Impact factor 4.659

Eurosurveillance

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

Vol. 19 | Weekly issue 32 | 14 August 2014

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Injectional anthrax - new presentation of an old disease

T Berger (tamaritayberger@gmail.com)[1,2], M Kassirer[1], A A Aran[1,3]
1. Israel Defense Force, Medical Corps, Ramat-Gan, Israel
2. Department of Internal Medicine D, Rabin Medical Center, Beilinson Hospital, Petah-Tikva, Israel
3. Department of Pediatric Intensive Care, The Edmond and Lily Safra Children's Hospital, The Chaim Sheba Medical Center, Tel-Hashomer, Ramat-Gan, Israel

Citation style for this article:
Berger T, Kassirer M, Aran AA. Injectional anthrax - new presentation of an old disease. Euro Surveill. 2014;19(32):pii=20877. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20877

Article submitted on 05 September 2013 / published on 14 August 2014

Bacillus anthracis infection (anthrax) has three distinct clinical presentations depending on the route of exposure: cutaneous, gastrointestinal and inhalational anthrax. Each of these can lead to secondary bacteraemia and anthrax meningitis. Since 2009, anthrax has emerged among heroin users in Europe, presenting a novel clinical manifestation, 'injectional anthrax', which has been attributed to contaminated heroin distributed throughout Europe; before 2009 only one case was reported. During 2012 and 2013, new cases of injectional anthrax were diagnosed in Denmark, France, Germany, and the United Kingdom. Here we present a comprehensive review of the literature and information derived from different reporting systems until 31 December 2013. Overall 70 confirmed cases were reported, with 26 fatalities (37% case fatality rate).The latest two confirmed cases occurred in March 2013. Thirteen case reports have been published, describing 18 confirmed cases. Sixteen of these presented as a severe soft tissue infection that differed clinically from cutaneous anthrax, lacked the characteristic epidemiological history of animal contact and ten cases required complimentary surgical debridement. These unfamiliar characteristics have led to delays of three to 12 days in diagnosis, inadequate treatment and a high fatality rate. Clinicians' awareness of this recently described clinical entity is key for early and successful management of patients.

Introduction

Anthrax is a worldwide endemic zoonotic disease, particularly of herbivores, caused by the bacterium *Bacillus anthracis*, a Gram-positive rod. The infective form is usually the spore, a stable form that can survive in certain environments for decades [1]. Humans usually acquire anthrax infection by occupational exposure to contaminated animals or animal products such as hides, wool, hair and bones or by ingesting contaminated meat. The spores may infect the host through different routes and lead to a variety of clinical presentations depending on their route of entrance: cutaneous (the most common form of infection), gastrointestinal and inhalational anthrax. Complications of these three infections are secondary bacteraemia and anthrax meningitis. Once the spores have penetrated the skin or mucosa, they germinate to the vegetative bacteria, which proliferate and produce the virulence factors, two exotoxins – lethal toxin and edema toxin –, causing the characteristic pathological findings: edema, haemorrhage and tissue necrosis with a relative lack of leukocytes in infected tissues [1,2].

Anthrax is a rare human infection in Europe and animal cases are sporadic and uncommon [3]. However, an outbreak of a fourth form of human anthrax infection, injectional anthrax, has been emerging among drug users in Europe since 2009 and has been attributed to contaminated heroin. Prior to 2009 only one case of injectional anthrax had been diagnosed, in Norway in 2000 [4]. The distribution of cases demonstrates a bimodal appearance of two clusters: over 100 cases were diagnosed between December 2009 and December 2010, the vast majority of them in Scotland [5-8], with no further cases documented until June 2012 when cases reemerged in England, Germany, Scotland and were diagnosed for the first time in Denmark, France and Wales [9,10].

Injectional anthrax infection is unnatural and more severe than cutaneous anthrax infection, distinctive in its clinical presentation, course and management. This has resulted in a delay in diagnosis, inadequate treatment and high fatality rate among reported cases [8-11].

This comprehensive literature review of all injectional anthrax cases reported so far, aims to present an inclusive view of this novel clinical entity in order to improve clinicians' awareness and knowledge base.

Search strategy and selection criteria

References for this review were identified through searches of PubMed for articles published until 31 December 2013, by using the terms 'anthrax AND heroin', '*Bacillus anthracis* AND heroin', '*Bacillus anthracis* AND drug user', 'anthrax AND drug user' and 'injectional anthrax'. Articles resulting from these searches and relevant references cited in those articles were

reviewed by TB starting with screening of abstracts followed by full text review where relevant. The Program for Monitoring Emerging Diseases (ProMED-mail) reporting system was searched by the terms 'anthrax AND heroin' until 31 December 2013 and by the term 'anthrax' from 1 December 2009 to 31 December 2013 and all relevant reports reviewed. ProMED-mail is an Internet-based reporting system, open to all sources, including media reports, official reports, online summaries, local observers, and other [10]. We also reviewed the Health Protection Scotland (HPS) 'National Anthrax Outbreak Control Team' report ('HPS report'), published in December 2011, on the anthrax outbreak among drug users in Scotland between December 2009 and December 2010 [8]. The European Centre for Disease Prevention and Control (ECDC) website [11] and Public Health England (PHE) website [9] were reviewed until 31 December, 2013. The ECDC website was searched for relevant information using the words 'anthrax AND heroin' and by reviewing the material published under the website heading Health Topic 'anthrax'. In addition, the ECDC's 2010–2013 annual epidemiological reports on communicable diseases in Europe were reviewed [11]. We searched the PHE website for the topic 'anthrax'. The findings under the topic were reviewed with the emphasis on information regarding the 2012–2013 outbreak. Articles, reports and data yielded by our search were included only if they were published in English.

Results

Overall 39 papers were retrieved through PubMed searches. Of those, 14 were not related to injectional anthrax and were excluded. Twenty five relevant papers, including references cited in these papers, were reviewed. Of those, 22 papers were included in the review and three papers excluded due to a lack of additional information.

A total of 70 laboratory-confirmed cases of injectional anthrax were reported among heroin users in Europe [4,6,8-11;14-26]. Apart from one case reported in the year 2000, all cases were reported starting as of December 2009. Incidents appeared during two distinct time periods, forming a bimodal distribution (Figure).

The first and largest cluster of cases took place during 2009–2010 and consisted of 126 cases (54 laboratory-confirmed cases, 35 probable cases and 37 possible cases). A majority of cases were recorded in Scotland. The main part of published data on injectional anthrax describe patients from this outbreak [6,8-11,16-22,24].

The second cluster of cases started with the reemergence of a total of 15 cases during June 2012 in six different European countries [9-11,14,15, 25,26]. The last case was reported in Scotland in March 2013 [10]. Only few case reports regarding five confirmed cases exist concerning this second cluster of cases. Hence, the information regarding the number of cases, their geographical distribution and demographic description (if present) mainly relies on a review of the ProMED-mail, ECDC and PHE websites.

Table 1

Number of laboratory-confirmed anthrax cases in drug users and fatalities among them by country, Europe, 2009 to 2013 (n=69)

	December 2009–December 2010		January 2012-December 2013	
	Laboratory-confirmed cases	Fatalities (case-fatality rate)	Laboratory-confirmed cases	Fatalities (case-fatality rate)
Scotland	47	13	2	1
England	5	4	5	4
Germany	2	1	4	1
Denmark	-	-	2	1
France	-	-	1	-
Wales	-	-	1	-
Total	**54**	**18 (33%)**	**15**	**7 (47%)**

Source: [8,10].

Course of events and epidemiological data

Ringertz et al. were the first to coin the phrase 'injectional anthrax' in the year 2000. They described a confirmed case of anthrax in a 49 year-old, human immunodeficiency virus (HIV)-negative, heroin-injecting drug user in Norway [4]. The patient's exceptional presentation resembled the effect of subcutaneous inoculation of *B. anthracis* in chimpanzees and shared similarities with an old case report of possible transmission of anthrax by injection [4].

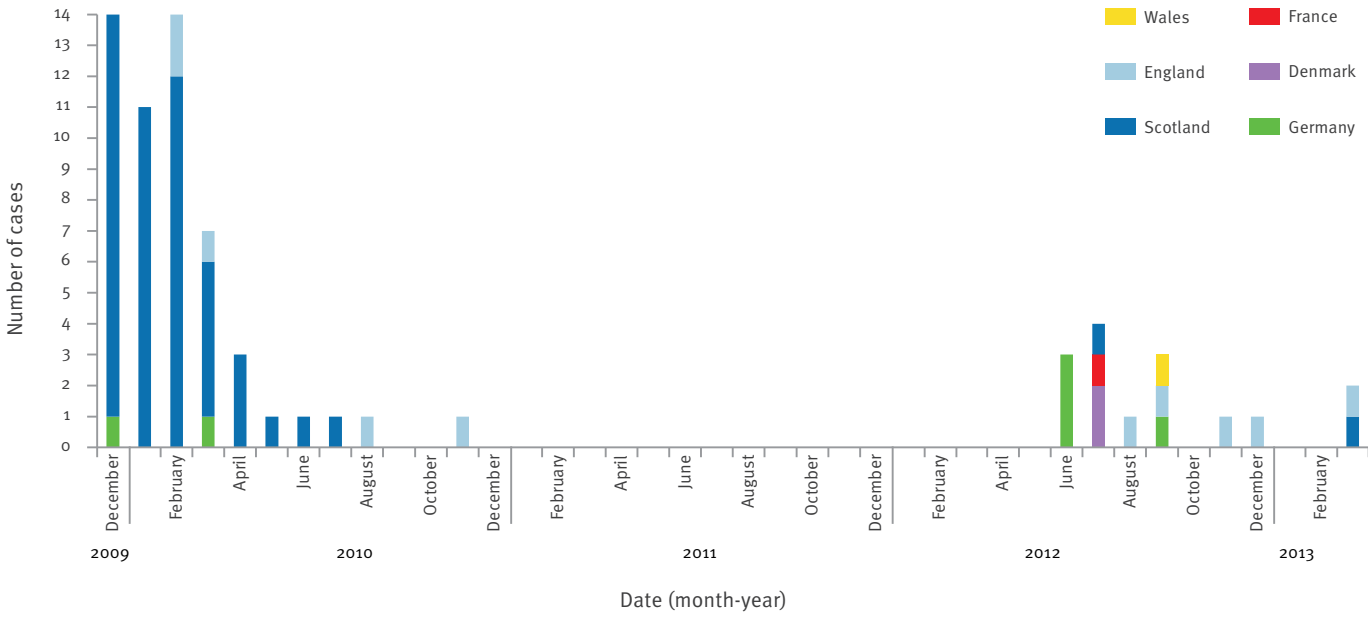
The following cases of confirmed 'injectional anthrax' were only reported in December 2009 among heroin users in Scotland and Germany [5-7], marking the start of the 2009–2010 injectional anthrax outbreak, that eventually included 126 cases (54 laboratory-confirmed cases, 35 probable cases and 37 possible cases) in Europe. The outbreak centered in Scotland with 119 cases: 47 laboratory-confirmed cases (13 fatal), 35 probable cases (1 fatal) and 37 possible cases. Case categories used by HPS are described in Table 2 [8].

Few cases were laboratory-confirmed in England (5 cases, 4 fatal) and Germany (2 cases, 1 fatal). The case fatality rate among confirmed cases was 33% (Table 1). The vast majority of confirmed cases appeared between December 2009 and March 2010. The last case occurred in England in November 2010 (Figure 1). The outbreak was declared over in December 2010 and the HPS published its concluding report in December 2011 [8-11].

