percentage of migrant TB cases increased from 13.6% to 21.8% between 2007 and 2013, while the overall number of cases of non-EU/EEA origin increased from 11,403 in 2007 to 14,975 in 2011 and slightly decreased thereafter to 14,050 in 2013. The increasing percentage of migrant TB among all notified TB cases is largely attributable to the decreasing numbers of native TB cases and cases with unknown origin. The highest mean annual increase in notifications was observed in TB cases originating from the Eastern Mediterranean and African Regions. The only decreasing trend was seen in cases originating from the European Region. Increasing trends in notified TB cases in migrants have also been observed in other high-income countries such as Australia, Canada, and the United States (US) [17-19].

TB cases originating from eight countries accounted for 51.6% of all TB cases in individuals of non-EU/EEA origin. This can be explained by the burden of TB in these countries [15] and the relatively high number of migrants from these countries to the EU/EEA [5,6]. Data from Australia, Canada and the US showed that the TB notification rate among migrants is strongly associated with the TB burden in the country of origin [18]. Among foreign-born and US-born cases in the US, the level of education, living conditions, low income and unemployment were associated with higher TB rates; this association was stronger in the foreign-born cases. According to the authors, these results support the hypothesis that the TB rates among foreign-born cases are more strongly influenced by experiences in their country of origin than by the environments in the host country [19]. Similarly to the situation in the EU/EEA, the 25 to 44 years-old age group was most represented in the US among foreign-born TB cases [20]. In the EU/EEA, the high proportions of males seen among cases originating from the Eastern Mediterranean and European Regions suggest that the majority of TB cases from these regions are migrant workers. This is supported by Eurostat data according to which, on average

29% of residence permits were issued in 2008–2012 due to employment and 28% due to family reasons [5].

Exposure to TB before immigration to the EU/EEA and when travelling back to the country of origin for family visits may result in relatively high latent TB infection rates in migrant populations [21-23]. Several studies suggest that the majority of cases among migrants occur due to TB infection or reinfection when travelling to their home country [20,24,25] or due to reactivation of latent TB [20,26,27]. However, TB in migrants might also be due to recent infection or reinfection in the host country after local exposure [27-30].

According to the Eurostat data, there are remarkable differences in the number of migrants received by different EU/EEA countries. The UK, Italy, Germany, France, the Netherlands and Spain received the highest number of non-EU/EEA migrants during the period 2007–2012 [4]. In most EU/EEA Member States, this migration peaked in 2010, which was probably largely attributable to the global financial crisis [4,31]. Both the geographical distribution of reported TB cases in individuals of non-EU/EEA origin and their overall trend over time appears to follow the general migration patterns described [5,20]. As the biggest reporting country of TB cases in individuals of non-EU/EEA origin, the UK saw the majority of these cases originating from India, Pakistan and Bangladesh. The same three countries were also among the top five countries contributing to the TB burden in the US [20].

The highest prevalence of MDR-TB and XDR-TB was observed among cases of non-EU/EEA European origin. In the US in 2007–2009, 1.5% of foreign-born cases with available DST results were reported with MDR-TB, and the highest percentage (9.3%) was also observed among cases of European origin [32]. Equally, in Canada, the highest percentage of MDR-TB cases (2.9%) among foreign-born TB cases originated from the European Region [33]. This reflects the high prevalence of drug resistance among TB cases in the non-EU/EEA European Region [15].

Extrapulmonary TB was more frequently reported in TB cases in individuals of non-EU/EEA origin. Since extrapulmonary TB (excluding laryngeal TB) is rarely infectious, these cases will not contribute to transmission in the host country but do have an impact on health service costs. Further, extrapulmonary TB can result in significant suffering [34] and the diagnosis is often challenging [35]. Therefore, healthcare workers need to have a relatively high level of suspicion when persons of non-EU/EEA origin present with unexplained signs and symptoms that might be caused by extrapulmonary TB.

As expected, given the global HIV situation [36], most HIV co-infections were observed among cases of African and Western Pacific origin.

