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Update: Multinational listeriosis outbreak due to 'Quargel', a sour milk curd cheese, caused by two different L. monocytogenes serotype 1/2a strains, 2009-2010

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We previously reported an outbreak of listeriosis in Austria and Germany due to consumption of 'Quargel' cheese. It comprised 14 cases (including five fatalities) infected by a serotype 1/2a *Listeria monocytogenes* (clone 1), with onset of illness from June 2009 to January 2010. A second strain of *L. monocytogenes* serotype 1/2a (clone 2) spread by this product could be linked to further 13 cases in Austria (two fatal), six in Germany (one fatal) and one case in the Czech Republic, with onset of disease from December 2009 to end of February 2010.

Clone 1

As reported earlier, the binational Austrian-German Consiliar Laboratory for Listeria in Vienna noticed a cluster of human isolates of *Listeria monocytogenes* serotype 1/2a in August 2009 with a new pulsed-field gel electrophoresis (PFGE) pattern [1]. Fourteen cases (12 Austrian and two German), including five with fatal outcome (two of them German), were identified. Onset of disease ranged from June 2009 to January 2010. An epidemiological investigation revealed 'Quargel' cheese produced by an Austrian manufacturer as the source of infection. The product was withdrawn from the Austrian, German, Slovakian and Czech markets on 23 January 2010 [1].

Microbiological investigations confirmed the presence of this new strain (clone 1) in 'Quargel' samples taken at the factory in 2010: Two of 64 isolates available for testing (44 isolates cultured from cheese produced in 2010 and provided by the manufacturer, 20 isolates cultured from samples officially gained during outbreak investigation) showed the new PFGE pattern associated with the outbreak.

Clone 2

The 62 remaining food isolates showed a different PFGE pattern, that had not previously been seen in Austrian isolates either (clone 2). It was indistinguishable from the pattern of a human isolate from a listeriosis patient hospitalised at the time, who claimed to have eaten 'Quargel' cheese. Only two of 46 human *L. monocytogenes* isolates documented at the Austrian Reference Centre in 2009 yielded this PFGE-pattern, both coming from patients with a food-history positive for 'Quargel'. Ultimately, this second outbreak clone of *L. monocytogenes* serotype 1/2a accounted for 13 Austrian cases (two with fatal outcome), six German cases (one death), and one Czech case; onset of disease ranged from December 2009 until end of February 2010. The epidemic curve shows all cases associated with the two different outbreak clones by onset of illness (Figure).

Outbreak analysis

In total, the outbreak involved 34 cases of invasive listeriosis: 25 outbreak cases originated from seven of nine Austrian provinces. Four of these patients presented with meningitis: two with clone 1 and two with clone 2. A further eight patients were from four of 16 German federal states, and one patient was from the Czech Republic. Eight of the 34 cases in this outbreak had a fatal outcome. The median age of the cases was 72 years (range: 57-89 years), and 26 patients were male. There were no materno-neonatal cases. Underlying diseases were not different from those generally described for patients with listeriosis [2].

A total of 63 food samples of the 'Quargel' cheese products were microbiologically analysed. 20 samples were found positive for *L. monocytogenes*. 11 of the 20

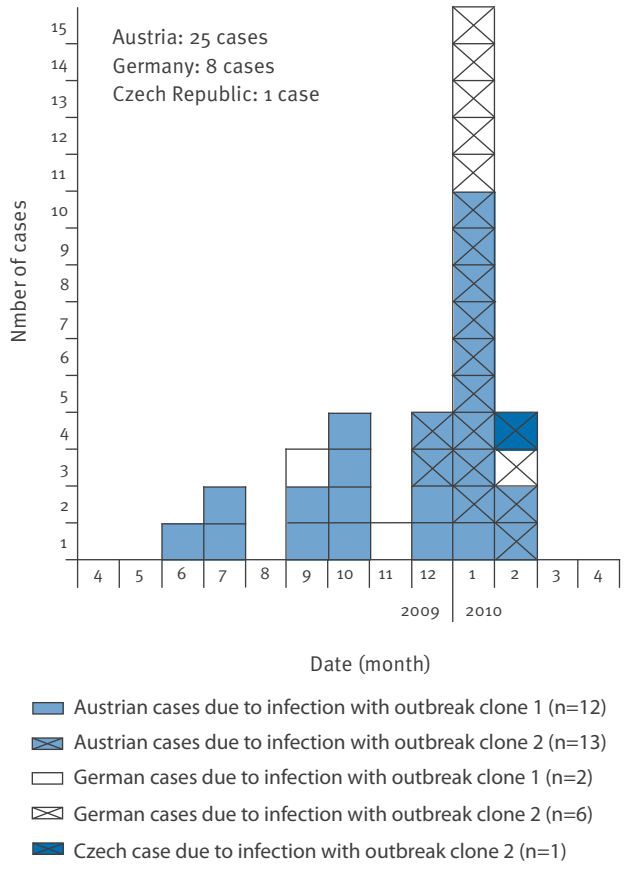
samples yielded less than 100 colony-forming units per gram (CFU/g), and nine samples harboured more than 100 CFU/g. All but one case can be explained by consumption of the contaminated product before it was withdrawn from the market on 23 January: one patient who was hospitalised for meningitis on 26 February 2010 had eaten the cheese (purchased before withdrawal from the market) on February 13. A leftover specimen, stored in the patient's refrigerator and sampled on 3 March, yielded 2,100,000 CFU/g of *L. monocytogenes*.

Case control study

The source of this outbreak was initially identified based solely on epidemiological findings [1]. We collected the cases' grocery receipts of purchases they made in December 2009, after their discharge from hospital, and compared them for matches. This generated a hypothesis to be tested by a case control study using case-case comparisons. For this study, a case was defined as a person in Austria from whom the *L. monocytogenes* outbreak clone 1 was isolated. Controls were patients from Austria with *L. monocytogenes* infections in 2009, whose isolates showed other profiles than the outbreak clone 1. Cases were asked about consumption of 12 cheese products in the six-month period prior to disease onset. Control persons were requested to provide information on consumption of the same cheese products in the year 2009. The overall response rate was 83.3% in the case group (ten of at the time 12 possible cases) and 72.2% in the control group (24 of at the time 33 possible controls, i.e. listeriosis-patients with isolates that showed other profiles than the outbreak clone 1). Consumption of the 'Quargel' cheese was identified as the only significant risk factor, highly associated with the illness in question. Nine of the ten cases with clone 1 had consumed the product; the tenth case provided no answer concerning this food item. Of 22 control cases (none with clone 2) all but two denied having eaten this specific cheese; the remaining two provided no answer concerning this food item. The computed odds ratio was 76.6 (95% confidence interval (CI): 9.3-infinity; P value <0.001).

Figure

Outbreak cases of listeriosis by onset of illness, Austria, Germany and Czech Republic, 2009-2010 (n=34)



Conclusions

The described outbreak provides some valuable lessons: Firstly, it underlines the considerable potential of molecular subtyping as a tool to identify outbreaks. Without routine PFGE typing of human isolates, this outbreak would have been missed. Secondly, it shows impressively that the waning of an outbreak (i.e. disappearance of an outbreak clone) does not necessarily imply that the underlying problem has disappeared. The shift to a different outbreak clone in December 2009/ January 2010 was probably caused by a change (in late November 2009) of the commercial ripening culture used in the cheese factory due to short supply of the original culture. Thirdly, our outbreak also emphasises the considerable potential of cross-border cooperation for elucidating chains of infections in multinational outbreaks. Industrial food production combined with international marketing of food and the low attack rate of *L. monocytogenes* hinder epidemiological outbreak investigations with traditional concepts [2]. Finally, the case of our patient with meningitis who had a leftover specimen of the causative food still in his refrigerator, underlines the importance of visiting households of listeriosis patients in order to obtain food samples and to advise other household members on precautionary measures. A single leftover food sample could prove an invaluable clue for elucidating the source of infection and thereby preventing further illness.

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Investigations and control measures following a non-travel-associated case of toxigenic Cornyebacterium diphtheriae, London, United Kingdom, December 2009-January 2010

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This article reports the investigation and control measures undertaken following the identification of a toxigenic strain of *Cornyebacterium diphtheriae* var *gravis*, designated ribotype 'Minsk', in a partially vaccinated teenager born in the United Kingdom with no recent history of travel or known contact with a case of diphtheria or a carrier. This case highlights the need for ongoing work to improve vaccine uptake rates to ensure children receive all scheduled vaccinations.

Introduction

Diphtheria is an acute bacterial disease that primarily affects the respiratory mucosa and skin, but more rarely has also been found in other mucous membranes such as conjunctivae, vagina and ear [1]. Three *Corynebacterium* spp. can potentially produce diphtheria toxin: *C. diphtheriae*, which has four biotypes: *gravis, mitis, intermedius* and *belfanti*, *C. ulcerans* and *C. pseudotuberculosis*. Toxin production occurs when bacteria are infected by corynebacteriophage containing the toxin gene tox [1].