The average age of the 119 injectional anthrax cases in Scotland was 34 years (range 18-55 years), with more males than females in all case categories [8]. It appeared that all injection routes of heroin, as well as smoking or snorting had been implicated during the

Figure

Timeline and geographical distribution of laboratory-confirmed injectional anthrax cases, Europe, December 2009–31 December 2013 (n=69)



Source: [8-11, 14-15]

outbreak, though the mode of abuse was not well documented in most reports. Of the 47 confirmed cases of anthrax in drug users in Scotland, only two denied injecting at all, lacked visible injection sites and reported using heroin only by smoking. One of those cases developed systemic anthrax. The clinical presentation of the latter two was not described in detail in the HPS report [8]. The report however, describes a number of anthrax cases that were admitted with advanced systemic infection; some without any history of injecting heroin but with a history of taking heroin by other routes, principally smoking, leading to the conclusion that there is a potential risk for systemic disease caused by snorting or smoking heroin contaminated with *B. anthracis* [8].

A case-control study investigating risk factors among 82 confirmed and probable cases in the Scottish outbreak of 2009–2010 found increased risk of injectional anthrax to be associated with longer injecting history (adjusted odds ratio (AOR), 2.43; 95% confidence interval (CI), 1.31–4.52), receiving opioid substitution therapy (AOR, 2.74; 95% CI, 1.40–5.37) and alcohol abuse (P = 0.09; AOR, 1.77; 95% CI, 0.91–3.47). Smoking heroin was associated with lower risk of infection – individuals who only smoked heroin in the past month were less likely to be a case (AOR 0.42; 95% CI, 0.20–0.86). The authors did not find an association between sharing injecting equipment and anthrax infection, aligned with the hypothesis that the heroin itself was the source of contamination and explaining the higher risk among patients with longer injecting history [12].

During 2011 there were no reports on new injectional anthrax cases. Moreover, a retrospective serological pilot study in two German regions conducted in 2011 failed to discover additional anthrax cases among 288 heroin users [27]. However, since June 2012 new cases have reemerged in Europe, including Denmark, France,

Table 2

Injectional anthrax case categories [8]

A drug user with a clinical syndrome compatible with anthrax[a] AND:	
Confirmed case	One or more of: • Growth of *Bacillus anthracis* from a clinical isolate confirmed by the reference laboratory • Evidence of *B. anthracis* DNA by PCR on multiple target genes • Demonstration of *B. anthracis* in a clinical specimen by immunohistochemistry • Serology with seroconversion on paired specimens • Demonstration of specific anthrax toxin in blood
Probable case	Gram-positive bacilli identified or bacterial colony growth (phenotypically resembling *B. anthracis*) from either a tissue specimen / swab of lesion or fluid/collection or blood culture
Possible case	Symptomatic individuals with an epidemiological link to a known confirmed or probable case

[a] Wide-ranging case presentation of anthrax occurred in heroin users. All possible presentations of anthrax need to be considered in anyone with a history of recent heroin use by any route: injection related soft tissue infections (chief presentation of injectional anthrax), severe sepsis and meningitis. Also potentially possible are inhalational and cutaneous anthrax from snorting or handling contaminated heroin [8].

Germany, and the United Kingdom (UK): England, Scotland, and Wales [9-11]. So far, 15 confirmed cases were reported, among them seven fatal, contributing to a 47% case fatality rate (Table 1, Figure 1). Less than half of case reports included epidemiological data such as age and sex, of those who did, the age range varied from 27 to 55 years [10,14,15,25,26].

To date, no person-to-person transmission was documented in injectional anthrax cases. There seems to be no risk for health workers or for the general population [4,6,8,10,14-26].

Source of infection

Since anthrax is rare in Europe and all cases occurred in drug users, it was assumed that the 2009–2010 outbreak was caused by a contaminated batch of heroin, even though microbiological analysis of heroin samples from a variety of sources in Europe failed to detect contamination with anthrax spores [8, 25]. The possibility of deliberate contamination was not eliminated but seemed unlikely due to the affected population [8]. Several recent studies [13-15,27,28] have conducted molecular analysis of *B. anthracis* isolates from the first reported case in Norway in 2000, from cases during the 2009–2010 outbreak and from cases in Denmark and Germany from the 2012–2013 outbreak. All isolates were closely related and it was concluded that they belong to the same *B. anthracis* strain despite small observed variations [13-15, 27]. This single anthrax strain was closely related to strains originating in Turkey. The findings suggested that the outbreak in Europe possibly derived from a single common source which was contaminated before the heroin was distributed. Accidental contamination with anthrax spores might have occurred along the drug manufacturing and trafficking route from soil, through an animal derived cutting agent (used to dilute the illicit drug) or animal hides used to smuggle heroin into Europe [13]. This is compatible with the forensic investigation findings of the 2009–2010 outbreak in the UK which estimated that 80 to 90% of heroin reaching the UK is supplied via criminal networks in Turkey and is being processed before trafficking [8,10]. Thus all analysed cases may be traced back to a single source of contamination that might be still circulating in Europe [27].

Clinical presentation

Clinical data from 13 case reports describing 18 confirmed cases is summarised in Table 3 [4,6,14-23,25] and presents several common features.

Injection site

Sixteen of the 18 published cases presented with severe soft tissue infection originating in the injection site one to ten days post injection [4,6,14-20,22,25]. Among laboratory-confirmed cases in Scotland from the 2009–2010 outbreak, whose symptoms were recorded as present or absent, 39 of 42 cases presented with skin or soft tissue involvement [8]. The estimated median time from the presumed 'culprit' injection to hospitalisation was three days [8]. Signs of skin infection varied among patients. The most consistent presenting sign, appearing in all 16 cases diagnosed with soft tissue infection, was substantial swelling or edema, at times defined as disproportionate to the skin lesion or extent of pain described. Erythema and pain were not essential features at presentation. Some reports described blistering or necrosis of skin (Table 3). Three cases were diagnosed initially with compartment syndrome or necrotising fasciitis [6,17,20].

None of the published cases showed the typical signs of cutaneous anthrax – a black crusted painless lesion, known as eschar [4,6,14-23,25]. According to the HPS report, only one confirmed case presented with eschar formation [8].

When documented, imaging and soft tissue exploration revealed edematous muscle and subcutaneous tissue (with or without necrosis) and serous discharge with no localised collection, pus or abscess formation [4,17-20,25]. Fever, leukocytosis and elevated C-reactive protein (CRP) were not consistent features of patients presenting with soft tissue infection [4,8,15,16,20,24,25]. The minority of reports provided patients' history of past blood-borne infections or immunological status including HIV status (Table 3 and HPS report [8]), conditions which may also have contributed to the diminished inflammatory response.

Gastrointestinal, respiratory and neurological symptoms

According to the HPS report, 22 of 40 laboratory-confirmed cases in Scotland described non-specific gastrointestinal symptoms such as nausea, vomiting and abdominal pain [8]. There are two reports on an unusual presentation of acute abdominal pain and peritonitis due to groin heroin injection [21,25].

Presenting with respiratory symptoms such as dyspnoea and pleuritic chest pain was extremely rare and was attributed to disseminated disease and sepsis in two cases, according to the HPS report [8]. Nausea and dyspnoea were also evident in one patient from Germany during the 2012–2013 outbreak [15].

Fourteen of 42 Scottish laboratory-confirmed cases reported neurological symptoms of variable degree [8]. A small number of injectional anthrax patients presented with signs of progressive systemic infection and meningitis. This was described in one case report of a patient with prolonged headache, confusion and coma [23]. This case and four more cases were diagnosed with intracranial or subarachnoid haemorrhage [8], and from available data, at least four of these five cases deteriorated rapidly and later died [23,24].

Clinical information about cases diagnosed since June 2012, is limited to three published case reports regarding five confirmed cases [14,15,25] (Table 3) and a brief

Table 3a

Summary of epidemiological and clinical data of published injectional anthrax cases, Europe, 2000- 2013 (n=18)

Reference	Age (years)	Sex	Report from, Date[a]	Clinical presentation	Clinical course and complications	Onset from injection	HIV, HBV, HCV status (-/+/NA)	Diagnosis	Treatment[d]	Outcome
Ringertz [4]	49	M	Norway, 4/2000	Painful infiltrate of the gluteal region, 5 cm in diameter, erythema. No eschar, no fever.	Septic shock, meningitis	NA	HIV – HBV,HCV: NA	Diagnosed after deterioration, positive blood culture, PCR	Dicloxacillin; post complications: high-dose penicillin, chloramphenicol, dexamethasone	Deceased
Radun [6]	42	M	Germany 12/2009	Leg swelling following drug injection to the popliteal fossa (drug unknown). Temperature: normal	Multi-organ failure	NA	NA	Anthrax was not clinically suspected, diagnosis post mortem by PCR from wound	Meropenem; surgical debridement	Deceased
Beaumont [16]	23	M	Scotland 2/2010	Diffuse swelling of the forearm and hand. Forearm numbness. No erythema, fever or pain.	Not mentioned	2 days	NA	Early diagnosis[e] due to awareness (no details)	"Treated as per a possible case of anthrax" , no details given	Recovered
Parcell [17]	28	F	Scotland, EP: 11/2010	Arm- and shoulder pain; massive swelling. No erythema or abscess. Compartment syndrome. Temperature 38°C, tachycardia, ↑[f] WBC.	Necrotising fasciitis, coagulopathy, bleeding, massive transfusions necessary	5 days	NA	Early diagnosis, 9 days post admission-Gram positive bacilli on histopathology, positive PCR on tissue	Benzylpenicillin, clindamycin, ciprofloxacin, metronidazole; two fasciotomies, six debridements, skin graft	Recovered
Powell [18]	32	M	Scotland, P: 1/2011	Swollen leg and purulent discharge from a chronic sinus in the groin, no abscess. Tachycardia, temperature 38.2°C, normal BP. ↑[f] WBC.	Cellulitis over the thigh and lower abdomen	NA	NA	Early diagnosis[e], positive blood cultures	Vancomycin, clindamycin, ciprofloxacin, gentamicin and metronidazole; thigh exploration; negative pressure wound therapy device	Recovered
Jallali [19]	53	F	England, EP: 7/2010	Painful and swollen lower limb, malaise.	Septic shock, skin necrosis, operation room- bleeding, massive transfusions	10 days	HIV+ HBV + HCV +	Positive blood cultures on day 5 post admission	No details regarding antibiotic treatment; limited debridement, fasciotomies, skin graft	Deceased
Jallali [19]	32	M	England, EP: 7/2010	Swollen scrotum and gluteus, skin necrosis over gluteal region.	Disproportionate operation room bleeding, pulmonary edema, pleural effusion	6 days	HIV - HBV + HCV +	Positive blood cultures and PCR of debrided tissues	No details regarding antibiotic treatment; i.v. AIG; debridement, skin graft	Recovered
Knox [20]	44	M	Scotland, P: 3/2011	Painful and swollen arm. Small erythema. Forearm: compartment syndrome. No fever. Normal WBC	Multi-organ failure requiring haemodialysis and ventilator support	10 days	HIV – HBV – HCV -	Positive tissue culture on day 5 of admission	Benzylpenicillin and flucloxacillin. Amended after diagnosis to iv ciprofloxacin, benzylpenicillin and clindamicin; fasciotomies; debridement	Recovered

AIG: anthrax immunoglobulins; BP: blood pressure; CRP: C-reactive protein; DIC: disseminated intravascular coagulation; EP: electronic publication; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; i.v.: intravenous; MALDI TOF MS: matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; NA: not available; P: published; PCR: polymerase chain reaction; WBC: white blood count.