In Japan, 63.4% foreign-born smear-positive TB cases had a successful treatment outcome in the period 2007–2010 [37]. The situation in the EU/EEA is much better with 77.4% of TB cases in individuals of non-EU/EEA origin having a successful treatment outcome 12 months after starting treatment. Among TB cases in individuals of non-EU/EEA origin notified in EU/EEA, 17.9% percent did not have treatment outcome data reported, while in Japan, treatment outcome was not available for 16.6% of foreign-born smear-positive cases [37]. In the EU/EEA, the lowest treatment success rate (70.4%) was observed in cases from the European Region. This is probably attributable to the high percentage of MDR TB and XDR TB cases which require more than 12 months of treatment and would therefore be reported as 'still on treatment' 12 months after starting treatment. Another reason may be the high percentage of non-evaluated cases (10.0%) which might mask the real number of cases lost to follow-up. The non-uniform use of treatment outcome categories such as 'lost to follow-up', 'transferred out', 'still on treatment' and 'unknown' across the EU/EEA Member States might contribute to the high number of cases with non-evaluated treatment outcome [38]. In contrast to an earlier publication from the year 2000 that covers the period 1993-1997, where origin from 'Eastern Europe' and 'Yugoslavia' were identified as risk factors for loss to follow-up [39], the percentage of this treatment outcome in our study was smaller in TB cases in individuals of non-EU/EEA origin than in cases of EU/EEA origin. The percentage was especially low in cases originating from the European Region outside the EU/EEA. The treatment success rate in TB cases in individuals of non-EU/EEA origin was higher compared with native TB cases (77.4% vs 74.6%), and the fatality rate was lower (3.2% vs 8.2%). The percentage of TB cases over 64 years of age was lower in migrants compared with native TB cases (8.9% vs 21.0%) which explains the treatment outcome results.

Limitations

This study is based on TB surveillance data submitted to ECDC by the EU/EEA countries. In the EU/EEA TB surveillance system, only a limited number of variables are collected. Also, not all reported information is complete, and data quality is primarily the responsibility of the individual country. The origin of 6.8% of TB cases notified between 2007 and 2013 was not reported. In addition, three countries did not report case-based drug resistance data, and four countries did not report case-based treatment outcome data for the whole period. Due to this missing information, our results might not provide the complete picture of TB epidemiology among cases of non-EU/EEA origin. Furthermore, TB rates among immigrants could not be calculated due to the unavailability of migrant population data.

The differences in reporting of country of origin (country of birth vs nationality) might affect the comparability of data between some countries. The burden of non-EU/EEA migrant TB cases might be underestimated

in countries reporting nationality, as the migrants might have obtained the citizenship of the host country before TB was diagnosed.

Italy, France and Spain are not reporting TB drug resistance data to TESSy. The exclusion of TB cases reported by these countries compromises the representativeness of laboratory results in this study as these three countries received a relatively high number of non-EU/EEA immigrants.

The laboratory confirmation rate has been shown to be below 50% in some major reporting countries EU/EEA MSs [1] which might lead to the underestimation of resistant TB cases.

The HIV testing coverage among TB cases is suboptimal and does therefore not allow for an in-depth analysis of the data. The low testing coverage might lead to under- or over estimation of TB/HIV co-infection in EU/EEA.

Conclusions

Migration from outside the EU/EEA contributes markedly to the TB burden in the EU/EEA. Targeted prevention and control efforts (e.g. access to healthcare for all migrants including undocumented migrants, avoiding interruption of treatment) and implementation of active case finding approaches (e.g. screening at entry point, screening for latent TB infection) focussed on non-EU/EEA migrants may be needed in order to diagnose cases early, provide adequate treatment and support and reduce the burden of TB among migrants.

*This designation is without prejudice to positions on status, and is in line with United Nations Security Council resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence.