In the United Kingdom (UK) toxigenic *C. diphtheriae* has become very rare (Table) due to national immunisation that has been in place since 1942 [2]. The small number of isolates identified are most commonly associated with travel to endemic countries. The *C. diphtheriae* biotype gravis "Minsk" ribotype, although uncommon in the UK, has been previously isolated in a UK immigrant during the 1990s. This ribotype was originally observed among diphtheria cases in Belarus during the period from 1993 to 2000. It was only prevalent in Belarus during the height of the diphtheria epidemics in the Newly Independent States of the former Soviet Union during the 1990s [Personal communication: Streptococcus and Diphtheria Reference Unit, Centre for Infection, Health Protection Agency, UK].

The last death in the UK from diphtheria was in an unvaccinated school-aged child in 2008 who had no recent history of travel. A public health investigation did not identify a source of infection [4]. A toxigenic *C. diphtheriae* var *mitis* was grown from a diagnostic bronchoalveolar lavage sample.

The UK vaccination schedule includes a primary course of diphtheria-containing vaccine given at two, three, and four months of age, with a pre-school booster given between three years and four months and five years of age. A school leavers' booster is then scheduled between the ages of 13 and 18 years [5].

Case report

On 31 December 2009 the case presented to a London hospital accident and emergency unit with a five-day history of severe sore throat, pustular tonsils and abdominal pain in the right upper quadrant. There was no history of travel and a pseudomembrane was not seen on clinical examination. A throat swab was taken. The case was admitted to a bay on a paediatric ward and treated with intravenous benzylpenicillin.

On 2 January 2010 a Monospot test was positive for Epstein-Barr virus (EBV). At this point intravenous antibiotics were stopped and a differential diagnosis of glandular fever was made. The case remained in hospital for four days (nine days after onset of disease) and was discharged on 4 January 2010 following clinical recovery. On 5 January 2010 the throat swab culture had grown group C beta-haemolytic *Streptococcus*.

The North East and North Central Health Protection Unit (HPU) were notified by the hospital medical microbiology registrar on 7 January 2010 that the throat swab had also grown *C. diphtheriae*. The sample was urgently couriered to the Health Protection Agency's (HPA) Streptococcus/Diphtheria Reference Unit to assess toxigenicity. It was identified as a toxigenic

C. diphtheriae var *gravis* strain by conventional and molecular methods [6].

On the evening of 8 January 2010 the HPU informed the family doctor of the results and advised of the need for medical assessment of the case and any close household contacts. The HPU also advised antibiotic treatment and prophylaxis for the case and household contacts as well as screening nose and throat swabs and ascertainment of their immunisation histories.

The case was prescribed a 14-day course of clarithromycin (500 mg twice a day). Three household contacts were identified by the family doctor: the parents and a younger sibling. Close household contacts were prescribed a seven-day course of erythromycin (dose based on their age and weight). The family doctor confirmed that the case had received a primary immunisation course at two, three and four months of age, but there was no record of a pre-school booster. The school records showed that the case had also received a dose of diphtheria-containing vaccine as part of the school-leavers' immunisation schedule in January 2009.

The case was clinically assessed by the family doctor, in consultation with HPU and the infectious disease physician at the local hospital following confirmation of the results and found to be well, not requiring re-admission to hospital. The family doctor was advised to offer a convalescent diphtheria booster vaccination to the case after January 2010 (i.e. 12 months after the last dose).

Contact investigations

It was assessed that the case may have been infectious since 19 December 2009 (i.e. seven days before onset of illness) and could have remained infectious until 8 January 2010 (i.e two weeks after onset of symptoms) which coincided with the start of antibiotic treatment with clarithromycin.

National guidelines were followed to identify close contacts at highest risk [2]. The contact investigations in this case included:

- Contacts in the hospital between 31 December 2009 and 4 January 2010. During the four days the case was hospitalised, they were cared for in a six-bedded open section of the ward. Investigations included risk assessment for hospital staff, patients and their visitors who stayed in the ward overnight.
- Contacts in the community between 19 December 2009 and 8 January 2010

In-depth interviews with the case and family confirmed that there was no recent travel history or contact with overseas visitors. There was no contact with other known cases.

Hospital contacts

A member of the HPU team was able to undertake a risk assessment with the hospital infection control team. Twelve healthcare workers were identified to have had contact with the case – none of them were involved in any aerosol generating procedures. All were fully immunised and were advised on self-surveillance. Two doctors involved with the case's care reported sore throats. They were swabbed, treated with clarithromycin and excluded from work until results of swabs were reported; both were negative.

Two patients were staying in the same section of the ward at the same time as the case: one child, whose mother stayed overnight, was in the same bay; mother and child were swabbed and given prophylactic antibiotics. The second child was in an adjacent bay and therefore not a high risk contact. The family doctors of both children and the parents were contacted and informed about their patients' exposure and advised to offer a booster dose of diphtheria-containing vaccine if the last dose received dated back more than 12 months. Both families were also informed about their exposure, and received health protection advice on signs and symptoms of diphtheria and their need for further immunisation.

Household and close contacts

Following the initial risk assessment of the case, three close household contacts (parents and a sibling) were identified. In-depth interviews with the case and family members identified a further seven contacts (family and friends), two of whom were considered to be close contacts. They were swabbed and provided with prophylaxis and were advised on signs and symptoms of the disease. All contacts were sent an advice sheet, and the family doctors were advised to review immunisa-

Table

Isolates of toxigenic corynebacteria, England and Wales, 1986-2009[a][3]

	Toxigenic *C. diphtheriae*			Toxigenic *C. ulcerans*		
	'Classical' diphtheria (with membrane)	Other[b]	Total	'Classical' diphtheria (with membrane)	Other[b]	Total
1986 - 1992	4	24	**28**	0	9	**9**
1993 - 1999	4	17	**21**	4	21	**25**
2000 - 2009	1	14	**15**	3	24	**27**
Total	**9**	**55**	**64**	**7**	**54**	**61**

[a] Data for 2009 are provisional

[b] Other includes respiratory diphtheria without a membrane, cutaneous diphtheria, and asymptomatic carriers

tion history and offer a booster dose if none had been received in the previous 12 months.

No school or work contacts were identified as the case was not at school during the infectious or incubation period. No other contacts were identified. All swabs from contacts were negative.

Discussion

Classical respiratory diphtheria is rare in the UK, and between 1986 and 2007 only eight cases of classical respiratory diphtheria caused by toxigenic *C. diphtheriae* were reported, all of whom had a history of travel to endemic countries. Countries with endemic diphtheria are Algeria, Angola, Egypt, Niger, Nigeria, Sudan, and sub- Saharan countries, Bolivia, Brazil, Colombia, Dominican Republic, Ecuador, Haiti, and Paraguay, Afghanistan, Bangladesh, Bhutan, Burma (Myanmar), Cambodia, China, India, Indonesia, Laos, Malaysia, Mongolia, Nepal, Pakistan, Papua New Guinea, Philippines, Thailand, and Vietnam, Iran, Iraq, Saudi Arabia, Syria, Turkey, and Yemen, Albania, Russia, and countries of the former Soviet Union [7]. In 2008 a fatal case of laryngeal diphtheria, caused by a toxigenic *C. diphtheriae* var *mitis* strain, was reported in London in an unimmunised child [4]. There was no history of recent travel and although one close contact had travelled to Africa a month before the child became unwell, the throat swab taken from this contact was negative.

The source of infection for the case discussed in this paper remains unknown; however, the association of the ribotype with Belarus implies that it may have been imported. There was no contact with known cases of diphtheria and no close contacts were identified as carriers of toxigenic *C. diphtheriae*. The case had no travel history, no contact with anyone who had recently travelled, and no recent contact with animals.

The case presented here was partially immunised against diphtheria, which may explain the less severe disease and non-classical presentation (i.e. lack of a pseudomembrane). While the incomplete vaccination did not protect the case against the infection, it probably protected them against the effects of toxin produced by the bacteria. Mild diphtheria is usually only diagnosed following laboratory confirmation of a toxigenic strain of corynebacteria. Throat swabs are routinely tested for corynebacteria at the hospital this case attended, but most UK laboratories will only screen for the organism if there is a clinical indication of diphtheria, and or contact with a known case. Other mild diphtheria cases therefore may be missed.

In this case there was some delay in the diagnosis of diphtheria. This was partly due to the low level of clinical suspicion as the disease is rare in the UK, a pseudo-membrane was not observed and the case was relatively well. Identification of EBV and a positive result for group C beta-haemolytic *Streptococcus* also complicated the diagnosis.

Some children may remain susceptible to diphtheria, particularly in London, as immunisation uptake rates are below the recommended level of 95% by two years of age. The most recent immunisation coverage data for London (October to December 2009) for children completing their primary immunisation by their second birthday in that quarter was 90.8%, compared with 95.3% in England [8]. However, the uptake rate for children who received their pre-school booster by their fifth birthday, evaluated in the same quarter was only 69.9% in London and 84.3% in England.