[a] Date: date of event, where not reported, date states the date of article publication (P) or electronic publication (EP).

[b] Accepted for publication on 8 May 2012 hence part of the 2009–2010 outbreak.

[c] Details taken also from ProMEDmail website [10] 21/6/2012 report.

[d] The treatment listed is the primary antibiotic regimen given to the patient. For some of the patients it was changed following confirmation of diagnosis, raising suspicion of anthrax infection or, when suspected, after receiving preliminary positive results of Gram-positive organisms in blood or tissue cultures. Also mentioned is the surgical treatment, if given along the admission.

[e] Early diagnosis is stated in cases diagnosed at admission due to clinical suspicion of injectional anthrax or preliminary positive results of Gram-positive organisms in cultures

[f] Elevated.

Table 3b

Summary of epidemiological and clinical data of published injectional anthrax cases, Europe, 2000- 2013 (n=18)

Reference	Age (years)	Sex	Report from, Date[a]	Clinical presentation	Clinical course and complications	Onset from injection	HIV, HBV, HCV status (-/+/NA)	Diagnosis	Treatment[d]	Outcome
Knox [20]	36	M	Scotland, P: 3/2011	Swollen forearm and hand. Restricted finger movements. No compartment syndrome. No fever. Normal WBC	Apyrexial throughout admission, fasciotomy revealed marked free fluid and edema	1-3 days	HIV – HBV – HCV -	Positive tissue culture on day 5 of admission	Benzylpenicillin and flucloxacillin. Amended after diagnosis to iv ciprofloxacin, benzylpenicillin and clindamicin; fasciotomies; debridement	Recovered
Knox [20]	32	F	Scotland, P: 3/2011	Spreading cellulitis and edema of the lower limbs. No fever, systemically well. Normal WBC.	During admission – swelling of hands (3 weeks post injection to palms)	NA	HIV – HBV – HCV -	Early diagnosis from tissue samples by PCR	Debridement, skin graft	Recovered
Johns [21]	24	M	Scotland, EP: 6/2011	Acute abdominal pain, chills, rigors, nausea. Temperature 37.7°C, BP: 95/64, tachycardia. Peritoneal signs on examination. Thigh swelling.	Multi-organ failure	2 days	NA	Positive culture from peritoneal fluid	No details regarding antibiotic treatment; i.v. AIG; repeated laparotomies, surgical exploration of the thigh;	Deceased
Meghji [22][b]	29	M	England, EP: 2/2013	Firm, erythematous swelling and stiffness of neck, dysphagia. Chest wall cellulitis, decreased air entry. Pyrexia.	Acute renal failure, ↑ inflammatory markers. Airway obstruction	NA	NA	No suspicion of anthrax on admission, positive Gram stain in tissue fluid	i.v. flucloxacillin, amended post deterioration to include ciprofloxacin, clindamycin, flucloxacillin	Deceased
Holzmann & Grunow [14,15]	Over 50	NA	Germany 6/2012	Worsening swelling and reddening at an injection site: upper arm. Nausea and dyspnea. No fever. Leukocytosis.	Respiratory failure, septic shock, multi-organ failure, DIC	NA	HIV , HBV: NA HCV+	Anthrax was not suspected, positive blood culture and PCR assay post death	NA	Deceased
Grunow [10,15][c]	40	F	Germany 6/2012	Purulent infection of neck. Second day: skin necrosis and blistering of legs, thorax and arms reddening; neck swelling. Fever.	Pneumonia, pleural effusion (no evidence of bacillus dissemination)	2-3 days	HIV, HBV: NA HCV+	Early diagnosis, positive blood cultures, MALDI TOF MS, and PCR on tissues	Clindamycin, metronidazole, cefazolin, upgraded to include penicillin and ciprofloxacin; surgical debridement	Recovered
Grunow [15]	NA	NA	Germany 6/2012	Progressive swelling, reddening, warmth, and pain of arm, spreading to the chest. Some blistering. No abscess. Fever.	No complications of infection	NA	HIV, HBV: NA HCV+	Suspicion of anthrax during treatment, positive serology, PCR from lesion	Levofloxacin and clindamycin. Surgical wound debridement	Recovered
Bannard-Smith [23]	54	F	England, P: 12/2012	Prolonged severe headache, confusion. On computed tomography scan: severe subarachnoid haemorrhage with mass effect and herniation.	Rapid deterioration to coma - intubation and ventilation	NA	NA	Anthrax was not suspected, positive blood cultures and PCR post mortem	Supportive care in the intensive care unit	Deceased

AIG: anthrax immunoglobulins; BP: blood pressure; CRP: C-reactive protein; DIC: disseminated intravascular coagulation; EP: electronic publication; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; i.v.: intravenous; MALDI TOF MS: matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; NA: not available; P: published; PCR: polymerase chain reaction; WBC: white blood count.

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[d] The treatment listed is the primary antibiotic regimen given to the patient. For some of the patients it was changed following confirmation of diagnosis, raising suspicion of anthrax infection or, when suspected, after receiving preliminary positive results of Gram-positive organisms in blood or tissue cultures. Also mentioned is the surgical treatment, if given along the admission.

[e] Early diagnosis is stated in cases diagnosed at admission due to clinical suspicion of injectional anthrax or preliminary positive results of Gram-positive organisms in cultures

[f] Elevated.

TABLE 3C

Summary of epidemiological and clinical data of published injectional anthrax cases, Europe, 2000- 2013 (n=18)

Reference	Age (years)	Sex	Report from, Date[a]	Clinical presentation	Clinical course and complications	Onset from injection	HIV, HBV, HCV status (-/+/NA)	Diagnosis	Treatment[d]	Outcome
Russell [25]	55	M	Denmark P:9/2013	Pain and swelling of thigh, abdominal pain, afebrile, normal CRP. Subcutaneous edema, no abscess on ultrasound.	Cardiac arrest, septic shock, multi-organ failure, pulmonary edema, abdominal compartment syndrome	1-4 days	HIV, HBV: NA HCV+	Anthrax was not suspected until positive blood and ascites fluid cultures on day 3	Post complication: cefuroxime and metronidazole, changed to meropenem, ciprofloxacin and metronidazole, later on clindamycin was added	Deceased
Russell [25]	39	M	Denmark P:9/2013	Swollen arm, fever, tachycardia, low BP. Somnolent. Soft tissue swelling on ultrasound.	Diffuse swelling and edema, fluctuating BP	NA	HIV,HBV: NA HCV+	Anthrax suspicion on day 2 with positive blood cultures and report of heroin injection	Cefuroxime amended to penicillin and ciprofloxacin	Recovered

AIG: anthrax immunoglobulins; BP: blood pressure; CRP: C-reactive protein; DIC: disseminated intravascular coagulation; EP: electronic publication; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; i.v.: intravenous; MALDI TOF MS: matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry; NA: not available; P: published; PCR: polymerase chain reaction; WBC: white blood count.

[a] Date: date of event, where not reported, date states the date of article publication (P) or electronic publication (EP).

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[d] The treatment listed is the primary antibiotic regimen given to the patient. For some of the patients it was changed following confirmation of diagnosis, raising suspicion of anthrax infection or, when suspected, after receiving preliminary positive results of Gram-positive organisms in blood or tissue cultures. Also mentioned is the surgical treatment, if given along the admission.

[e] Early diagnosis is stated in cases diagnosed at admission due to clinical suspicion of injectional anthrax or preliminary positive results of Gram-positive organisms in cultures.

[f] Elevated.

description of the single case diagnosed in France [26]. It appears that the clinical presentation and course were similar to those described in the 2009–2010 outbreak [9-11,14,15,25,26] (Table 3).

Clinical course and complications

Among 16 reported cases presenting with soft tissue infection, four patients demonstrated haemodynamic stability [16,18,20], one of them despite evidence of *B. anthracis* bacteraemia [18]. However, nine patients showed progressive soft tissue infection and systemic complications such as coagulopathy, rapid deterioration to septic shock, toxaemia and multi-organ failure [4,6,14,15,17,19,20,22,25]. An important complication occurring intra- or post debridement procedures of soft tissue infections was disproportionate bleeding and oozing (at times, despite correction of coagulopathy) requiring massive transfusions [8]. This was documented in three of the reported cases [17,19].

Patients lacking signs of soft tissue infection, who presented with other signs of systemic infection, meningitis or peritonitis were usually misdiagnosed and rapidly deteriorated due to lack of appropriate treatment [21,23,24] (Table 3).

Following admission, two of the reported cases developed pleural effusions, one associated with pulmonary edema [19] and the other associated with pneumonia [15]. In both cases there was no evidence of *B. anthracis* in the pleural fluid cultures. Besides these two cases, diagnosed outside of Scotland, the HPS report found that a small pleural effusion which did not cause symptoms was detected in ten of 41 patients reported. In some cases anthrax bacilli were recovered from respiratory samples, either from pleural fluid or in two cases from bronchial tissues or exudate. The HPS report states this may be an evidence of smoking or snorting contaminated heroin [8]. Complications are presented in Table 3.

Diagnosis, management and outcome

Diagnosis of injectional anthrax was based on traditional microbiological methods as Gram stain and cultures of tissue, blood, peritoneal fluid or cerebrospinal fluid (CSF) and by polymerase chain reaction (PCR). Evidence of scanty pus cells was a common finding in tissue histopathology [4,6,14-23].

In the first cases from Denmark and Germany in 2012, a matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry method or serology testing for antibodies to the *B. anthracis* protective antigen were also utilised [14,15,25,26].

Patient management was inconsistent. When reported, most cases presenting with soft tissue infection were treated with supportive treatment and antibiotic regimen with or without surgical intervention. The antibiotic regimen varied among cases: anthrax infection was not clinically suspected in eight patients and treatment covered other, more common, causes of severe

soft tissue infections [4,6,15,20,22,25]. This is possibly due to these patients being among the first injectional anthrax cases in different countries. In six of those cases, antibiotic regimens were amended upon clinical suspicion or after preliminary results have suggested anthrax infection [15,20,22,25]. The diagnosis of anthrax infection at presentation was raised in three patients, who were treated with a combination of four or five antibiotics covering both *B. anthracis* infection and more common pathogens causing soft tissue infections. These three patients were part of the 2009–2010 outbreak in Scotland, hence early detection might have been due to clinicians' awareness of the ongoing outbreak [17,18,20] (Table 3). Surgical procedures were reported in ten of 15 cases presenting with soft tissue infection [6,15,17-20] and included explorative surgery, fasciotomy and tissue debridement as well as occasional skin grafting. Four patients required multiple debridement procedures [15,17,20] (Table 3).

In addition, few patients were treated with intravenous anthrax immunoglobulin (i.v. AIG) provided by the United States' Centers for Disease Control and Prevention (CDC) as an investigational new drug (IND). According to the HPS report on the 2009–2010 outbreak, of the 119 Scottish cases, 14 were treated with i.v. AIG [8]. Outside of Scotland, only one case report, from England, stated i.v. AIG usage [19]. Detailed information regarding these patients is lacking, though according to the CDC guidelines for anthrax post-exposure prophylaxis and treatment, of the 15 injectional anthrax cases treated with i.v. AIG, 10 survived and all cases appeared to tolerate the antitoxin [29].