Acknowledgements

The authors acknowledge the nominated national operational contact points for tuberculosis (in alphabetical order): Peter Henrik Andersen, Delphine Antoine, Trude Margrete Arnesen, Thorsteinn Blondal, Domnica Ioana Chiotan, Edita Davidavičienė, Irene Demuth, Raquel Duarte, Sabine Erne, Walter Haas, Alexander Indra, Jerker Jonsson, Ourania Kalkouni, Maria Koliou, Maria Korzeniewska – Kosela, Gábor Kovács, Joan O'Donnell, Analita Pace Asciak, Maria Grazia Pompa, Elena Rodríguez Valín, Alexandar Simuniovic, Erika Slump, Hanna Soini, Ivan Solovič, Petra Svetina, Lucy Thomas, Tonka Varleva, Piret Viiklepp, Kate Vulāne, Jiří Wallenfels and Maryse Wanlin, who provided the surveillance data used in this analysis.

No specific sources of funding were used for this study. The authors performed the study as part of their job at ECDC.

Conflict of interest

None declared.

Authors' contributions

CK contributed to the study design, performed the data analysis, and wrote the first draft of the manuscript, PZ contributed to the study design, and contributed to further versions of the manuscript and approved the final version before submission, MvdW contributed to the study design and data analysis, and contributed to further versions of the manuscript and approved the final version before submission.

References

1. European Centre for Disease prevention and Control (ECDC)/WHO Regional Office for Europe. Tuberculosis surveillance and monitoring in Europe, 2015. Stockholm: ECDC; 2015. Available from: http://ecdc.europa.eu/en/publications/Publications/tuberculosis-surveillance-monitoring-Europe-2015.pdf
2. Abarca Tomás B, Pell C, Bueno Cavanillas A, Guillén Solvas J, Pool R, Roura M. Tuberculosis in migrant populations. A systematic review of the qualitative literature.PLoS One. 2013;8(12):e82440. DOI: 10.1371/journal.pone.0082440 PMID: 24349284
3. Millet JP, Moreno A, Fina L, del Baño L, Orcau A, de Olalla PG, et al. Factors that influence current tuberculosis epidemiology. Eur Spine J. 2013;22(S4) Suppl 4;539-48. DOI: 10.1007/s00586-012-2334-8 PMID: 22565801
4. Eurostat. Databases. [Accessed 25 July 2014] Available from: http://ec.europa.eu/eurostat/en/data/database
5. Eurostat. European social statistics. 2013; [Accessed 15 August 2014]. Available from: http://ec.europa.eu/eurostat/documents/3930297/5968986/KS-FP-13-001-EN.PDF/6952d836-7125-4ff5-a153-6ab1778bd4da
6. Klinkenberg E, Manissero D, Semenza JC, Verver S. Migrant tuberculosis screening in the EU/EEA: yield, coverage and limitations.Eur Respir J. 2009;34(5):1180-9. DOI: 10.1183/09031936.00038009 PMID: 19880618
7. Kruijshaar ME, Abubakar I, Stagg HR, Pedrazzoli D, Lipman M. Migration and tuberculosis in the UK: targeting screening for latent infection to those at greatest risk of disease.Thorax. 2013;68(12):1172-4. DOI: 10.1136/thoraxjnl-2013-203254 PMID: 23828120
8. Tafuri S, Martinelli D, Melpignano L, de Palma M, Quarto M, Prato R, et al. Tuberculosis screening in migrant reception centers: results of a 2009 Italian survey. Am J Infect Control. 2011;39(6):495-9. DOI: 10.1016/j.ajic.2010.10.013 PMID: 21570737
9. Alvarez GG, Clark M, Altpeter E, Douglas P, Jones J, Paty MC, et al. Pediatric tuberculosis immigration screening in high-immigration, low-incidence countries. Int J Tuberc Lung Dis. 2010;14(12):1530-7.PMID: 21144237
10. Kik SV, Mensen M, Beltman M, Gijsberts M, van Ameijden EJ, Cobelens FG, et al. Risk of travelling to the country of origin for tuberculosis among immigrants living in a low-incidence country. Int J Tuberc Lung Dis. 2011;15(1):38-43.PMID: 21276294
11. Pace-Asciak A, Mamo J, Calleja N. Tuberculosis among undocumented boat migrants to Malta: implications for a migrant tuberculosis policy.Int J Tuberc Lung Dis. 2013;17(8):1065-70. DOI: 10.5588/ijtld.12.0622 PMID: 23827031
12. Wolfheze Transborder Migration Task Force,Dara M, de Colombani P, Petrova-Benedict R, Centis R, Zellweger JP, Sandgren A, et al. . Minimum package for cross-border TB control and care in the WHO European region: a Wolfheze consensus statement.Eur Respir J. 2012;40(5):1081-90. DOI: 10.1183/09031936.00053012 PMID: 22653772
13. World Health Organization (WHO). Framework towards tuberculosis elimination in low-incidence countries. Geneva: WHO; 2014. Available from: http://www.who.int/tb/publications/elimination_framework/en/
14. European Commission. Commission Decision of 28 April 2008 amending Decision 2002/253/EC laying down case definitions for reporting communicable diseases to the Community network under Decision No 2119/98/EC of the European Parliament and of the Council. Brussels: European Commission. 28 Apr 2008. Available from: http://ec.europa.eu/health/ph_threats/com/docs/1589_2008_en.pdf
15. World Health Organization (WHO). Global Tuberculosis Report 2014. Geneva: WHO; 2014. Available from: http://apps.who.int/iris/bitstream/10665/137094/1/9789241564809_eng.pdf
16. International Organization for Standardization. Codes for the representation of names of countries and their subdivisions -- Part 2: Country subdivision code. 15 Nov 2013. Available from: http://www.iso.org/iso/home/store/catalogue_tc/catalogue_detail.htm?csnumber=63546