Conclusions

Diphtheria can occur in children and adults with incomplete immunisation history, in whom the disease can be difficult to diagnose. Investigation of these cases can have considerable implications for health services. The case presented here required extensive investigations and risk assessment in hospital and community, which identified nine high risk contacts who received prophylactic antibiotic. Furthermore it highlights the need for ongoing work to improve vaccine uptake rates to ensure children receive all scheduled vaccinations. This case report also emphasises the importance of maintaining microbiological surveillance, expertise and awareness of these organisms among public health specialists, microbiologists and clinicians.

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Prevalence and characteristics of meticillin-resistant Staphylococcus aureus in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009

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A total of 2,662 samples, collected from March to September 2009 in Switzerland, were tested for the presence of meticillin-resistant *Staphylococcus aureus* (MRSA). The collection comprised nasal swabs from 148 pig farmers, 133 veterinarians, 179 slaughterhouse employees, 800 pigs, 300 calves, 400 cattle, 100 pooled neck skin swabs from chicken carcasses, and 460 food samples of animal origin. Moreover, 142 *S. aureus* strains, isolated from bovine mastitis milk, were included in the study. Twenty samples (< 1%; four veterinarians, 10 pigs, three calves, one young bull, and two mastitis milk samples) tested positive for MRSA. Genotyping of the MRSA strains was performed by multilocus sequence typing, *spa*- and SCC*mec*-typing, and revealed ST398 (n=18), ST8 (n=1), ST 1 (n=1), *spa* types t011 (n=7), t034 (n=11), t064 (n=1), t127 (n=1), and SCC*mec* types IV (n=4) and V (n=16). The 20 MRSA strains were subjected to antibiotic susceptibility testing and pulsed-field gel electrophoresis using the restriction enzyme *Eag*I. Supplementary PCR reactions were performed to investigate the presence of Panton-Valentine leukocidin and staphylococcal enterotoxins A to D.

Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA) has become a pathogen of increasing importance in hospitals, the community, and in recent years also in livestock. MRSA associated with livestock (LA-MRSA) have been reported worldwide in many species, but mainly in pigs, with sequence type (ST) 398 found most frequently [1-3]. With regard to humans in contact with farm animals, Voss *et al*. described in 2005 that Dutch pig farmers were at a 760-fold risk of being colonised with MRSA compared to the general Dutch population [4]. In an international study, Wulf *et al*. found MRSA in 12.5% of veterinarians originating from all over the world [5]. These studies strongly suggest that people working with livestock are at a potential risk of becoming MRSA carriers and hence are at an increased risk of infections caused by MRSA. To date, there is no comprehensive data on the situation of LA-MRSA in Switzerland. The aim of this study was to evaluate the occurrence of MRSA in people in contact with livestock, in farm animals, and in food of animal origin, and to investigate genotypic characteristics as well as phenotypic resistance data of isolated strains.

Methods

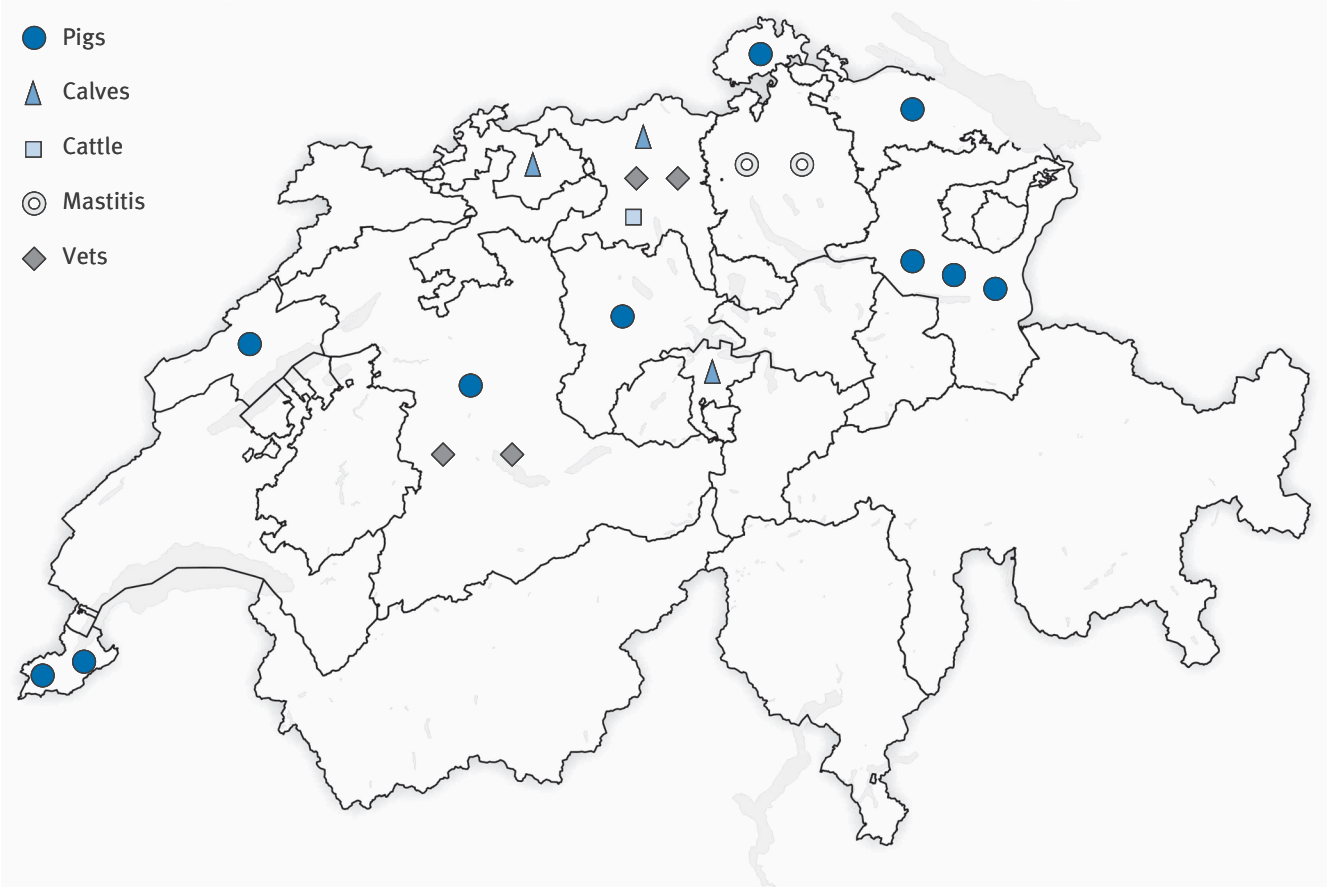
From March to September 2009, we collected and analysed a total of 2,662 samples from humans, livestock, and food of animal origin. In terms of humans with contact to farm animals, we analysed nasal swabs from 148 pig farmers attending meetings on swine breeding, 133 veterinarians participating in a course on castration of piglets, and 179 slaughterhouse employees working in two different abattoirs. Livestock was sampled at slaughter: Nasal swabs from pigs (n=800), calves (n=300), and cattle (n=400) were collected, as well as neck skin samples (n=100) from chicken carcasses, pooled by flock. Sampled animals originated from more than 830 farms distributed throughout Switzerland. In terms of food, 100 samples of bulk tank milk (BTM), 200 samples of raw-milk cheese, and 160 minced pork and beef samples were tested. Furthermore, 142 *S. aureus* strains from clinical cases of bovine mastitis were integrated in the study.

After a two-step enrichment procedure in Mueller-Hinton broth supplemented with 6.5% NaCl (24 h at 37°C) and in phenol red mannitol broth supplemented with 75 mg/L aztreonam and 5 mg/L cefoxitin (24 h at 37°C), the samples were plated onto Oxoid Brilliance MRSA Agar (Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 37°C. In addition, 142 *S. aureus* strains isolated from bovine mastitis milk were directly streaked onto Oxoid Brilliance MRSA Agar. Presumptive positive colonies were confirmed as *S. aureus* by species-specific 23S rDNA PCR [6] and as MRSA by PCR detection of *mec*A gene [7], before further characterisation

by multilocus sequence typing (MLST) [8], *spa* typing [9], and determination of staphylococcal cassette chromosome *mec* (SCC*mec*) type [10] was performed. Moreover, strains were tested by PCR for *luk*S-PV and *luk*F-PV [11] encoding Panton-Valentine leukocidin (PVL), and for *sea* to *sed* [12] encoding staphylococcal enterotoxins (SE) A to D. To demonstrate phenotypic properties, strains were tested for their antibiotic resistance patterns using the disk diffusion method (BD BBL Sensi-Disc; Becton, Dickinson and Company, Sparks, MD, US). The following disks were used: ampicillin, cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamicin, oxacillin, penicillin, rifampicin, sulphamethoxazole/trimethoprim, tetracycline, and vancomycin. Susceptibility testing with cefoxitin is recommended by Witte *et al.*, especially for low-level resistant MRSA [13]. Etest (AB Biodisk, Solna, Sweden) was additionally used for cefoxitin and oxacillin resistance testing. The results of antibiotic susceptibility testing were interpreted according to the guidelines of