As described, in many cases anthrax was not suspected or was diagnosed with delay. When reported, delay ranged from three to 12 days post admission to hospital [6,19,25] or a first visit to a clinic [4]. This probably contributed to the adverse outcome of eight fatalities among 18 published cases [4,6,14,15,19,21-23,25]. Mortality was higher among the 15 cases arising since June 2012 in comparison to the 2009–2010 outbreak (Table 1). One contributing factor to the difference in mortality rate might have been enhanced awareness and knowledge of injectional anthrax among care givers during the Scottish outbreak leading to earlier adequate treatment.

Discussion

Injecting drug users (IDUs) are at higher risk of developing diverse forms of infections, among those, soft tissue infection is a well-known complication of parenteral drug use, though anthrax infection of injection sites was considered very rare before the 2009–2010 injectional anthrax outbreak [30,31]. Hope et al. have estimated the differences in rates of severe infections among IDUs in Europe, between 2000–2009, caused by four spore-forming bacteria that have been associated with contaminated heroin – *Clostridium botulinum, C. tetani*, *C. novyi*, and *B. anthracis*. They identified 367 infections over the 10-year period. Of these cases, 300 occurred in the UK; only 13 of those were caused by *B. anthracis*. These 300 infections led to an overall rate of 1.9 to 2.1 infections per 1,000 IDUs over 10 years (among those 0.083–0.09 anthrax infections per 1,000 IDUs). The rates of infection with spore-forming bacteria among IDUs in other European countries (Germany, Greece, Italy, the Netherlands and Norway) were much lower than those in the UK, with the exception of a higher infection rate in Ireland (3.9–6.6 infections/1,000 IDUs/10 years) [32].

Thus, although the 2009–2010 injectional anthrax outbreak has constituted the largest cluster of severe bacterial infection among IDUs in Scotland in a decade, with a rate of 1.96 confirmed anthrax infections per 1,000 heroin users in Scotland [8], overall *B. anthracis* is an uncommon cause of soft tissue infection among IDUs and other more common pathogens as staphylococci, streptococci and anaerobes [31] must be considered first in the differential diagnosis and management of patients. Nevertheless, injectional anthrax should be considered in the differential diagnosis of a heroin user presenting with severe soft tissue infection at an injection site, with a progressive course, that does not respond to customary antimicrobial agents aimed to treat most common pathogens causing soft tissue infections, especially in the context of epidemiological link to other injectional anthrax cases in the area.

Another unprecedented outbreak among injecting heroin users took place predominantly in Scotland between April and August 2000. This was mainly attributed to *C. novyi* type A infection, manifesting with a very similar presentation to injectional anthrax i.e. marked swelling and pain at the intramuscular injection site and/or multi-organ failure, presenting within 10 days of injection. As opposed to injectional anthrax infection, this outbreak had a higher case-fatality rate, of 87% (20 of 23 definite cases), and a higher number of cases had necrotising fasciitis (15/23) and pleural effusion (13/23). One of its characteristic features was a leukaemoid reaction – a markedly raised white cell count with left shift and a median white cell count of 60X109/L (normal range ~ 3.54–9.06x109/L), which is not a common feature of injectional anthrax [33].

Both *C. novyi* and *B. anthracis* outbreaks have occurred in clusters, which were attributed to specific batches of contaminated heroin [34], both outbreaks necessitated comprehensive measures of containment.

During the 2009—2010 outbreak informative materials were distributed to drug addicts, recommending drug users to stop using heroin and to ask for medical help in case of symptoms. Clinicians were alerted and the police reinforced its attempts to interrupt the heroin distribution networks [8]. The reoccurrence of 15 injectional anthrax cases since June 2012, most likely caused by the same *B. anthracis* strain as the 2009–2010 outbreak [13-15,25,27] affirmed that contaminated heroin probably still circulates in at least

Table 4

Comparison of clinical presentation, clinical course, diagnosis, treatment and outcome of cutaneous versus injectional anthrax cases, Europe, 2000–2013

	Cutaneous anthrax	Injectional anthrax[a]
Classical presentation	Papule progresses to a vesicle and to a black eschar. Painless lesion without purulence +/- marked non-pitting edema. Numerous bacilli, paucity of leukocytes on staining [1,2].	Soft tissue infection with marked edema, painless at times, without pus, scarce leukocytes on staining. No eschar. Changes are attributed to bacterial virulence factors including edema and lethal toxins (Table 3).
Mortality rate	<1% when treated, 10-20% without treatment [1,2].	37% (26/70 confirmed cases), higher rate of septicaemia (Table 1).
Diagnosis	Gram stain and culture from lesion usually sufficient [1,2] [b]	Mainly Gram stain, culture and PCR confirmation from blood, tissue, pus or other body fluid. Other methods included silver and immunohistochemical staining, serum samples for toxin levels, MALDI TOF MS and serology testing (Table 3).
Antibiotic regimen	Naturally acquired cutaneous anthrax: For localised, uncomplicated cases oral ciprofloxacin or doxycycline for seven to 10 days. For severe cases with signs of systemic involvement, extensive edema, or lesions of head and neck – i.v. therapy for 7–10 days [29, 35].	**Soft tissue infection** – i.v. combination of five antimicrobial agents to cover both *B. anthracis*, and other more common causes of severe soft tissue infections: ciprofloxacin, clindamycin, penicillin, flucloxacillin, metronidazole. **Systemic anthrax** without soft tissue infection - therapy as inhalational anthrax with potential CNS involvement: i.v. ciprofloxacin and clindamycin with at least one other active drug against *B. anthracis* - penicillin or vancomycin (in patients allergic to penicillin) [36].[c]
Additional treatment	No information.	Exploration surgeries and tissue debridement for the removal of toxin reserve and devitalised tissue. Fasciotomy for compartment syndrome. i.v. AIG when indicated for confirmed or probable case (positive Gram stain) [36].

AIG: anthrax immunoglobulin; CNS: central nervous system; i.v.: intravenous; MALDI TOF MS - Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry.

[a] More comparable with systemic anthrax regarding fatality rate, diagnosis and antimicrobial treatment.

[b] Diagnostic procedures should preferably be performed prior to initiation of antibiotics, as vesicular fluid and dermal tissue are quickly sterilized after initiation of antibiotics [1].

[c] Other agents with anti-anthrax activity include rifampicin, imipemem, meropenem, chloramphenicol and gentamicin. For the duration of therapy – refer to text. Ciprofloxacin is the preferred choice over doxycycline in cases of severe disease. Clindamycin is included for its bacterial toxin synthesis inhibition ability and the third antimicrobial drug is included for its adequate activity against *B. anthracis* and CNS penetrance properties [29,35,36].

six European countries and further appearance of new injectional anthrax cases might be expected.

Injectional anthrax presents a challenge for physicians often due to lack of evident case clusters, unfamiliar clinical presentation and severe course of disease. Unlike cutaneous anthrax, injectional anthrax is typically a systemic infection with high mortality rate (Table 4). This may be attributed to the deeper and greater inoculation of spores, higher rates of septicaemia, delayed diagnosis and to factors specific to drug addicts including delayed medical consultation, malnutrition, presence of concomitant diseases such as HIV infection and defective immune response. The weight of these contributing factors could not be assessed in the absence of a detailed database and a lack of a control group exposed to the pathogen through the same route.

The predominantly systemic nature of injectional anthrax requires different management than cutaneous anthrax. In cases of naturally occurring cutaneous anthrax, oral antibiotic treatment with fluoroquinolones (ciprofloxacin, levofloxacin, and moxifl\oxacin) or doxycycline is usually sufficient [29, 35]. However, the progressive clinical course of injectional anthrax, frequent systemic involvement and high fatality rate necessitates early and aggressive management. Most reported cases with soft tissue infection required surgical procedures such as debridement or fasciotomy in cases of compartment syndrome. These interventions were made in addition to i.v. antibiotic therapy, adjuvant treatment with i.v. AIG in some cases and supportive critical care management [6,15,17-20].

In March 2010 the HPS published interim clinical guidance for the management of suspected anthrax in drug users [36]. In accordance to it, the appropriate management of cases with soft tissue infection should include i.v. application of a combination of antimicrobial agents. As for the duration of treatment, the guidance suggest reviewing antimicrobial therapy after ten

to 14 days of i.v. therapy, and either continuation of therapy, modification of agent or its route of administration, or discontinuation of therapy, depending on the clinical course of the individual patient. When therapy has ceased, patients must be monitored closely for worsening of symptoms necessitating renewal of therapy and re-evaluation of the need for further surgical debridement [36].

According to the US CDC guidelines for anthrax post-exposure prophylaxis and treatment, systemic illness cases that were exposed to aerosolised spores should be treated for 60 days due to the potential for delayed germination of *B. anthracis* spores [29,35]. Inhalational exposure to aerosolised spores should be considered in cases of smoking or snorting of contaminated heroin.

Complementary treatment with corticosteroids may be beneficial for patients with edema, especially of the head or neck, with evidence of anthrax meningitis or of vasopressor-resistant shock [29].

Due to insufficient clinical data there are no general recommendations for the use of i.v. AIG for the treatment of severe anthrax infections. The US CDC offers AIG under emergency IND protocol on a patient by patient basis [29,35]. The HPS interim clinical guidance recommends the consideration of i.v. AIG if the patient fits the clinical definition and is a confirmed or a probable case based on a Gram stain demonstrating Gram-positive bacilli [36].

The main constraint of this review arises from the incomplete information, a limited number of case reports and the variable degree of clinical description that do not allow additional analysis or prognostic indicators assessment regarding clinical presentation and recommended management. However, this review of injectional anthrax cases aspires to serve as a source of knowledge on this recently described and distinctive infection, in order to encourage early recognition, diagnosis and management of future cases and allow further evaluation of diagnostic methods and management of this important pathogen.

Conclusion

The erratic emergence and severe clinical course of injectional anthrax present a challenge for clinicians. The optimal management of injectional anthrax necessitates a high index of suspicion and prompt treatment. Clinicians should be extra vigilant when examining a heroin user presenting with either severe soft tissue infection at an injection site, signs of severe sepsis or meningitis with or without evidence of soft tissue infection, evidence of intracranial bleeding on a computed tomography scan or symptoms of inhalational anthrax. It is important to be aware of the progressive clinical course of disease. When suspected, diagnostic tests should be performed and appropriate antibiotic treatment be commenced promptly along with surgical debridement and critical care management when indicated.

Conflict of interest

None declared

Authors' contributions

Tamar Berger contributed to the literature search, data collection, analysis and interpretation and writing of the first draft.
Adi Avniel Aran and Michael Kassirer contributed to the data interpretation and writing of the first draft.