17. Watkins RE, Plant AJ. Predicting tuberculosis among migrant groups.Epidemiol Infect. 2002;129(3):623-8. DOI: 10.1017/S0950268802007604 PMID: 12558347
18. Watkins RE, Plant AJ, Gushulak BD. Tuberculosis rates among migrants in Australia and Canada.Int J Tuberc Lung Dis. 2002;6(7):641-4.PMID: 12102305
19. Olson NA, Davidow AL, Winston CA, Chen MP, Gazmararian JA, Katz DJ. A national study of socioeconomic status and tuberculosis rates by country of birth, United States, 1996-2005.BMC Public Health. 2012;12(1):365. DOI: 10.1186/1471-2458-12-365 PMID: 22607324
20. Ricks PM, Cain KP, Oeltmann JE, Kammerer JS, Moonan PK. Estimating the burden of tuberculosis among foreign-born persons acquired prior to entering the U.S., 2005-2009.PLoS One. 2011;6(11):e27405. DOI: 10.1371/journal.pone.0027405 PMID: 22140439
21. Pareek M, Abubakar I, White PJ, Garnett GP, Lalvani A. U.K. immigrant screening is inversely related to regional tuberculosis burden.Thorax. 2011;66(11):1010. DOI: 10.1136/thx.2010.152280 PMID: 21325664
22. Pareek M, Abubakar I, White PJ, Garnett GP, Lalvani A. Tuberculosis screening of migrants to low-burden nations: insights from evaluation of UK practice.Eur Respir J. 2011;37(5):1175-82. DOI: 10.1183/09031936.00105810 PMID: 21071474
23. Pareek M, Watson JP, Ormerod LP, Kon OM, Woltmann G, White PJ, et al. Screening of immigrants in the UK for imported latent tuberculosis: a multicentre cohort study and cost-effectiveness analysis. Lancet Infect Dis. 2011;11(6):435-44. DOI: 10.1016/S1473-3099(11)70069-X PMID: 21514236
24. Lobato MN, Hopewell PC. Mycobacterium tuberculosis infection after travel to or contact with visitors from countries with a high prevalence of tuberculosis.Am J Respir Crit Care Med. 1998;158(6):1871-5. DOI: 10.1164/ajrccm.158.6.9804106 PMID: 9847280
25. Ormerod LP, Green RM, Gray S. Are there still effects on Indian Subcontinent ethnic tuberculosis of return visits?: a longitudinal study 1978-97.J Infect. 2001;43(2):132-4. DOI: 10.1053/jinf.2001.0872 PMID: 11676520
26. Codecasa LR, Porretta AD, Gori A, Franzetti F, Degli Esposti A, Lizioli A, et al. Tuberculosis among immigrants from developing countries in the province of Milan, 1993-1996. Int J Tuberc Lung Dis. 1999;3(7):589-95.PMID: 10423221
27. Lillebaek T, Andersen AB, Bauer J, Dirksen A, Glismann S, de Haas P, et al. Risk of Mycobacterium tuberculosis transmission in a low-incidence country due to immigration from high-incidence areas. J Clin Microbiol. 2001;39(3):855-61. DOI: 10.1128/JCM.39.3.855-861.2001 PMID: 11230395