Figure

Origin of isolated meticillin-resistant *Staphylococcus aureus* strains, Switzerland, March-September 2009 (n=20)



Table

Characterisation of meticillin-resistant *Staphylococcus aureus* strains, Switzerland, March-September 2009 (n=20)

Origin	Number of isolates	Sequence type (ST)	*spa* type	SCC*mec* type	PVL	SE	Resistance
Pig NS	8	398	t034	V	-	-	Amp, Cef, Cli, Ery, Oxa, Pen, Tet
Pig NS	1	398	t034	V	-	-	Amp, Cef, Cli, Ery, Gen, Oxa, Pen, SxT, Tet
Pig NS	1	398	t034	V	-	-	Amp, Cef, Oxa, Pen
Calf NS	3	398	t011	V	-	-	Amp, Cef, Cli, Ery, Oxa, Pen, Tet
Cattle NS	1	1	t127	IV	-	-	Amp, Cef, Ery, Oxa, Pen, Tet
Vet NS	1	398	t034	V	-	-	Amp, Cef, Cip, Cli, Ery, Pen, Tet
Vet NS	1	398	t011	IV	-	-	Amp, Cef, Oxa, Pen, Tet
Vet NS	1	398	t011	IV	-	-	Amp, Cef, Gen, Oxa, Pen, Tet,
Vet NS	1	8	t064	IV	-	A, B	Amp, Cef, Pen, SxT, Tet
Mastitis milk	2	398	t011	V	-	-	Amp, Cef, Cli, Ery, Oxa, Pen, Tet

Amp: ampicillin, Cef: cefoxitin, Cip: ciprofloxacin, Cli: clindamycin, Ery: erythromycin, Gen: gentamicin, NS: nasal swab, Oxa: oxacillin, Pen: penicillin, PVL: Panton-Valentine leucocidin, SE: staphylococcal enterotoxins; SCC: staphylococcal cassette chromosome, SxT: sulphamethoxazole/trimethoprim, Tet: tetracycline Vet: veterinarian.

the Clinical and Laboratory Standards Institute (CLSI). Furthermore, macrorestriction profiling with pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *Eag*I was performed [2].

Results

A total of 20 MRSA strains were detected (Table). They derived from samples from four (3.0%) of 133 veterinarians, 10 (1.3%) of 800 pigs, three (1.0%) of 300 calves, one (0.3%) of 400 cattle, and from two (1.4%) of 142 mastitis milk samples (Figure). In contrast, MRSA were not found in pig farmers, slaughterhouse employees, poultry, and in food samples such as BTM, raw milk cheese, and minced meat.

The four strains isolated from veterinarians belonged to ST8 and ST398 (Table). The strain of ST8 harbored *spa* type t064, SCC*mec* type IV, was negative for PVL, and positive for SEA and SEB. This strain of ST8 from a veterinarian was thus the only one harbouring genes encoding staphylococcal enterotoxins. The three strains belonging to ST398 tested negative for PVL and SE. One was *spa* type t034 and SCC*mec* type V. The other two strains belonged to *spa* type t011 and SCC*mec* type IV. The 10 MRSA strains isolated from pigs originated from eight different farms in seven regions of Switzerland, and all belonged to ST398, *spa* type t034, SCC*mec* type V and tested negative for PVL and SE. These characteristics are the same as those found in one strain from a veterinarian. The results obtained from strains of calves were similar, with the only difference that all three strains were grouped into *spa* type t011. Two strains from veterinarians were also of ST398 and *spa* type t011 but belonged to SCC*mec* type IV. The strain found in a young bull showed different characteristics. It belonged to ST1, *spa* type t128, SCC*mec* type IV, and was negative for SE and PVL. The two strains isolated from mastitis milk both belonged to ST398, *spa* type t011, SCC*mec* type V and were negative for PVL and SE. According to these typing results, the strains isolated from mastitis are identical to the ones isolated from calves.

Digestion with *Eag*I as restriction enzyme provided uniform band patterns for nine of 10 strains isolated from pigs. The three strains from calves and the two strains from mastitis milk showed uniform patterns as well and were related to the ones from pigs. The three MRSA strains of type ST398 isolated from veterinarians showed different patterns.

All 20 MRSA strains were susceptible to vancomycin and rifampin. All but two strains were susceptible to gentamicin and sulphamethoxazole/trimethoprim and all but one were susceptible to ciprofloxacin (Table). Of the 16 strains isolated from animals (livestock and mastitis milk), all were resistant to four beta-lactams (ampicillin, cefoxitin, oxacillin, penicillin), 15 were resistant to erythromycin and tetracycline, and 14 to clindamycin. Of the four MRSA strains isolated from veterinarians, two strains were phenotypically susceptible to oxacillin but resistant to cefoxitin, with the disk diffusion as well as the Etest method, and therefore were low-level resistant MRSA.

Discussion

Our results show that MRSA, and ST398 in particular, are present in Swiss livestock but still occur in low numbers. Compared to the herd level prevalence of 81% in Dutch pigs [1], the herd level prevalence of MRSA ST398 of 2.9% in pigs and 1.6% in calves found in our samples was low. In view of the small proportion of MRSA-positive animals found in Swiss livestock, the related risk of food contamination and transmission of MRSA to people in contact with livestock does currently not seem of particular importance in Switzerland. In our study, MRSA prevalence in veterinarians was 3%. This finding is favourable compared to results published by Wulf *et al*. [5], who found 12.5% of veterinarians attending an international congress on pig health to be MRSA carriers. Contrary to what was recently reported by De Boer *et al*. [14], we found no MRSA in meat samples. Our findings in raw-milk cheese and BTM are in good accordance with recently published data from the United States [15]. The fact that we detected no MRSA in poultry, pig farmers, slaughterhouse employees, and food samples is especially noteworthy since these results are different to findings published for other countries [3,4,13,16].

Possible explanations for the low MRSA prevalence in Switzerland may be the restrictive and controlled use of antibiotics in farming, a good health status of pig herds compared to many countries in the European Union, and the fact that the importation rate of live pigs in Switzerland is very low (<1%) [17].

The two non-ST398 strains with ST1 (young bull) and ST8 (veterinarian) found in our study are of sequence types usually considered as community-associated MRSA. Juhász-Kasanyitzky *et al*. reported MRSA of ST1, *spa* type t127, SCC*mec* type IV in humans and bovines [18]. Moreover, such MRSA were also isolated from horses and horse personnel in Austria [19]. The presence of MRSA ST8, *spa* type t064, SCC*mec* type IV was recently reported in horses by Weese and van Duijkeren [20].

All LA-MRSA of ST398 found in our study, belonged to two *spa* types (t034, t011), which represent the most common *spa* types in European LA-MRSA. Among our samples, *spa* type t011 was associated with bovine strains, whereas *spa* type t034 was associated with strains isolated from pigs. The two MRSA strains we isolated from mastitis milk were of ST398 and *spa* type t011, which is comparable to the recent results of Vanderhaeghen *et al*. [21]. It seems quite understandable that veterinarians can carry both, *spa* type t011 and t034, since veterinarians in Switzerland usually visit pig and cattle facilities. Visiting many different farms per day can also be an explanation for the higher

percentage of MRSA carriers among veterinarians compared to pig farmers.

Conclusion

MRSA, and especially LA-MRSA ST398, have entered Swiss farming operations but to date occur in low numbers. This low prevalence suggests that at the moment there is only a limited risk of MRSA transmission from livestock to humans and to food of animal origin. To maintain this situation, further efforts within the field of veterinary public health are of major importance and it is necessary to establish a monitoring system for further trend analysis.

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West Nile virus circulation in Emilia-Romagna, Italy: the integrated surveillance system 2009

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Following a large West Nile virus (WNV) epidemic in north-eastern Italy in 2008, human and animal surveillance activities were implemented in Emilia Romagna. Human surveillance was performed by serology or genome detection on blood and cerebrospinal fluid for all suspected cases suffering from acute meningoencephalitis in the regional territory. Animal surveillance consisted of passive and active surveillance of horses and active surveillance of wild birds and mosquitoes. Between 15 June and 31 October 2009, nine of 78 possible cases of West Nile neuroinvasive disease were confirmed (three fatal). From May to October, 26 cases of neurological West Nile disease were confirmed among 46 horses. The overall incidence of seroconversion among horses in 2009 was 13%. In 2009, 44 of 1,218 wild birds yielded positive PCR results for WNV infection. The planned veterinary and entomological surveillance actions detected WNV activity from the end of July 2009, about 2-3 weeks before the onset of the first human neurological case. Passive surveillance of horses seems to be an early and suitable tool for the detection of WNV activity, but it will be less sensitive in the future, because an intensive programme of horse vaccination started in June 2009.

Regional integrated surveillance system

In Italy a national veterinary plan for the surveillance of West Nile virus (WNV) circulation was set up in 2001 under the coordination of the National Reference Centre for Exotic Diseases of animals (CEntro Studi Malattie Esotiche; CESME).