References

1. Martin GJ, Friedlander AM. Bacillus anthracis (Anthrax). In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 7th ed. Philadelphia: Elsevier Inc. 2010. Chapter 208. p. 2715–2725. http://dx.doi.org/10.1016/B978-0-443-06839-3.00208-3
2. Purcell BK, Worsham PL, Friedlander AM. Anthrax. In: Dembek ZF, editor. Medical Aspects of Biological Warfare. Washington, DC: Office of the Surgeon General, US Army Medical Department Center and School; Borden Institute, Walter Reed Army Medical Center; 2007. p. 69–90.
3. European Centre for Disease Prevention and Control (ECDC). Annual Epidemiological Report 2011. Reporting on 2009 surveillance data and 2010 epidemic intelligence data. Stockholm: ECDC; 2011. Available from: http://www.ecdc.europa.eu/en/publications/publications/1111_sur_annual_epidemiological_report_on_communicable_diseases_in_europe.pdf
4. Ringertz SH, Høiby EA, Jensenius M, Maehlen J, Caugant DA, Myklebust A, et al. Injectional anthrax in a heroin skin-popper. Lancet. 2000; 356(9241):1574–5. http://dx.doi.org/10.1016/S0140-6736(00)03133-0
5. Ramsay CN, Stirling A, Smith J, Hawkins G, Brooks T, Hood J, et al. An outbreak of infection with Bacillus anthracis in injecting drug users in Scotland. Euro Surveill. 2010;15(2): pii= 19465.
6. Radun D, Bernard H, Altmann M, Schöneberg I, Bochat V, van Treeck U, et al. Preliminary case report of fatal anthrax in an injecting drug user in North-Rhine-Westphalia, Germany, December 2009. Euro Surveill. 2010;15(2):pii=19464.
7. Christie B. Heroin contaminated with anthrax has killed 11 people. BMJ. 2010;340:c937. http://dx.doi.org/10.1136/bmj.c937
8. Health Protection Scotland (HPS). National Anthrax Outbreak Control Team: An outbreak of anthrax among drug users in Scotland, December 2009 to December 2010. Glasgow: HPS; 2011.
9. Public Health England website - Health Protection topics – Anthrax: Information on 2012 outbreak. [Accessed 26 January 2014]. Available from: http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/Anthrax/
10. ProMED-mail. [Accessed 26 Jan 2014]. Available from: http://www.promedmail.org/
11. European Centre for Disease Prevention and Control (ECDC). [Accessed 26 Jan 2014]. Available from: http://www.ecdc.europa.eu/en/Pages/home.aspx
12. Palmateer NE, Ramsay CN, Browning L, Goldberg DJ, Hutchinson SJ. Anthrax infection among heroin users in Scotland during 2009-2010: a case-control study by linkage to a national drug treatment database. Clin Infect Dis. 2012;55(5):706–10. http://dx.doi.org/10.1093/cid/cis511
13. Price EP, Seymour ML, Sarovich DS, Latham J, Wolken SR, Mason J, et al. Molecular epidemiologic investigation of an anthrax outbreak among heroin users, Europe. Emerg Infect Dis. 2012;18(8):1307–13. http://dx.doi.org/10.3201/eid1808.111343
14. Holzmann T, Frangoulidis D, Simon M, Noll P, Schmoldt S, Hanczaruk M, et al. Fatal anthrax infection in a heroin user from southern Germany, June 2012. Euro Surveill. 2012;17(26): pii=20204
15. Grunow R, Verbeek L, Jacob D, Holzmann T, Birkenfeld G, Wiens D, et al. Injection anthrax-a new outbreak in heroin users. Dtsch Arztebl Int. 2012;109(49):843–8.

16. Beaumont G. Anthrax in a Scottish intravenous drug user. J Forensic Leg Med. 2010;17(8):443–5. http://dx.doi.org/10.1016/j.jflm.2010.09.008
17. Parcell BJ, Wilmshurst AD, France AJ, Motta L, Brooks T, Olver WJ. Injection anthrax causing compartment syndrome and necrotising fasciitis. J Clin Pathol. 2011;64(1):95–6. http://dx.doi.org/10.1136/jcp.2010.082586
18. Powell AG, Crozier JE, Hodgson H, Galloway DJ. A case of septicaemic anthrax in an intravenous drug user. BMC Infect Dis. 2011;11:21. http://dx.doi.org/10.1186/1471-2334-11-21
19. Jallali N, Hettiaratchy S, Gordon AC, Jain A. The surgical management of injectional anthrax. J Plast Reconstr Aesthet Surg. 2011 Feb; 64(2):276–7. http://dx.doi.org/10.1016/j.bjps.2010.06.003
20. Knox D, Murray G, Millar M, Hamilton D, Connor M, Ferdinand RD, et al. Subcutaneous anthrax in three intravenous drug users. J Bone Joint Surg Br. 2011;93(3):414–7. http://dx.doi.org/10.1302/0301-620X.93B3.25976
21. Johns N, Cooper D, Terrace J. An unusual case of peritonitis in an intravenous drug user. Gastroenterology. 2011;141(2):435–6, 780–1. http://dx.doi.org/10.1053/j.gastro.2011.02.076
22. Meghji S, Judd O, Carr E. Fatal cutaneous anthrax in a heroin user. J Laryngol Otol. 2013;127(4):423-5. http://dx.doi.org/10.1017/S0022215112003210
23. Bannard-Smith J, Carroll I, Nichani R, Sharma R. Anthrax infection in an intravenous drug user. Intensive Care Med. 2013;39(3):530. http://dx.doi.org/10.1007/s00134-012-2772-0
24. Booth MG, Hood J, Brooks TJ, Hart A. Anthrax infection in drug users. Lancet 2011;375(9723):1345–1346. http://dx.doi.org/10.1016/S0140-6736(10)60573-9
25. Russell L, Pedersen M, Jensen AV, Sřes LM, Hansen AB. Two anthrax cases with soft tissue infection, severe oedema and sepsis in Danish heroin users. BMC Infect Dis. 2013;13:408. http://dx.doi.org/10.1186/1471-2334-13-408
26. European Centre for Disease Prevention and Control (ECDC). Risk assessment: Anthrax cases among injecting drug users Germany, June-July 2012 - Update, 13 July 2012. [Accessed 6 Feb 2013]. Available from: http://ecdc.europa.eu/en/publications/publications/rapid-risk-assessent-anthrax-idus-july-13.pdf
27. Grunow R, Klee SR, Beyer W, George M, Grunow D, Barduhn A, et al. Anthrax among heroin users in Europe possibly caused by same Bacillus anthracis strain since 2000. Euro Surveill. 2013 Mar 28;18(13): pii=20437
28. Rückert C, Licht K, Kalinowski J, Espírito Santo C, Antwerpen M, Hanczaruk M et al. Draft genome sequence of Bacillus anthracis UR-1, isolated from a German heroin user. J Bacteriol. 2012;194(21):5997-8. http://dx.doi.org/10.1128/JB.01410-12
29. Hendricks KA, Wright ME, Shadomy SV, Bradley JS, Morrow MG, Pavia AT et al. Centers for disease control and prevention expert panel meetings on prevention and treatment of anthrax in adults. Emerg Infect Dis. 2014;20(2). http://dx.doi.org/10.3201/eid2002.130687
30. Kaushik KS, Kapila K, Praharaj AK. Shooting up: the interface of microbial infections and drug abuse. J Med Microbiol. 2011;60 (Pt 4):408–22. http://dx.doi.org/10.1099/jmm.0.027540-0
31. Lavender TW, McCarron B. Acute infections in intravenous drug users. Clin Med. 2013;13(5):511-3. http://dx.doi.org/10.7861/clinmedicine.13-5-511
32. Hope VD, Palmateer N, Wiessing L, Marongiu A, White J, Ncube F et al. A decade of spore-forming bacterial infections among European injecting drug users: pronounced regional variation. Am J Public Health. 2012;102(1):122-5. http://dx.doi.org/10.2105/AJPH.2011.300314
33. McGuigan CC, Penrice GM, Gruer L, Ahmed S, Goldberg D, Black M et al. Lethal outbreak of infection with Clostridium novyi type A and other spore-forming organisms in Scottishinjecting drug users. J Med Microbiol. 2002;51(11):971-7.
34. Palmateer NE, Hope VD, Roy K, Marongiu A, White JM, Grant KA et al. Infections with spore-forming bacteria in persons who inject drugs, 2000-2009. Emerg Infect Dis. 2013;19 (1):29-34. http://dx.doi.org/10.3201/eid1901.120044
35. Stern EJ, Uhde KB, Shadomy SV, Messonnier N. Conference Report on Public Health and Clinical Guidelines for Anthrax. Emerg Infect Dis. 2008;14(4). http://dx.doi.org/10.3201/eid1404.070969
36. Health Protection Scotland (HPS). Interim clinical guidance for the management of suspected anthrax in drug users: version 12.1. [Accessed 5 Feb, 2013]. Available from: http://www.hps.scot.nhs.uk/anthrax/documents/clinical-guidance-for-use-of-anthraximmune-globulin-v12-1-2010-03-19.pdf

Increase of pertussis incidence in 2010 to 2012 after 12 years of low circulation in Spain

V Sizaire (vsizaire@isciii.es)[1,2], M Garrido-Estepa[1], J Masa-Calles[1,3], M V Martinez de Aragon[1,3]
1. National Centre for Epidemiology, Institute of Health Carlos III, Madrid, Spain
2. European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden
3. CIBER-Epidemiología y Salud Pública (CIBERESP), Institute of Health Carlos III, Madrid, Spain

Citation style for this article:
Sizaire V, Garrido-Estepa M, Masa-Calles J, Martinez de Aragon MV. Increase of pertussis incidence in 2010 to 2012 after 12 years of low circulation in Spain. Euro Surveill. 2014;19(32):pii=20875. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20875

Article submitted on 08 May 2013 / published on 14 August 2014

In Spain, whole cell pertussis vaccination started in 1975, with three doses before the age of 6–7 months. Doses at 15–18 months and 4–6 years were introduced in 1996 and 2001, respectively. Spain switched to an acellular vaccine in 2005. From 1998 to 2009, pertussis incidence rates remained ≤1.5 cases/100,000 inhabitants but increased from 2010 to 7.5 cases/100,000 in 2012. Data from 1998 to 2012 were analysed to assess disease trends and susceptible populations. We defined four epidemic periods: 1998–2001 (reference), 2002–05, 2006–09 and 2010–12. In 2002–05, the incidence rate increased in individuals aged 15–49 years (IRR: 1.41 (95% CI: 1.11–1.78)) and ≥50 years (IRR: 2.78 (95% CI: 1.78–4.33)) and in 2006–09 increased also in infants aged <3 months (IRR: 1.83 (95% CI: 1.60–2.09)). In 2010–12, the incidence rate increased notably in all age groups, with IRRs ranging between 2.5 (95% CI: 2.3–2.8) in 5–9 year-olds and 36.0 (95% CI: 19.4–66.8) in 20–29 year-olds. These results, consistent with the country's vaccination history, suggest a progressive accumulation of susceptible individuals due to waning immunity after years of low incidence. Further vaccination strategies should be assessed and implemented to prevent pertussis in pre-vaccinated infants, in whom the disease is more severe.

Introduction

Pertussis, commonly known as whooping cough, is a highly transmissible respiratory infectious disease caused by *Bordetella pertussis*. Worldwide, it is estimated that there are 30–50 million pertussis cases and 300,000–400,000 deaths due to the disease per year [1,2]. In non-vaccinated populations, pertussis occurs seasonally, with variations in time and place, displaying epidemic cycles of two to five years [3]. The epidemic cycles continue to be observed even when high vaccination coverage (≥90% coverage with three doses in infants [4]) is achieved, possibly because pertussis vaccination can protect well against severe forms of disease but not so well against infection [5-8]. Although a dramatic decrease in incidence occurs after the introduction of vaccination against pertussis, elimination of the disease has never been achieved, even in countries with decades of high vaccination coverage [3,9,10].