28. Dahle UR, Sandven P, Heldal E, Caugant DA. Continued low rates of transmission of Mycobacterium tuberculosis in Norway.J Clin Microbiol. 2003;41(7):2968-73. DOI: 10.1128/JCM.41.7.2968-2973.2003 PMID: 12843028
29. Diel R, Rüsch-Gerdes S, Niemann S. Molecular epidemiology of tuberculosis among immigrants in Hamburg, Germany.J Clin Microbiol. 2004;42(7):2952-60. DOI: 10.1128/JCM.42.7.2952-2960.2004 PMID: 15243044
30. Kamper-Jørgensen Z, Andersen AB, Kok-Jensen A, Kamper-Jørgensen M, Bygbjerg IC, Andersen PH, et al. Migrant tuberculosis: the extent of transmission in a low burden country. BMC Infect Dis. 2012;12(1):60. DOI: 10.1186/1471-2334-12-60 PMID: 22423983
31. Eurostat. Migration and migrant population statistics 2014. [Accessed 25 August 2014]. Available from: http://ec.europa.eu/eurostat/statistics-explained/index.php/Migration_and_migrant_population_statistics
32. Taylor AB, Kurbatova EV, Cegielski JP. Prevalence of anti-tuberculosis drug resistance in foreign-born tuberculosis cases in the U.S. and in their countries of origin.PLoS One. 2012;7(11):e49355. DOI: 10.1371/journal.pone.0049355 PMID: 23145161
33. Minion J, Gallant V, Wolfe J, Jamieson F, Long R. Multidrug and extensively drug-resistant tuberculosis in Canada 1997-2008: demographic and disease characteristics.PLoS One. 2013;8(1):e53466. DOI: 10.1371/journal.pone.0053466 PMID: 23326436
34. N A, Ahmad F, Huda N. Osteoarticular tuberculosis-a three years' retrospective study.J Clin Diagn Res. 2013;7(10):2189-92. PMID: 24298472
35. Solovic I, Jonsson J, Korzeniewska-Koseła M, Chiotan DI, Pace-Asciak A, Slump E, et al. Challenges in diagnosing extrapulmonary tuberculosis in the European Union, 2011. Euro Surveill. 2013;18(12):20432.PMID: 23557946
36. World Health Organization (WHO). The global health sector strategy on HIV/AIDS 2011-2015: an interim review of progress: abridged report. Geneva: WHO; 2014. Available from: http://apps.who.int/iris/bitstream/10665/112790/1/9789241507295_eng.pdf?ua=1
37. Uchimura K, Ngamvithayapong-Yanai J, Kawatsu L, Ohkado A, Yoshiyama T, Shimouchi A, et al. Characteristics and treatment outcomes of tuberculosis cases by risk groups, Japan, 2007-2010. Western Pac Surveill Response J. 2013;4(1):11-8. DOI: 10.5365/wpsar.2012.3.4.016 PMID: 23908950
38. Falzon D, Scholten J, Infuso A. Tuberculosis outcome monitoring--is it time to update European recommendations?Euro Surveill. 2006;11(3):20-5.PMID: 16567878
39. Borgdorff MW, Veen J, Kalisvaart NA, Broekmans JF, Nagelkerke NJ. Defaulting from tuberculosis treatment in The Netherlands: rates, risk factors and trend in the period 1993-1997.Eur Respir J. 2000;16(2):209-13. DOI: 10.1034/j.1399-3003.2000.16b05.x PMID: 10968493