During the late summer of 2008 a large epidemic of WNV infection occurred in north eastern Italy over an area exceeding 7,000 km^2, in three regions, including Emilia-Romagna. Twenty-three horse cases and three human cases of the neuroinvasive form of West Nile disease (WND) were confirmed by laboratory tests [1,2]. After the first evidence of WNV circulation in horses was found, additional surveillance on horses, birds and mosquitoes was activated.

In Emilia-Romagna the WNV surveillance plan 2009 adopted locally the surveillance measures indicated by the national plan. In particular, among the surveillance activities, the choice was to monitor wild non-migratory birds, such as corvids (the crow family), considered the most sensitive indicators among birds, which can be captured easily. As regards equine surveillance, the regional plan emphasised the education of veterinary practitioners, focusing on the inclusion of WNV in differential diagnosis and the achievement of rapid reporting. A major feature of this plan was to establish an extremely sensitive system of passive surveillance. In addition to passive surveillance, active monitoring of horses was implemented in the area involved in the 2008 outbreak, including Ferrara and the neighbouring provinces [3].

Evidence of WNV circulation in 2008 was found in animals [1,4], humans [5], and mosquitoes. This highlighted the need to implement an integrated surveillance system which would describe the phenomenon comprehensively. Such a system facilitates the collection of data to evaluate spatial distribution and time trends of viral circulation and shares information.

For this reason the 2009 Regional Surveillance Plan implemented activities beyond those of the National Plan, revised the surveillance system of human cases, activated intensive entomological monitoring, and enlarged the surveillance area to involve all the provinces along the Po River.

Human surveillance

The aim of the human surveillance system was the early detection of infection in humans and the estimation of its diffusion through the systematic analysis of newly emerging clinical cases, in order to manage specific interventions.

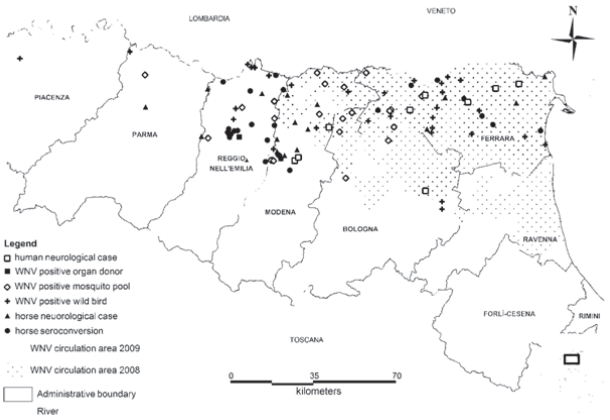
The surveillance was performed throughout the regional territory, from 15 June to 31 October 2009, corresponding to the period of vector activity in Emilia-Romagna and adjusted locally according to weather conditions and vector activity reports. In 2009, the 2008 case definition [6] was extended to include cases of all ages and not only those over 15 years of age.

The human surveillance activity was performed by serology or genome detection on blood and cerebrospinal fluid for all suspected cases suffering from acute meningoencephalitis in the regional territory. Active surveillance of people who live or work in areas of documented viral circulation was also performed. In addition blood and cerebrospinal fluid samples from subjects living or staying for at least one night in the regional area were sent to the Regional Reference Centre for Microbiological Emergencies (Centro di Riferimento Regionale per le Emergenze Microbiologiche; CRREM). In selected cases, positive specimens were confirmed by the National Health Institute (Istituto Superiore di Sanità; ISS) and by the National Institute for Infectious Diseases (Istituto Nazionale Malattie Infettive; INMI).

From October 2008 to April 2009 a serosurvey performed on 9,177 healthy blood donors living in the province of Ferrara detected a total of 62 IgG positive subjects, corresponding to a seroprevalence of 0.68%.

Figure 1

Map of municipalities with confirmed West Nile virus circulation and localisation of human West Nile neuroinvasive disease cases by probable infection site, Emilia-Romagna, Italy, 2009



WNV: West Nile virus

Animal surveillance

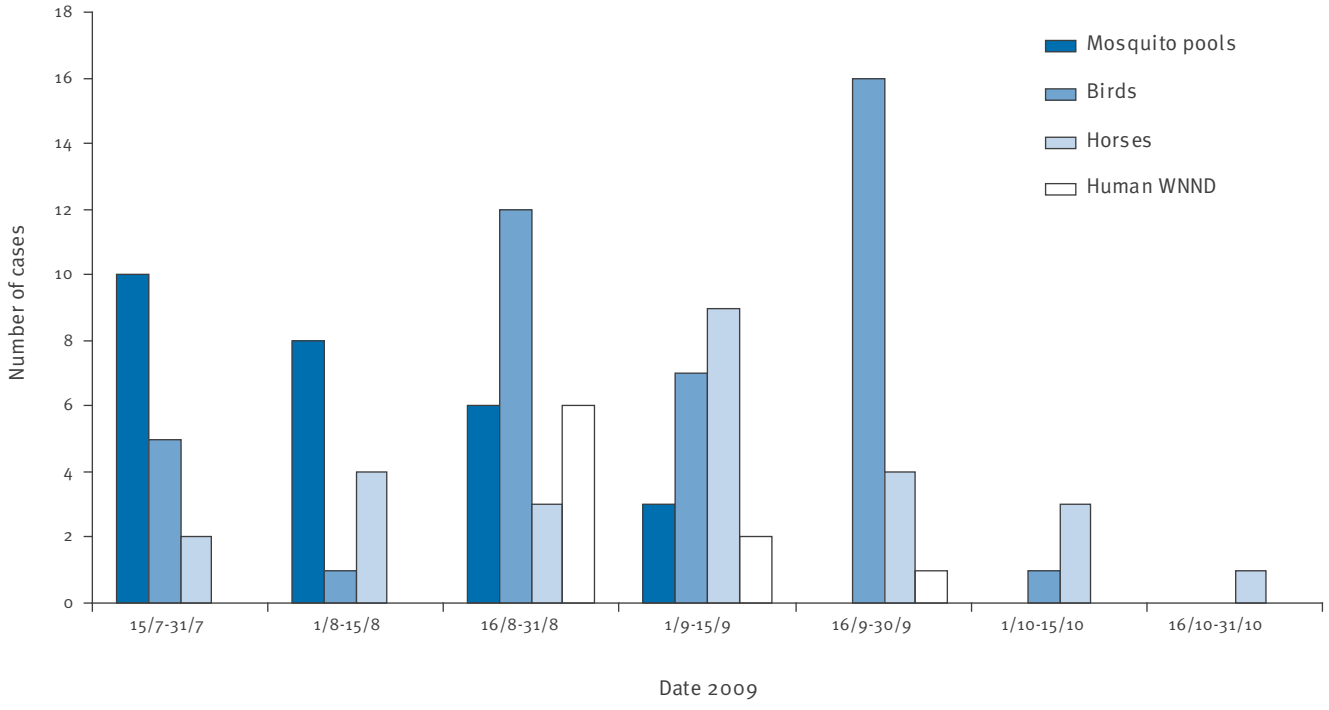
The regional veterinary WNV surveillance system was activated from May to October, performing passive and active surveillance on horses and on non-migratory wild birds.

Horse passive surveillance

In Italy all suspected signs of WND in horses must be notified to the official veterinary services. Suspected cases were confirmed if resulted positive by reverse transcription – polymerase chain reaction (RT-PCR) performed on central nervous system [7] or to a WNV virus

Figure 2

Distribution of West Nile virus confirmed cases (mosquito pools, birds, horses, and human West Nile neuroinvasive disease) by date, Emilia-Romagna, Italy, July-October 2009



WNND: West Nile neuroinvasive disease

Note: The figure does not include a magpie (found in early May) or a jay (found in early November).

neutralisation (VN) test (cut-off titre 1:10) in microtitre plates and to IgM enzyme linked immunosorbent assay (ELISA) [8,9].

Horse active surveillance

In the provinces of Ferrara, Bologna, Modena, Ravenna, and Reggio Emilia, every 1,600 km², 28 seronegative unvaccinated equine sentinels, sufficient to detect an incidence above 10% (CI 95%), were selected in the spring of 2009. They were serologically tested twice after the selection, at the beginning of August and the beginning of September. Samples collected were screened by a home-made competitive ELISA [10]. Positive samples were confirmed by VN and IgM ELISA at the CESME in Teramo. A seroconversion was confirmed if VN titre was at least 1:10 and there was evidence of IgM antibodies.

Wild bird surveillance

Monitoring was carried out in all the provinces along the Po River, in the plain area of Emilia-Romagna. Every 1,600 km², a monthly sample of about 40 wild birds caught or shot within specific wildlife population control programmes was collected. Samples of organs (brain, heart, and kidney) of each bird were pooled and examined by RT-PCR [7].