Recently, a resurgence of the disease has been described in several countries with high vaccine coverage, in North America (the United States [11,12] and Canada [10]) and in Europe (e.g. the Netherlands [13], Norway [14], Germany [15], the United Kingdom [16,17] and Slovenia [18]).
Pertussis resurgence in a context of sustained high vaccination coverage may be explained by the following: (i) better diagnosis and reporting, especially of patients with milder and/or non-specific forms of the disease, in whom notification has been considered underestimated [19]; (ii) waning of natural and vaccine-induced immunity [19]; (iii) loss of vaccine effectiveness due to an antigenic shift of *B. pertussis* strains [13]; and (iv) lower vaccine effectiveness of acellular pertussis vaccine (aP) compared with wP [11,20] and less sustainable immunity, at least when aP is administered to preschool children (aged 0–6 years) [9,21,22].

In Spain, the whole cell vaccine against pertussis combined with diphtheria and tetanus toxoids (wP) was commercialised in the 1960s and administered in two annual campaigns to infants (aged <1 year). In 1975, wP was included in the national childhood immunisation schedule, with three doses (at 3, 5 and 7 months of age). In 1996, the vaccination schedule was changed, reducing the age of administration of the three doses, to 2, 4 and 6 months of age, and included an additional fourth dose at 15–18 months of age. In 2001, a fifth dose was added to the vaccination schedule, at 4–6 years of age. In 2005, wP vaccine was replaced by a three-component aP.

Notification by general practitioners of the weekly number of pertussis cases to the national surveillance system has been mandatory in Spain since 1982. In 1996, case-based reporting was implemented, with inclusion of individual basic epidemiological data (sex, age, case classification and history of vaccination) and

became fully functional in 1998. Pertussis notification remains based on a standard clinical case definition according to criteria of the World Health Organization (WHO) [23] and European Centre for Disease Prevention and Control (ECDC) [24]. A pertussis case was defined as any person with cough lasting at least two weeks, in the absence of another apparent cause, with at least one of the following symptoms: paroxysms of coughing, inspiratory stridor or convulsive post-tussive vomiting. Cases were classified as: (i) suspected – i.e. a person with clinical criteria; (ii) confirmed by an epidemiological link – i.e. a person with clinical criteria and an epidemiological link to a laboratory-confirmed case; and (iii) laboratory confirmed – isolation of *B. pertussis* from a clinical specimen or *B. pertussis* nucleic acid detected by polymerase chain reaction (PCR) or *B. pertussis*-specific antibody response.

Vaccination coverage in Spain increased progressively during 1982 to 2012 and since 1998 has remained above 95% for the three basic doses [25]. Vaccination coverage for the four-dose schedule has been between 93% and 95% since 1996, and for the five-dose schedule has remained around 80–90% since 2001 [25].

After 12 years of low incidence (between 0.8 and 1.5 per 100,000 inhabitants annually from 1998 to 2009 [26]) except for a peak in 2000, when the rate increased to 2.3/100,000, reported data showed an increase of pertussis incidence during 2010 to 2012 (from 1.9 to 7.5 per 100,000 inhabitants annually). We present here results of pertussis epidemiology in Spain from 1998 to 2012, to describe incidence overall and by age and hospitalisation trends and to identify the susceptible age groups most affected by the increased incidence. This should help to understand the resurgence of the disease in a context of sustained high vaccination coverage in Europe and provide health authorities with information that will help to define the best vaccination policies.

Methods

To analyse the general trend of pertussis incidence in Spain in parallel with three-dose vaccine coverage, data on incident cases were obtained from the National Epidemiological Surveillance Network, from 1982 to 1997 as weekly aggregated data and from 1998 onwards as case-based data and data on vaccine coverage for the three-doses schedule were obtained from the Ministry of Health, Social Services and Equality [27]. Since fully functioning case-base notification in 1998, cases without information on age were attributed to the different age groups, assuming a similar distribution by age group for cases with known age in the same year, although from 2005 onwards, only 0.4% of cases (40/9,960) were reported without age information.

Hospitalisation data since 1998, including patient age and length of stay, were available from the national registry of hospitalisations of the Ministry of Health. The cause of hospitalisation was considered to be pertussis when the ninth *International classification of diseases* [28] code 033 was recorded by the hospital as the main diagnosis. Data on mortality due to pertussis were obtained from the national mortality registry of the National Institute of Statistics, as were annual population data, used as denominator. Data on national vaccination coverage within the Spanish national immunisation programme since 1982 were available from the Ministry of Health [27].

We included all notified cases reported to the National Epidemiological Surveillance Network in our analysis, including suspected cases, in order to analyse the overall changes in pertussis incidence. Suspected cases were included because the proportion of cases confirmed by the laboratory or an epidemiological link remained constant during our study period (1998–2012).

We describe here the overall annual pertussis incidence rates, number of deaths due to pertussis and annual hospitalisation rates due to pertussis from 1998 to 2012. To analyse the evolution of the incidence by age, we calculated the annual incidence rates and hospitalisation rate by age group (<1, 1–4, 5–9, 10–14, 15–49 and ≥50 years) from 1998 to 2012. We also assessed changes in incidence in subgroups of notified cases aged less than 1 year (<1, 1, 2, 3–5, 6–8 and 9–11 months).

We defined four periods according to the observed epidemic waves and changes in pertussis vaccination: 1998–2001 (reference period), 2002–05 (following introduction of fifth dose of wP at 4–6 years of age), 2006–09 (following switch to aP for all five vaccine doses) and 2010–12 (pertussis resurgence wave), in order to identify possible changes in pertussis epidemiology related to vaccination changes. Period incidence rate ratios (IRRs) and hospitalisation rate ratios (HRRs) were calculated by Poisson regression, taking the 1998–2001 epidemic wave as reference, and expressed as the ratio between the period rates (overall or by age group) and the rate for 1998–2001 in the same group. IRRs or HRRs >1 ($p<0.05$) were considered as statistically significant increases in the rate and IRRs or HRRs <1 ($p<0.05$) as significant decreases. Confidence intervals were calculated using a Poisson distribution. All statistical analyses were performed using Stata version 12.

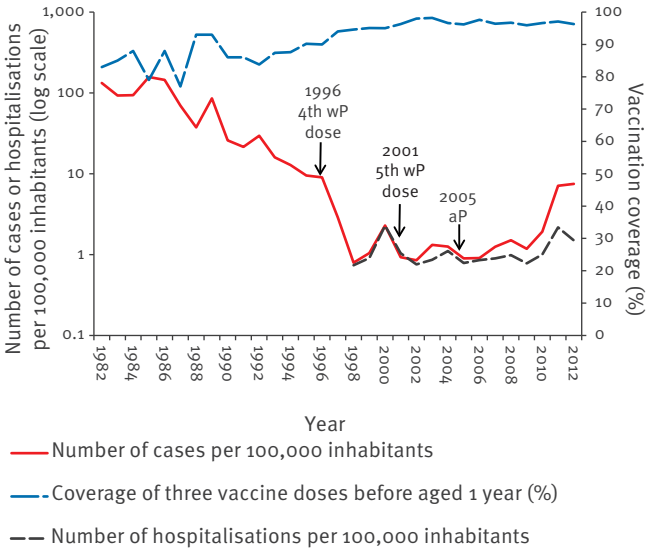
Results

Pertussis annual incidence rates and hospitalisation rates

Annual vaccination coverage (for the three-dose schedule) and pertussis incidence rates and pertussis hospitalisation rates from 1998 to 2012 are shown, along with those from 1982 to 1997 (Figure 1), 1982 being the start of mandatory notification.

Figure 1

Pertussis incidence rates, hospitalisation rates and vaccine coverage, Spain, 1982–2012



aP: acellular vaccine against pertussis combined with diphtheria and tetanus toxoids. wP; whole cell vaccine against pertussis combined with diphtheria and tetanus toxoids.

Source:

Cases: National Epidemiological Surveillance Network: National Centre for Epidemiology, Institute of Health Carlos III.

Hospitalisations and vaccine coverage: Ministry of Health, Social Services and Equality.

Pertussis incidence decreased significantly from the mid-1980s to the end of the 1990s ($p<0.001$) and the decrease was more pronounced after the inclusion of the fourth wP dose in 1996 (Figure 1).

From 1982 to 1997, the mean annual incidence was 58.9 cases/100,000 inhabitants (standard deviation (SD): 13.1). From 1998 to 2009, except for a peak in 2000 (2.3/100,000), the incidence remained between 0.79 and 1.5 cases/100,000. However, after 2009, the incidence started to increase, with 1.92 cases/100,000 in 2010 and reached 7.5 cases/100,000 in 2012.

The mean pertussis hospitalisation rate during the study period was 1.11/100,000 inhabitants (SD: 0.14), ranging between 0.74 and 2.28/100,000. Similar to incidence rates, after a peak observed in 2000 (2.28/100,000), the rate remained between 0.9 and 1.5/100,000 until 2009, when it began to increase, reaching another peak in 2011 (2.2/100,000), with a lower rate in 2012 (1.5/100,000).

Although the hospitalisation and incidence rates followed the same pattern from 1998 to 2012, the incidence rates were slightly higher than the hospitalisation rates between 1998 and 2006 (incidence rate/hospitalisation rate: 1.10; $p<0.001$), but after 2006, the increases in incidence rates were higher than those in hospitalisation rates (incidence rate/hospitalisation rate: 2.60; $p<0.001$).

Pertussis incidence rates by age group

The increase in pertussis incidence seen during 2010 to 2012 affected all age groups. From 1998 to 2012, incidence rates increased from 33.4/100,000 in infants (aged <1 year) in 1998 to 177.7/100,000 in 2012, with a peak of 214.0/100,000 in 2011; from 5.6 to 39.0 cases/100,000 children aged 1–4 years; from 2.3 to 21.2 cases/100,000 children aged 5–9 years; from 2.3 to 19.3/100,000 children aged 10–14 years; and from 0.04–2.27/100,000 individuals aged ≥15 years (Figure 2).

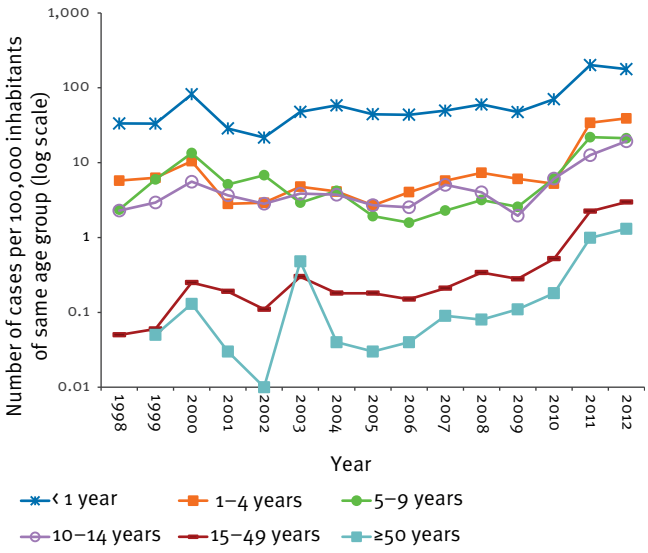
Among infants, a similar increasing trend was observed in all ages, but was particularly clear in children aged <1, 1 and 2 months of age, i.e. children too young to be vaccinated. Between 1998 and 2012, the incidence increased from 14.5 to 706.1/100,000 infants aged <1 month; from 121.3 to 391.8/100,000 infants aged 1 month; from 86.6 to 422.6/100,000 infants aged 2 months; from 24.4 to 140.4/100,000 infants aged 3–5 months; from 14.8 to 30.6/100,000 infants aged 6–8 months; and from 20.5 to 24.6/100,000 infants aged 9–11 months (Figure 3).