License and copyright

National Bulletins

Austria

Mitteilungen der Sanitätsverwaltung
Bundesministerium für Gesundheit Familie und Jugend, Vienna
Monthly, print only. In German.
http://www.bmgfj.gv.at/cms/site/thema.html?channel=CH0951

Belgium

Vlaams Infectieziektebulletin
Department of Infectious Diseases Control, Flanders
Quarterly, print and online. In Dutch, summaries in English.
http://www.infectieziektebulletin.be

Bulletin d'information de la section d'Epidémiologie
Institut Scientifique de la Santé Publique, Brussels
Monthly, online. In French.
http://www.iph.fgov.be/epidemio/epifr/episcoop/episcoop.htm

Bulgaria

Bulletin of the National Centre of Infectious and Parasitic Diseases, Sofia
Print version. In Bulgarian.
http://www.ncipd.org/

Cyprus

Newsletter of the Network for Surveillance and Control of Communicable Diseases in Cyprus
Medical and Public Health Services, Ministry of Health, Nicosia
Biannual, print and online. In Greek.
http://www.moh.gov.cy

Czech Republic

Zpravy CEM (Bulletin of the Centre of
Epidemiology and Microbiology)
Centrum Epidemiologie a Mikrobiologie Státního
Zdravotního Ústavu, Prague
Monthly, print and online. In Czech, titles in English.
http://www.szu.cz/cema/adefaultt.htm

EPIDAT (Notifications of infectious diseases in the Czech Republic)
http://www.szu.cz/cema/epidat/epidat.htm

Denmark

EPI-NEWS
Department of Epidemiology, Statens Serum Institut, Copenhagen
Weekly, print and online. In Danish and English.
http://www.ssi.dk

Finland

Kansanterveyslaitos
Department of Infectious Disease Epidemiology, National Public Health Institute, Helsinki
Monthly, print and online. In Finnish.
http://www.ktl.fi/portal/suomi/osastot/infe/tutkimus/tartuntatautien_seuranta/tartuntatautilaakarin_kommentit/

France

Bulletin épidémiologique hebdomadaire
Institut de veille sanitaire, Saint-Maurice Cedex
Weekly, print and online. In French.
http://www.invs.sante.fr/beh/default.htm

Germany

Epidemiologisches Bulletin
Robert Koch-Institut, Berlin
Weekly, print and online. In German.
http://www.rki.de/DE/Content/Infekt/EpidBull/epid__bull__node.html

Greece

HCDCP Newsletter
Hellenic Centre for Disease Control and Prevention (HCDCP/KEELPNO), Athens
Monthly, online. In English and Greek.
http://www2.keelpno.gr/blog/?lang=en

Hungary

Epinfo (az Országos Epidemiológiai Központ epidemiológiai információs hetilapja)
National Center For Epidemiology, Budapest
Weekly, online. In Hungarian.
http://www.oek.hu/oek.web?to=839&nid=41&pid=7&lang=hun

Iceland

EPI-ICE
Landlæknisembættið
Directorate Of Health, Seltjarnarnes
Monthly, online. In Icelandic and English.
http://www.landlaeknir.is

Ireland

EPI-INSIGHT
Health Protection Surveillance Centre, Dublin
Monthly, print and online. In English.
http://www.hpsc.ie/hpsc/EPI-Insight

Italy

Notiziario dell'Istituto Superiore di Sanità
Istituto Superiore di Sanità, Reparto di Malattie Infettive, Rome
Monthly, online. In Italian.
http://www.iss.it/publ/noti/index.php?lang=1&tipo=4

Bolletino Epidemiologico Nazionale (BEN)
Istituto Superiore di Sanità, Reparto di Malattie Infettive, Rome
Monthly, online. In Italian.
http://www.epicentro.iss.it/ben

Latvia

Epidemiologijas Bileteni
Sabiedribas veselibas agentura
Public Health Agency, Riga
Online. In Latvian.
http://www.sva.lv/epidemiologija/bileteni

Lithuania

Epidemiologijos žinios
Užkreciamuju ligu profilaktikos ir kontroles centras
Center for Communicable Disease Prevention and Control, Vilnius
Online. In Lithuanian.
http://www.ulac.lt/index.php?pl=26

Netherlands

Infectieziekten Bulletin
Rijksinstituut voor Volksgezondheid en Milieu
National Institute of Public Health and the Environment, Bilthoven
Monthly, print and online. In Dutch.
http://www.rivm.nl/infectieziektenbulletin