Entomological surveillance

The surveillance system was based on the weekly to monthly (frequency depends on local resources) collection of mosquitoes in fixed stations and in the sites where birds, humans, or horses signalled WNV activity. Mosquito collections for WNV screening were conducted in six provinces: Ferrara, Ravenna, Bologna, Modena, Reggio Emilia, and Parma, using 92 CO_2 baited traps positioned in fixed stations. Moreover, mosquito collections were performed promptly using CO_2 and gravid traps in sites where positive horses and human cases had been detected.

The surveillance system was activated in the period 15 April to 10 October. Collected mosquitoes were pooled (maximum 200) by species, date, and site of collection and examined by RT-PCR [7]. In addition, overwintered mosquito females were collected during the period 3 March to 8 April by manual aspirator in rural buildings in the area where WNV was active in 2008.

Virological analysis

Human samples

The detection of WNV genome in human plasma, cerebrospinal fluid, and serum samples obtained from patient suffering from clinical symptoms of meningoencephalitis was performed by an RT-PCR assay based on specific TaqMan probes [7].

Animal samples

In horses RNA was extracted starting from 200 µl of serum or whole blood with EDTA as anticoagulant. In birds RNA was extracted from 200 µl of phosphate buffer saline homogenate (about 20% tissue g / buffer ml) of pooled brain, heart and kidney of each analysed bird. In mosquitoes RNA was extracted from 200 µl of a maximum of 200 pooled mosquitoes manual grinded by using copper stained beads, in 500-800 µl of PBS.

cDNA was submitted to RT-PCR according to the method of Tang and colleagues [7]. Positive samples were confirmed by sequencing of partial nucleocapsin and premembrane protein M amplified according to [11]. Finally from each RT-PCR positive sample WNV was isolated on Vero and RK13 cell lines.

Results

Results of the integrated surveillance system are mapped in figure 1, with the sequence of events summarised in figure 2 (July-October), and discussed below.

Human cases

As of 31 October 2009, nine out of 78 possible cases of West Nile neuroinvasive disease (WNND) notified in Emilia-Romagna have been confirmed (8/9 males; median age 72 years, range: 62-78). Three patients

Table 1

Species distribution of wild birds tested for West Nile virus (n=1,218), Emilia-Romagna, Italy, May-October 2009

Species	Birds tested	WNV RT-PCR positive	% WNV positive
European magpie (*Pica pica*)	607	27	4.4
Carrion crow (*Corvus corone cornix*)	350	5	1.4
European starling (*Sturnus vulgaris*)	98	5	5
Eurasian jay (*Garrulus glandarius*)	96	2	2
Common blackbird (*Turdus merula*)	30	0	-
Strigiformes	11	2	18
Charadriiformes	8	3	38
Other Passeriformes	7	0	-
Other bird orders	5	0	-
Piciformes	4	0	-
Columbiformes	2	0	-
Total	**1,218**	**44**	**3.6**

RT-PCR: reverse transcription-polymerase chain reaction; WNV: West Nile virus

Table 2

Species of mosquitoes tested for West Nile virus (n=190,516), Emilia-Romagna, Italy, May-October 2009

Province	Bologna			Forlì			Ferrara			Modena			Piacenza			Parma			Ravenna			Reggio Emilia			Total		
Species	Mos-quito	Pool	Pool +	Mos-quito	Pool	Pool +	Mos-quito	Pool	Pool +	Mos-quito	Pool	Pool +	Mos-quito	Pool	Pool +	Mos-quito	Pool	Pool +	Mos-quito	Pool	Pool +	Mos-quito	Pool	Pool +	Mos-quito	Pool	Pool +
Ae. albopictus	169	13		11	4		392	31		227	23		58	11		228	6					142	20		1,227	108	0
Ae. caspius	1,713	51		14	5		9,953	114		16,915	121		2	1		12	2		606	11		68	9		29,283	314	0
Ae. detritus																1	1					4	1		5	2	0
Ae. dorsalis										13	1														13	1	0
Ae. geniculatus										6	2											2	1		8	3	0
Ae.vexans	2	1		11	2		84	3		4,090	41		1	1		363	3					46	9		4,597	60	0
An. maculipennis	4	1					59	3		14	5		1	1								4	4		82	14	0
An. plumbeus										2	2														2	2	0
Cx. modestus	7	3					114	10		117	12											8	1		246	26	0
Cx. pipiens	84,225	645	9	158	17		52,973	396	5	6,664	78	7	331	13		6,926	50	1	911	10		2,865	50	5	155,053	1,259	27
Total	**86,120**	**744**	**9**	**194**	**28**	**0**	**63,575**	**557**	**5**	**28,048**	**285**	**7**	**393**	**27**	**0**	**7,530**	**62**	**1**	**1,517**	**21**	**0**	**3,139**	**95**	**5**	**190,516**	**1,789**	**27**

died, two living in Ferrara Province and one in Modena. In addition, not reported in figures, the local health units of Parma and Modena notified two other confirmed cases, both 72 year-old women resident in Mantua province (Lombardy region), treated in hospital in Emilia-Romagna. Another case of infection was that of a 78 year-old female liver donor. Before her death, she had spent two weeks visiting relatives in the WNV affected area (Reggio Emilia).

Horse cases

Passive surveillance

From May to October, 26 cases of neurological WND were confirmed among 46 horses in which it was suspected. Four of the eight provinces involved in the regional surveillance system had WND cases in horses. The first symptoms in horses were detected in the second half of July in the provinces of Ferrara and Reggio Emilia, but the most cases were notified between mid-August and mid-September (figure 2).

Active surveillance

Seroconversions in sentinel horses were detected in three provinces (Ferrara, Modena, and Reggio Emilia). Early seroconversions were registered among the controls examined at the beginning of August. Serological controls around the stables with WND cases also confirmed recent WNV infections in the province of Parma. The overall incidence of seroconversion among horses in 2009 was 13% (95% CI: 10% to 16%), but in Ferrara it was 28% (95% CI: 19% to 39%).

Wild birds

Six of the eight provinces that took part in the regional surveillance system reported positive birds. In 2009, 44 wild birds out of 1,218 (tested by PCR) yielded positive results for WNV infection. With the exception of a magpie caught in May, positive wild birds were detected from the end of July (figure 2). Most of the infected wild birds were corvids (*Pica pica, Corvus corone cornix, Garrulus glandarius*), collected within population control programmes in August and September, but WNV was detected also in other species, mainly found dead in wildlife recovery centres (table 1).

Table 1. Species distribution of wild birds tested for West Nile V (n=1,218), Emilia-Romagna, Italy, May-October 2009

Mosquitoes

About 190,000 mosquitoes were collected, pooled and tested using PCR (1,789 pools of ≤200 individuals/pool). Culex pipiens were the most abundant species (81.4%) followed by *Aedes caspius* (15.4%) and Aedes vexans (2.4%). Other collected species are shown in table 2.

Twenty-seven pools, all consisting of *Culex pipiens*, yielded positive results for WNV. Early positive pools were collected in the province of Reggio Emilia at the end of July. Minimum infection rates (MIR: (no. of

positive pools/no. of mosquitoes tested) x 1,000) [12] were calculated, with higher MIR values recorded in August in the provinces of Reggio Emilia (3.08) and Modena (1.44).

Referring to the collection of overwintering mosquitoes, three mosquito species were collected: *Culex pipiens* (516 females, 52%), *Anopheles maculipennis* s.l. (475 females, 48%), *Culiseta annulata* (4 females, <1%): all specimens were tested and yielded negative results.

Conclusions

The planned veterinary and entomological surveillance actions detected WNV activity from the end of July 2009, about 2-3 weeks before the onset of the first human neurological case. Figure 2 shows that mosquitoes and birds were the first indicators of WNV circulation. The same figure makes it clear that human cases occurred later in the season, as reported elsewhere [6]. Passive surveillance of horses also seems to be an early and suitable tool for the detection of WNV activity, but it will be less sensitive in the future, because an intensive programme of horse vaccination started in June 2009.

More human cases of WNND occurred in 2009 than in 2008, and three were fatal. It is important to note that in 2008 the epidemic became evident in the late summer (beginning of September). In 2009 the first human cases were detected earlier than 2008. It is likely that increased attention of clinicians to this emerging disease improved the surveillance system sensitivity in 2009.

The circulation of WNV in a large area of the Po plain in two consecutive years shows that this territory is becoming suitable to support WNV establishment and possible endemicity. This indicates a need to organise standard surveillance measures to detect WNV activity early and assess risk to public health.

The results of the entomological surveillance confirm that the CO_2 trap is a reliable and valuable tool for early detection of WNV. *Culex pipiens*, the most abundant mosquito species in the region, is the only vector species incriminated, since no other species collected in the field were found to be infected.

The quick and intensive spread of WNV in the past two years suggests that the whole Po plain may be affected in the future. In forthcoming years, surveillance of wild birds and insects will be used to forecast the extension and spread of WNV. The information gathered will be used to direct or optimise actions intended to prevent virus transmission, such as vector monitoring and control, information campaigns to improve personal protection, and deploy screening tests on blood, tissue, and organs for transplant.