Pertussis hospitalisation rates by age group

From 1998 to 2009, the annual rate of pertussis hospitalisation tended to decrease in all age groups, except in infants aged <1 year, in whom it remained essentially stable (Figure 4), but after 2009, an

Figure 2

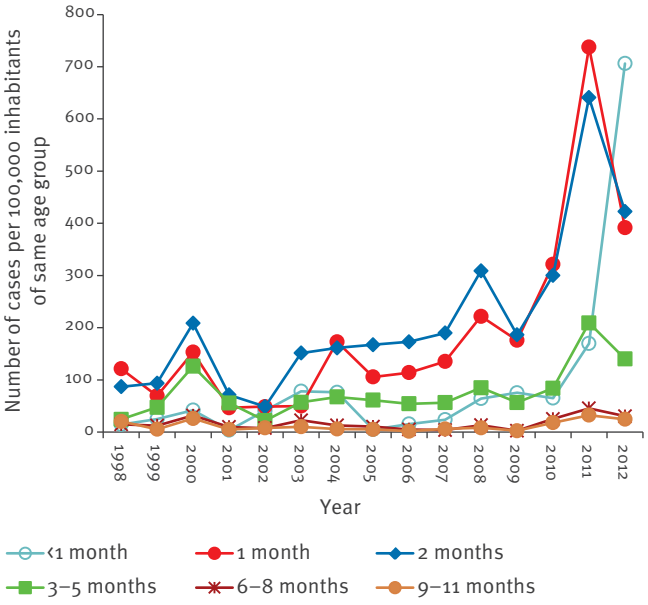
Pertussis annual incidence rates by age group, Spain, 1998–2012



Source: National Epidemiological Surveillance Network.

Figure 3

Pertussis annual incidence rates in infants aged <1 year by age subgroup, Spain, 1998–2012



Source: National Epidemiological Surveillance Network.

increase was observed in infants aged <1 year, with a peak in 2011 of 378 hospitalisations/100,000 infants. In 2012, the hospitalisation rate decreased to 131.4 hospitalisations/100,000.

Among infants, annual pertussis hospitalisation rates peaked in 2000 and showed no clear changes in the rates until 2010, when rates started to increase, especially in infants of 1 month of age (in 2011, 923.0/100,000), followed by that in those aged 2 months (in 2011, 658.5/100,000) and <1 month (in 2011, 218.8/100,000) (Figure 5). However in 2012, the hospitalisation rate decreased in these age subgroups (625.3, 434.2/100,000 and 143.7, respectively).

Pertussis incidence and hospitalisation rate ratios during the epidemic waves

Taking the 1998–2001 epidemic wave as the reference period, we evaluated changes in the incidence and hospitalisation rate ratios in the three successive epidemic waves in 2002–05, 2006–09 and 2010–12 (Tables 1 and 2).

The overall IRR of the disease was <1 during 2002–05 and 2006–09 ($p<0.001$). In 2010–12, the overall IRR was 4.3 ($p<0.001$). The overall HRR followed the same pattern as the IRR, with a HRR <1 during 2002–05 and 2006–09; in 2010–12, it was 1.2 ($p<0.001$) (Table 1).

Pertussis incidence and hospitalisation rate ratios by age group

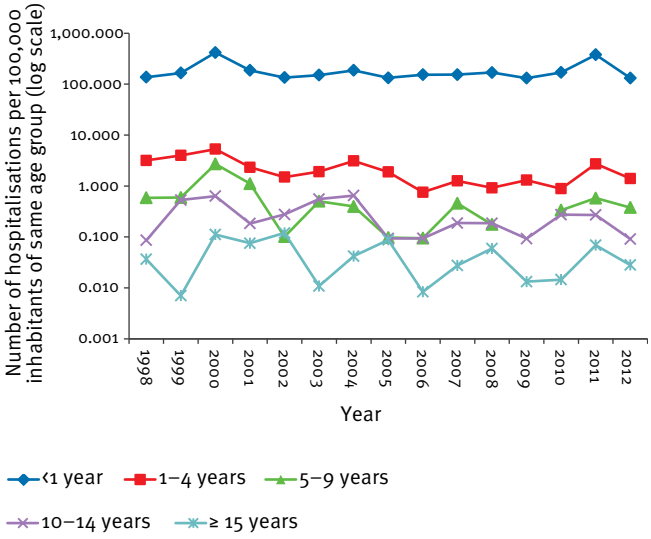
During 2002 to 2005, children aged 1–4 years (IRR: 0.58, $p<0.001$) and 5–9 years (IRR: 0.59, $p<0.001$) showed a significant decrease in the incidence rate (compared with the reference period). In infants aged under <1 year and children aged between 10–14 years, there was a decrease in the incidence rate, but this was not statistically significant. However, individuals aged ≥15 years had a higher incidence rate, when compared with 1998 to 2001. During 2002 to 2005, the hospitalisation rate decreased in those aged <1 year , 1–4 years and especially in the 5–9 year-olds, for whom rates of hospitalisation were reduced nearly by 80%, while those aged over 9 years had a higher rate of hospitalisation (Table 1).

In 2006 to 2009, after the switch to aP, the overall IRR was similar to that of the previous epidemic wave (2002–05). In children aged 5–9 years and in adults ≥50 years-old, the IRR was lower than in the preceding wave, while in infants aged <1 year, children aged 1–4 years and individuals aged 15–49 years, the IRR increased. On the other hand, the HRR for all age groups was lower than that in 2002–2005, except for infants aged under <1 year, in whom the HRR remained similar to that of the previous wave (Table 1).

In 2010 to 2012, an increase in incidence was seen in all age groups: the IRR varied between 2.5 and 5.7 in the age groups between 0 and 19 years; in individuals aged 15–49 years, the IRR was 14 and 17 in adults aged ≥50 years. During 2010 to 2012, in contrast to IRRs,

Figure 4

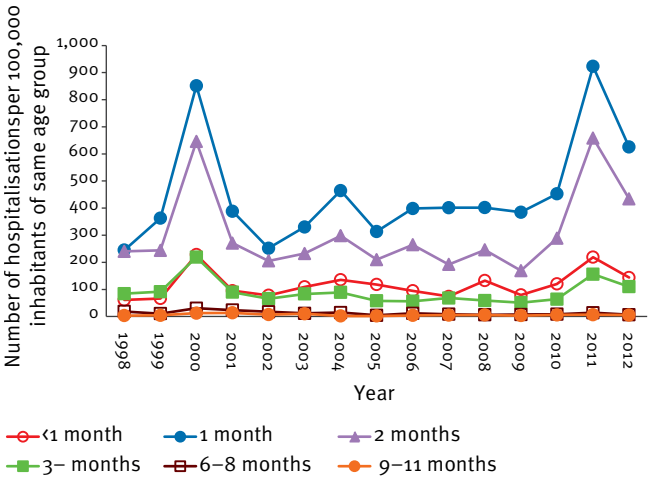
Pertussis hopitalisation rates per 100,000 inhabitants by age group, Spain, 1998–2012



Source: National Epidemiological Surveillance Network.

Figure 5

Pertussis hopitalisation rates in infants aged <1 year by age subgroup, Spain, 1998–2012



Source: National Epidemiological Surveillance Network.

HRRs remained below 1 (p<0.05) in the age groups 1–4, 5–9 and 10–14 years, whereas the HRR was >1 in the age groups <1 year (HRR: 1.16), 15–49 years (HRR: 1.92) and ≥50 years (HRR: 1.24) (Table 1).

Among infants, a statistically significant IRR of >1 (p<0.001) in 2002–05 was seen only in those aged <1 month; in 2006–09, the IRR was >1 (p<0.001) in those at age of pre-vaccination (<1, 1 and 2 months). In 2010–12, the IRR was >1 for all age subgroups; this was particularly pronounced in those aged <1 month (IRR: 14.8, p<0.001) (Table 2). Overall, the HRR was <1 in 2002–05 and 2006–09 and although it became >1 (p<0.001) in 2010–12 (Table 1), this increase affected only infants who had yet not completed the first three doses (i.e. those aged <3 months; IRR: 1.83 (95% CI: 1.60–2.09)), 73% (1,556/2,136) of all hospitalisations between 2010 and 2012.

Duration of hospital stay

Data from the national registry of hospitalisations showed that the mean hospitalisation stay was 7.9 days (SD: 10.2) for all cases from 1998 to 2012. The length of stay was longer (p<0.001) in infants aged <3 months (mean: 8.7; SD: 11.9) than in those aged 3–11 months (mean: 6.5; SD: 5.5) and in individuals ≥1 year of age (mean: 5.7; SD: 4.77).

A significant (p<0.001) decrease in stay was observed over time: in 1998 to 2001, the mean duration of stay was 8.9 days (SD: 7.7) while in 2010–12, it was 6.8 days (SD: 6.1)

Pertussis-related mortality from 1982 to 2012

Data from the national mortality registry of the National Institute of Statistics showed that from 1982 to 1987, between one and four deaths due to pertussis were observed every year. From 1988 to 1996, there were no pertussis-related deaths, but in 1997, pertussis-related deaths reappeared and increased from 1–2 to 3–5 annual deaths between 2007 and 2010. In 2011, there were eight pertussis-related deaths and six in 2012. Since 1997, all deaths but one (in a person aged 41 years) were reported in infants younger than 3 months.

Discussion

Our results show that in Spain, pertussis incidence decreased from 133.2/100,000 in 1982 to 0.79/100,000 inhabitants in 1998 after reaching high vaccination

Table 1

Pertussis incidence and hospitalisation rate ratios by age group, Spain, 2002–12[a]

Age group in years	Incidence rate ratio[a] (95% CI)			Hospitalisation rate ratio[a,b] (95% CI)		
	2002–05	2006–09	2010–12	2002–05	2006–09	2010–12
<1	0.98 (0.88–1.08)	1.13 (1.03–1.25)	3.35 (3.07–3.65)	0.70 (0.65–0.75)	0.68 (0.63–0.72)	1.16 (1.09–1.23)
1–4	0.58 (0.49–0.68)	0.92 (0.80–1.06)	4.25 (3.79–4.75)	0.60 (0.45–0.81)	0.29 (0.20–0.41)	0.56 (0.41–0.76)
5–9	0.59 (0.51–0.68)	0.36 (0.31–0.42)	2.51 (2.27–2.79)	0.23 (0.13– 0.44)	0.14 (0.07–0.30)	0.44 (0.26–0.73)
10–14	0.91 (0.78–1.07)	0.94 (0.80–1.10)	3.60 (3.16–4.10)	1.43 (0.75–2.72)	0.39 (0.15–1.01)	0.67 (0.28–1.57)
15–19	0.63 (0.38–1.03)	1.38 (0.92–2.07)	5.65 (4.03–7.92)	–	–	–
20–29	4.81 (2.48–9.35)	4.82 (2.47–9.38)	36.03 (19.42–66.84)			
30–39	1.19 (0.80–1.77)	1.54 (1.06–1.77)	13.16 (9.63–17.98)			
40–49	1.87 (1.09–3.20)	1.91 (1.12–3.23)	21.47 (13.64–33.81)			
15–49	1.41 (1.11–1.78)	1.77 (1.42–2.21)	14.06 (11.63–16.99)	1.76 (0.70–4.42)	1.03 (0.37–2.84)	1.92 (0.75–4.96)
≥50	2.78 (1.78–4.33)	1.57 (0.97–2.53)	16.91 (11.40–25.09)	1.01 (0.47–2.14)	0.53 (0.22–1.28)	1.24 (0.59–2.62)
Total	**0.86 (0.80–0.91)**	**0.81 (0.76–0.86)**	**4.34 (4.13–4.55)**	**0.75 (0.70–0.80)**	**0.72 (0.67–0.77)**	**1.23 (1.16–1.31)**

[a] The reference period was 1998 to 2001.