Norway

MSIS-rapport
Folkehelseinstituttet, Oslo
Weekly, print and online. In Norwegian.
http://www.folkehelsa.no/nyhetsbrev/msis

Poland

Meldunki o zachorowaniach na choroby zakazne i zatruciach w Polsce
Panstwowy Zaklad Higieny,
National Institute of Hygiene, Warsaw
Fortnightly, online. In Polish and English.
http://www.pzh.gov.pl

Portugal

Saúde em Números
Ministério da Saúde,
Direcção-Geral da Saúde, Lisbon
Sporadic, print only. In Portuguese.
http://www.dgs.pt

Romania

Info Epidemiologia
Centrul pentru Prevenirea si Controlul Bolilor Transmisibile, National Centre of Communicable Diseases Prevention and Control, Institute of Public Health, Bucharest
Sporadic, print only. In Romanian.
Sporadic, print only. In Romanian.
http://www.insp.gov.ro/cnscbt/index.php?option=com_docman&Itemid=12

Slovenia

CNB Novice
Inštitut za varovanje zdravja, Center za nalezljive bolezni, Institute of Public Health, Center for Infectious Diseases, Ljubljana
Monthly, online. In Slovene.
http://www.ivz.si

Spain

Boletín Epidemiológico Semanal
Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Madrid
Fortnightly, print and online. In Spanish.
http://revista.isciii.es

Sweden

Folkhälsomyndighetens nyhetsbrev
Folkhälsomyndigheten, Stockholm
Weekly, online. In Swedish.
http://www.folkhalsomyndigheten.se/

United Kingdom

England and Wales

Health Protection Report
Public Health England, London
Weekly, online only. In English.
https://www.gov.uk/government/collections/health-protection-report-latest-infection-reports

Northern Ireland

Communicable Diseases Monthly Report
Communicable Disease Surveillance Centre, Northern Ireland, Belfast
Monthly, print and online. In English.
http://www.cdscni.org.uk/publications

Scotland

Health Protection Scotland Weekly Report
Health Protection Scotland, Glasgow
Weekly, print and online. In English.
http://www.hps.scot.nhs.uk/ewr/

European Union

"Europa" is the official portal of the European Union. It provides up-to-date coverage of main events and information on activities and institutions of the European Union.
http://europa.eu

European Commission - Public Health

The website of European Commission Directorate General for Health and Consumer Protection (DG SANCO).
http://ec.europa.eu/health/

Health-EU Portal

The Health-EU Portal (the official public health portal of the European Union) includes a wide range of information and data on health-related issues and activities at both European and international level.
http://ec.europa.eu/health-eu/

European Centre for Disease Prevention and Control

European Centre for Disease Prevention and Control (ECDC)
The European Centre for Disease Prevention and Control (ECDC) was established in 2005. It is an EU agency with aim to strengthen Europe's defences against infectious diseases. It is seated in Stockholm, Sweden.
http://www.ecdc.europa.eu

Articles published in *Eurosurveillance* are indexed in PubMed/Medline.

The *Eurosurveillance* print edition is a selection of short and long articles previously published on the *Eurosurveillance* website. The full listing of all *Eurosurveillance* articles can be found in the Archives section of the website.

TQ-AD-16-005-EN-C

Eurosurveillance

Visit our website at **www.eurosurveillance.org**

The *Eurosurveillance* print edition is a compilation of short and long articles that have previously been published on our website.

All the articles in this issue are available online: you can print each page separately or download the whole quarterly in pdf format.

The website archives all articles since 1995, and offers a search facility.

To receive *Eurosurveillance's* free **electronic releases** and e-alerts by email, please subscribe on our website.

Papers published in the former monthly release are indexed for MEDLINE since January 2001, and papers published in the weekly release from January 2005 (with the exception of short, non-scientific notices) are also indexed for MEDLINE.

The Index Medicus abbreviation for *Eurosurveillance* is Euro Surveill.

Follow us on Twitter : #eurosurveillanc

Contributions to Eurosurveillance are welcomed. Full instructions to authors are available on our website www.eurosurveillance.org

ISSN 1025 496X