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Changes to the varicella and pertussis immunisation schedule in Germany 2009: Background, rationale and implementation

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In July 2009, the German Standing Committee on Vaccination (STIKO) modified its recommendations for varicella and pertussis vaccination, based on newly available data on disease epidemiology, vaccine effectiveness (VE) and safety, and an evaluation of the feasibility of the recommended immunisation strategy. The recommendation for varicella vaccine now includes a routine two-dose schedule with the administration of the first dose at the age of 11 to 14 months and the second dose at the age of 15 to 23 months, with a minimum interval of four weeks between these doses. Furthermore, STIKO recommended adding a one-time pertussis booster to the adult vaccination schedule to expand the cocoon strategy in place since 2004. The recommendation of a booster vaccination with an acellular pertussis vaccine every 10 years for persons employed in the care of pre-school children and for healthcare personnel in paediatric, gynaecologic and obstetric health facilities was extended to persons employed in schools and in other institutions caring for older children, and to all healthcare personnel. These recommendations were based on available epidemiological data showing an increase in incidence from 7-10 cases per 100,000 inhabitants in 2002-2004 to over 30 by 2007. Moreover, the high burden of pertussis in infants at 94 hospitalised cases per 100,000 infants in 2007 suggested that the previous cocoon strategy was insufficient.

Introduction

The German Standing Committee on Vaccination (STIKO) is a board of honorary experts in the fields of vaccinology, infectious disease epidemiology, microbiology and virology, paediatrics, evidence-based medicine and others. STIKO members are appointed by the Federal Ministry of Health every three years. STIKO reviews its recommendations for vaccination on an annual basis. Its decision-making process is based on published data on disease epidemiology, vaccine effectiveness (VE), and safety, and on the feasibility of the recommended immunisation strategy. STIKO is not responsible for a health economic assessment of the effect of vaccination. In this paper we communicate the major modifications to the immunisation schedule in Germany implemented in July 2009 and we detail the scientific rationale for the changes [1]. The main changes were the addition of a second dose of varicella vaccine to the routine childhood vaccination schedule and the recommendation of a single pertussis booster for adults.

So far, Germany is the only country in Europe to have introduced a nationwide recommendation for routine childhood varicella vaccination. Since the introduction of, initially, a one-dose varicella vaccination in children, a significant reduction of varicella cases in children was observed in a nationwide sentinel surveillance system, as recently published by Siedler *et al.* [2]. This positive experience with varicella vaccination in Germany and the deliberations of the STIKO on the introduction of a two-dose varicella vaccination schedule might be helpful for other countries in Europe in their decision making process.

Second dose of varicella vaccine in children

In 2004, STIKO recommended routine varicella vaccination for all children older than 11 months with the aim of reducing varicella associated morbidity and the reduction of the burden of disease [3]. A single dose of monovalent varicella vaccine was recommended at 11 to 14 months of age and at that time, two licensed vaccines were available for this purpose in Germany, Varilrix and Varivax. In 2006, the measles-mumps-rubella-varicella (MMRV) vaccine (Priorix-Tetra) was licensed for administration in two doses at least four weeks apart [4]. In 2008, the licensed immunisation schedule of both monovalent varicella vaccines changed to a two-dose scheme for all children [5,6].

Based on epidemiological data from Germany and the United States (US) as well as newly available data on varicella zoster virus (VZV) vaccine immunogenicity and effectiveness after one and two doses of VZV-containing vaccines, STIKO decided to follow

the change of vaccine licensures and recommended a routine two-dose vaccination strategy in children. The aim of this recommendation is to reduce the number of varicella outbreaks, the number of cases with varicella breakthrough illness (BI) and the transmission of VZV to susceptible persons with a higher risk for severe varicella disease such as pregnant women and immunocompromised patients.

Epidemiological data from Germany and the US

Varicella is not notifiable in Germany. Therefore, a country-wide sentinel project on varicella and zoster epidemiology was established in Germany in April 2005 [7,8]. The constitution of the sentinel system and its first results covering the time frame April 2005 to March 2009 have been published recently [2]. Since sentinel surveillance cannot provide population-based data, varicella incidence and vaccination coverage cannot be calculated directly. However, from 2005 to 2009, physicians reporting to the sentinel diagnosed successively fewer children with varicella with a significantly decreasing trend over time, while they administered a rapidly increasing number of varicella vaccines for first doses and since 2008, second doses of VZV-containing vaccine as well. A stable number of sentinel physicians reported around 60% fewer varicella cases in the 2008-9 season than in the 2005-6 season. Concurrently, a rising number of varicella BI was reported over the observation period [2,8].

Available information on vaccine coverage for the federal state of Schleswig-Holstein shows that coverage in children under 24 months of age with one dose of VZV containing vaccine, increased from 11% in 2006 to 83% in 2008 [personal communication Hans-Martin Bader and Maik Ludwig].

In 2008 and 2009, the RKI investigated seven varicella outbreaks in day-care centres including 631 children with available varicella vaccination information [9]. The attack rate was 13% in vaccinated children and 48% in unvaccinated children, resulting in an overall VE of 71% [95% confidence interval (CI): 57-81] in the multivariable analysis [9]. VE differed significantly by disease severity and number of doses administered. VE of one dose and two doses of VZV-containing vaccines was estimated to be 62% [95% CI: 43-75] and 94% [95% CI: 75-98], respectively. Furthermore, results of outbreak investigations in Germany suggest that VE and the risk of varicella BI are not uniform for the different varicella vaccine products available in Germany [9].

The US varicella vaccination programme implemented in 1996 recommended one dose of monovalent varicella vaccine at the age of 12 to 18 months and achieved over 90% reduction in varicella morbidity in children between 1995 and 2005 [10,11]. Since varicella outbreaks continued to occur and an increasing number of cases with varicella BI was observed in active surveillance areas, the US Advisory Committee on Immunization Practices (ACIP) recommended a second dose of varicella vaccine for all children aged four to six years in 2007 [12]. However, when applying these data to the German situation, it must be kept in mind that there is only one vaccine (Varivax) available in the US and a high vaccination coverage over 90% in children under three years old has been achieved for a prolonged period of time [11].

Effectiveness, immunogenicity, and safety of VZV-containing vaccines

VE after one dose Varivax was estimated at 84.5% (range: 44-100) in a recent review [13]. Estimates from three epidemiological studies on the VE of one dose of Varilrix range from 20 to 92% [14-16]. So far no data on VE after two doses of Priorix-Tetra have been published.

In 2008, the licensed immunisation schedule of both monovalent varicella vaccines changed to a two-dose scheme for all children [5,6]. The regulatory authorities based this change of licensure on immunogenicity data after one and two doses of VZV containing vaccines and on a large epidemiological study by Kuter *et al*. conducted in the US in the 1990s using Varivax, although at a higher potency than available licensed vaccines [17-19]. In this study the risk of developing varicella 42 days after vaccination was 3.3-fold lower in children who received two doses of Varivax compared with those who received only one dose ($p<0.001$). The estimated vaccine efficacy for the 10-year observation period for one or two doses Varivax was 94.4% and 98.3%, respectively ($p<0.001$). This efficacy estimate was based on comparison with historical data of annual varicella incidences in unvaccinated susceptible children. In a randomised controlled trial in 10-21 month-old VZV seronegative children an over 20-fold higher geometric mean titre (GMT) of VZV antibodies was observed in children with two doses of Priorix-Tetra compared with children vaccinated with only one dose of a monovalent varicella vaccine [17]. However, the study by Kuter *et al*. showed that GMTs decreased to similar levels within several years regardless of the number of doses (one or two) applied [19]. Therefore, the reliability of VZV antibody levels as a correlate of protection after vaccination remains unclear.

A good safety profile of VZV-containing vaccines available in Germany has been reported [4-6]. However, for the MMRV vaccine ProQuad, which is only available in the US, febrile seizures were observed more frequently post-vaccination than after simultaneous administration of MMR and monovalent varicella vaccines in children under two years of age. [20].

Feasibility of routine two-dose varicella vaccination in children

The first dose of VZV-containing vaccine is recommended at the age of 11 to 14 months and the second dose at the age of 15 to 24 months. Thus, varicella vaccination can be administered simultaneously with the

measles-mumps-rubella (MMR) vaccination using a monovalent varicella vaccine plus an MMR vaccine or using the licensed MMRV vaccine. However, varicella vaccination can be offered to any child aged 11 months or older. A second dose is also recommended for all children who thus far received only one dose of VZV-containing vaccine.

Pertussis vaccination of adults

In July 2009, STIKO recommended adding a single pertussis booster to the adult vaccination schedule against pertussis, in order to expand the cocoon strategy which recommended pertussis vaccination for all adults with close contact to infants since 2004. The 2004 recommendations also included a booster vaccination with an acellular pertussis (ap) vaccine every 10 years for persons employed in the care of pre-school children and for healthcare personnel in paediatric, gynaecologic and obstetric health facilities. These were expanded to include persons employed in schools and in other institutions caring for older children, as well as all healthcare personnel. The new recommendations were based on careful analysis of available epidemiological data on the disease burden in adults as well as data on immunogenicity, effectiveness and safety of available vaccines. The aim of the updated recommendations is to decrease pertussis-related morbidity in adults as well as in non-immune, unprotected contacts, particularly infants [21;22].

Epidemiological data from Germany for adults

Pertussis is a notifiable disease in five of the 16 German federal states. Despite increasing vaccination coverage among children at school entry to over 93%, the incidence of pertussis in adults in the states with statutory surveillance increased from 7-10 cases per 100,000 inhabitants in 2002-2004 to a maximum of 32 in 2007, decreasing only slightly to 30 in 2008 [22;23]. The average age of notified cases increased from 15 years in 1995 to 42 years in 2008, with a concomitant increase in the proportion of cases older than 19 years (adult cases) from 20 to 75%. Sentinel surveillance performed from 2000-2004 [24] estimated a similar annual incidence in an east and a west German city, 169 and 160 pertussis cases per 100,000 adult inhabitants, respectively, translating to a nationwide estimated incidence of 160. However, pertussis incidence increased according to statutory surveillance in the years following conclusion of this sentinel study. These figures thus suggested a marked under-ascertainment in the statutory surveillance system. In addition, the burden of pertussis in infants remains high with an incidence of 94 hospitalised cases per 100,000 infants in 2007, implying that the cocoon strategy implemented in 2004 was insufficient.

The majority of pertussis outbreaks notified from 2002-2007 in the five German federal states with statutory pertussis surveillance involved children older than nine years (70% of cases in outbreaks with more than five cases), which was the rationale for expanding the recommendation for pertussis vaccination to persons employed in schools and other institutions involved in the care of older children. A Canadian study calculated that healthcare personnel had a 1.7-fold elevated risk for pertussis compared with the general population [25]. Numerous pertussis outbreaks among healthcare personnel involved in the care of adults have been published [26] and a modelling study in the United Kingdom estimated a high incidence of pertussis in hospitalised elderly patients [27].

Adults with pertussis often fail to present with the full-blown clinical picture, but nonetheless suffer significant morbidity. In the above mentioned surveillance study by Riffelmann *et al*. [24], the median duration of the primary symptom, cough, was 48 days (maximum: 72 weeks) and on average, patients consulted their general physician 5.4 times and 27% were referred to a specialist. Antibiotics were prescribed for 53% of patients and 13% received steroids. In various case series between 23% and 28% of all adult patients with pertussis suffered complications, including sinusitis, otitis media, incontinence, weight loss, rib fractures, syncope and pneumonia [24;25;28;29]. Hospitalisation was reported in 1 to 3% of adult pertussis cases [25;29-31].

Effectiveness, immunogenicity and safety of pertussis vaccines

Effectiveness of vaccination with an ap vaccine containing three pertussis antigens was demonstrated in adults in one randomised controlled trial, which was, however, based on the observation of only 10 pertussis cases in total: one case amongst 1,391 tetanus-diphtheria-acellular pertussis (Tdap)-vaccinated participants, and nine pertussis cases amongst 1,390 hepatitis A vaccinated participants [34]. Vaccine efficacy was estimated at 92% (95% CI 32-99). Numerous studies have demonstrated immunogenicity of pertussis vaccines in adults equivalent to that observed in studies in children in which clinical efficacy was shown [32]. These studies have also demonstrated that the safety and reactogenicity of ap vaccines is similar to that of Td (tetanus-diphtheria) vaccines.

Cost-effectiveness

Lee *et al*. [33] performed a cost-effectiveness analysis for vaccination of adults in Germany using conservative estimates of pertussis incidence and including direct costs as well as indirect costs due to loss of work by means of a Markov model. This suggested that one time vaccination of around 62% of adults would be cost-effective and possibly cost-saving, at a cost of 160 Euros per pertussis case prevented. Two other models tailored to the US situation came to similar conclusions [21;34;35].

Feasibility of implementing a one-time adult pertussis booster

Implementation of an adult booster is considered feasible. Telephone surveys in Germany revealed a high

acceptance of tetanus vaccination, with 75% of a population based sample of adults reporting having received a tetanus-containing vaccination in the past 10 years (unpublished data, RKI). However, it is essential that Tdap vaccines are made widely available for tetanus post-exposure immunisation in emergency settings. In addition, efforts are needed to improve vaccination coverage in adolescents, which remains low at 36% [36].

Conclusions

Varicella vaccination

Germany is currently the only country in Europe that includes varicella vaccination in the routine immunisation schedule for children. Results from sentinel surveillance show that the recommendation of routine varicella vaccination for all children older than 11 months in 2004 has had a positive effect on the overall burden of varicella in Germany [2]. Based on immunogenicity data after one and two doses VZV-containing vaccines (indirect evidence) and based on the findings of the study of Kuter *et al*., licensures of both monovalent varicella vaccines changed to a two-dose schedule [17-19]. With respect to recent epidemiological findings in Germany and from the US, where varicella vaccines are included in the routine immunisation programmes since 1996 [2,11], STIKO decided to follow the change of licensure and recommended a second dose for all children in July 2009. As published VE estimates vary widely, and one VZV vaccine (ProQuad) has been associated with an increased risk of febrile seizures in children less than 2 years of age [13-16,20], there is a need for additional post-marketing data on safety and effectiveness of VZV-containing vaccines. A population-based VZV surveillance system should be established in Germany to evaluate the effect of routine varicella vaccination in children and to monitor varicella and herpes zoster incidences in all age groups.

Pertussis vaccination

As herd protection has been demonstrated after implementation of pertussis vaccination in children [37;38], it seems reasonable to assume that vaccination of adults can lead to reduction of disease in unprotected individuals, particularly infants. Furthermore, a modelling study has estimated that around 34% of all transmission of pertussis to infants occurs via less close contacts [39]. Both natural and vaccine-induced immunity is not lifelong [40], waning immunity in the face of decreased natural boosting in conjunction with increasing vaccination coverage in children [41] – is likely the most important factor leading to high disease burden in adults. The aim of the addition of a pertussis booster to the adult vaccination schedule is thus to reduce the overall pertussis disease burden. The implementation of the changes to the adult pertussis vaccination schedule will be evaluated in regular population-based telephone interviews soliciting data on vaccination status, and through analysis of routine surveillance data available from the former East German states, hospital discharge statistics and the established laboratory surveillance of molecular genetic characteristics of *B. Pertussis*.

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Occurrence of haemagglutinin mutation D222G in pandemic influenza A(H1N1) infected patients in the West of Scotland, United Kingdom, 2009-10

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To the editor: Kilander *et al*. (2010) [1] have previously reported that in some cases of patients with severe or fatal pandemic influenza A(H1N1), an amino acid substitution from aspartic acid to glycine occurs at position 222 (D222G) of the HA1 subunit of haemagglutinin (HA). In their study 11 (18%) of 61 patients with severe disease had the mutation, in contrast to 0 of 205 patients with mild disease.

Since the original report [1] several countries have detected this mutation [2]. This data has been summarised in a recent World Health Organization (WHO) review, which reported that the overall prevalence of D222G was <1.8% in contrast to a rate of 7.1% in fatal cases [2]. The WHO paper also reports on the occurrence of other mutations at this amino acid, D222E and D222N, although their significance is unclear. A group in Hong Kong have also analysed this amino acid in severe and non-severe cases of pandemic influenza A(H1N1) [3]. In this study nine (4.1%) of 219 severe or fatal cases of pandemic influenza A(H1N1) had the D222G mutation, in contrast to 0 of 239 non-severe cases.

We sequenced the HA1 subunit of the HA gene from a number of West of Scotland cases, both community cases and severely ill. Furthermore we subdivided the severely ill into those who had died and those who recovered after hospitalisation. We found an increased incidence of D222G in those patients who died (2/23 - 8.7%) compared to both community and hospitalised patients (0/35 - 0%). We also detected an increased incidence (2/32 - 6.2% cf 0/26 - 0%) of D222N (aspartic acid to asparigine) in severely ill patients and those who had died. The significance of this mutation is unclear. There was a low level of D222E (aspartic acid to glutamic acid) present in both severely ill and community cases with no significant difference between the two. The results are summarised in the Table.

Interestingly, in one of the patients who died and had the D222G mutation, the original sequence had a mixed base in the D222 codon giving D222D/G. On resequencing two more samples from this patient, we obtained a pure D222G on one occasion and a pure wildtype D222 on the other, showing that this patient had a mixed population of virus. This confirms the finding in Kilander's paper [1] of the co-existence of mutant and wildtype virus.

Table

Prevalence of mutations at amino acid D222 of haemagglutinin of influenza A(H1N1), Scotland, United Kingdom, 2009-2010

	Number of patients	D222G	D222N	D222E
All cases	58	2 (3.4%)	2 (3.4%)	4 (6.9%)
Patients who died	23	2 (8.7%)	0	1 (4.3%)
Seriously ill patients	9	0	2 (22%)	1 (11%)
Community patients	26	0	0	3 (11%)

References

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