[b] Hospitalisation rate ratios were not calculated for the age groups 15–19, 20–29, 30–39 and 40–49 years, as the number of hospitalisations in these groups was small. Instead, these age groups were combined, with the hospitalisation rate ratio calculated for individuals aged 15–49 years.

Source: National Epidemiological Surveillance Network.

Table 2

Pertussis incidence and hospitalisation rate ratios in infants aged under 1 year, by age subgroup, Spain, 2002–12[a]

Age group in months	Incidence rate ratio[a] (95% CI)			Hospitalisation rate ratio[a] (95% CI)		
	2002–05	2006–09	2010–12	2002–05	2006–09	2010–12
<1	2.38 (1.54–3.67)	2.17 (1.41–3.34)	14.78 (10.08–21.68)	0.98 (0.79–1.22)	0.83 (0.67–1.04)	1.32 (1.07–1.63)
1	1.00 (0.79–1.27)	1.65 (1.33–2.04)	4.68 (3.85–5.68)	0.75 (0.66–0.84)	0.85 (0.76–0.96)	1.38 (1.24–1.54)
2	1.18 (0.96–1.46)	1.54 (1.27–1.87)	3.45 (2.88–4.13)	0.67 (0.59–0.77)	0.64 (0.56–0.73)	1.23 (1.10–1.40)
3–5	0.83(0.70–0.99)	0.88 (0.75–1.04)	2.07 (1.78–2.40)	0.61 (0.53–0.70)	0.49 (0.42–0.57)	0.89 (0.78–1.02)
6–8	0.80 (0.56–1.13)	0.30 (0.19–0.47)	1.82 (1.34–2.46)	0.58 (0.41–0.83)	0.42 (0.29–0.62)	0.48 (0.32–0.71)
9–11	0.51 (0.33–0.79)	0.26 (0.15–0.44)	1.57 (1.12–2.21)	0.60 (0.35–1.04)	0.54 (0.32–0.93)	0.65 (0.37–1.14)

[a] The reference period was 1998 to 2001.

Source: National Epidemiological Surveillance Network.

coverage against pertussis, and remained around 1.0/100,000, with a cyclical pattern of epidemic waves every three to five years as described in the literature [3]. However, after 12 years with an incidence ≤1.5/100,000 (except for a peak of 2.3/100,000 inhabitants in 2000), there was a significant increase that started in 2010, with 1.92 cases/100,000 and reached 7.5/100,000 in 2012, affecting especially individuals >14 years and infants <3 months of age. Other countries in North America and Europe with consolidated high vaccination coverage have observed similar re-emergence of pertussis [10,13-18,29].

The PCR method, introduced during the mid-1990s, was already widely used by all laboratories and hospitals in Spain by the 2000s. If better diagnosis and increased notification have played a role in the increased incidence, it would probably be due to increased awareness among clinicians. However, the fact that the proportion of cases confirmed by the laboratory or an epidemiological link has remained constant over time, the increase in pertussis-related deaths since 2007 and the different trends by age group compatible with the history of vaccination in Spain observed in our results suggest a real increase in pertussis incidence. As would be expected from the vaccination history, in 2002–05 there was already a decrease in incidence and pertussis-related hospitalisation in infants aged >2 months. We also observed a decrease in incidence in children of 1–4 and 5–9 years of age – more evident in those aged 5–9 years – after the introduction of the fourth and fifth doses in 1996 and 2001. However, in the last period of our study (2010–12), these age groups presented a marked increased incidence as well.

During 1998 to 2012, the highest reported pertussis incidence was observed among infants <1 year-old. However, looking at the trends by age group, reported pertussis incidence started increasing first among individuals aged >19 years during 2002–05, while children aged between 3 months and 19 years continued to be protected. During the last period of our study (2010–2012), although reported incidence increased significantly in all age groups, the most important increase was observed in those aged ≥15 years. This may be explained not only by better diagnosis and improved reporting but also by the progressive accumulation of susceptible individuals, including unvaccinated people who were born before there was high vaccination coverage, and loss of population immunity (natural or acquired by vaccination), after years of low incidence, which reduced the opportunities for boosting immunity with the natural disease [30].

Immunity is known to wane in around 7–20 years after pertussis infection and 5–6 years after immunisation [30]. Regarding post-vaccine waning immunity, among children aged 10–14 years, the increase of pertussis incidence started only in the last period of our study (2010–12) and was less than that observed among the ≥15 year-olds, in contrast to reports from other countries in Europe, where the increase in pertussis incidence has been seen more in the 10–14 years age group [31]. In our study, lower incidence in this age group (compared with that seen in those aged ≥15 years) is probably associated with the fifth-dose effect at the age of 4–6 years.

In addition to those aged ≥15 years, the other group affected by an increase in pertussis incidence during 2006–09 were infants, specifically those too young to have received the first three doses of pertussis vaccine. This can be explained as a consequence of the infants being infected at home by siblings, parents or grandparents [32]. The important increase in pertussis incidence, hospitalisation and mortality among infants is consistent with ongoing transmission of *B. pertussis* in Spain, independent of a possible increase in notification during the last period of our study.

The most recent change in the vaccine calendar was the switch from whole cell to acellular vaccine in 2005. Although aP causes fewer side effects than wP [12], it has been suggested that it has reduced effectiveness compared with wP [11,20]. Moreover, protection of aP does not last as long as that of wP [11,22,33].

Consistent with the characteristics of aP, our data suggest an increasing incidence rate in children born after the switch from wP to aP in 2005 (during 2006–09 compared with 2002–05). This was shown by the fact that the incidence rate started increasing in those aged 1–4 years, already in 2006–09, while in those aged 5–9 years, who had received the five wP doses, the incidence rate was still decreasing. In the subsequent period of our study (2010–12), the IRR became significantly higher in this after-2005 birth cohort, which included both the 1–4 and the 5–9 year-old children. This is also compatible with our observations that in infants not yet vaccinated, both incidence and hospitalisation rates increased while in vaccinated infants and children, the incidence increased in the last period but the hospitalisation rates continued to decrease, because the vaccine would protect more against severe disease than against infection itself [1]. Consistent with that, we also found a reduction in duration of hospitalisation stay over the study period, which may be associated with the occurrence of less severe forms of the disease. Specific studies will be needed in order to verify these results and estimate vaccine effectiveness.

The main limitations of our study were firstly that the vaccination status of the diagnosed patients was not included in the analysis. However, as pertussis vaccination coverage has remained above 95% since 1998 for the three basic doses, vaccination coverage for the four-dose schedule has been between 93% and 95% since 1996, and although coverage for the five-dose schedule has remained around 80–90% since 2001, we can assume that the ratio of vaccinated to non-vaccinated pertussis cases remained constant throughout the study period. However, during 2010 to 2012, a notable increase in incidence was seen even in younger age groups with constant vaccination coverage.

Secondly, only some diagnosed patients were laboratory confirmed. However, the proportion of laboratory-confirmed cases remained stable since 2005, so we do not consider that the increased incidence was a result of increased laboratory confirmation. Additionally, because laboratory-confirmed cases are more comparable with hospitalised cases, inclusion of all cases in our study allowed us to describe changes in the overall pertussis incidence rate and this probably would be biased if using only laboratory-confirmed cases had been included.

In conclusion, the last epidemic wave of pertussis seen in 2010–12 shows an increasing trend of the disease, with its main public health consequences affecting pre-vaccinated infants. This will require further reinforcement of pertussis surveillance in Spain, with an emphasis on infants, and assessment of other targeted vaccination strategies, such as cocooning, consisting of selective immunisation of mothers and close family contacts of newborns, as in Switzerland [34] and a maternal vaccination strategy, consisting of administering pertussis vaccine during pregnancy, as proposed in England [35] and Canada [36] in order to prevent infection in infants. Neonatal immunisation has also been suggested, but the effectiveness of one dose in newborns has been questioned given the possible interference with maternal antibodies and the vulnerability of newborns [37]. Discussions about different strategies are being carried out at national level by the Spanish Inter-territorial Health Council. However, to date, only specific recommendations for outbreak situations have been adopted [31].

Acknowledgements

The authors would like to thank all the epidemiologists and health officers who have participated in pertussis surveillance in the National Epidemiological Surveillance Network.

Conflict of interest

None declared.

Authors' contributions

Vinciane Sizaire: data analysis and writing as main author M. Garrido Estepa: support for the statistical analysis and reviewer. J. Masa Calles: revised drafts. MV Martínez de Aragon: mentoring and writing.

References

1. Centers for Disease Control and Prevention (CDC). Pertussis (whooping cough). Atlanta, GA: CDC. Updated 11 Jun 2012. [Accesssed 10 Apr 2013]. Available from: http://www.cdc.gov/pertussis/countries.html
2. Crowcroft NS, Stein C, Duclos P, Birmingham M. How best to estimate the global burden of pertussis? Lancet Infect Dis. 2003;3(7):413-8. http://dx.doi.org/10.1016/S1473-3099(03)00669-8
3. Crowcroft NS, Pebody RG. Recent developments in pertussis. Lancet. 2006;367(9526):1926-36. http://dx.doi.org/10.1016/S0140-6736(06)68848-X
4. Pertussis vaccines: WHO position paper. Wkly Epidemiol Rec. 2010;85(40):385-400.
5. Cherry JD. Historical review of pertussis and the classical vaccine. J Infect Dis. 1996;174 Suppl 3:S259-63. http://dx.doi.org/10.1093/infdis/174.Supplement_3.S259
6. Deville JG, Cherry JD, Christenson PD, Pineda E, Leach CT, Kuhls TL, et al. Frequency of unrecognized Bordetella pertussis infections in adults. Clin Infect Dis. 1995;21(3):639-42. http://dx.doi.org/10.1093/clinids/21.3.639
7. Gay NJ, Miller E. Pertussis transmission in England and Wales. Lancet. 2000;355(9214):1553-4. http://dx.doi.org/10.1016/S0140-6736(05)74603-1
8. Schmitt-Grohé S, Cherry JD, Heininger U, Uberall MA, Pineda E, Stehr K. Pertussis in German adults. Clin Infect Dis. 1995;21(4):860-6. http://dx.doi.org/10.1093/clinids/21.4.860
9. Clark TA, Messonnier NE, Hadler SC. Pertussis control: time for something new? Trends Microbiol. 2012;20(5):211-3. http://dx.doi.org/10.1016/j.tim.2012.03.003
10. Skowronski DM, De Serres G, MacDonald D, Wu W, Shaw C, Macnabb J, et al. The changing age and seasonal profile of pertussis in Canada. J Infect Dis. 2002;185(10):1448-53. http://dx.doi.org/10.1086/340280
11. Cherry JD. Epidemic pertussis in 2012--the resurgence of a vaccine-preventable disease. N Engl J Med. 2012;367(9):785-7. http://dx.doi.org/10.1056/NEJMp1209051
12. Shapiro ED. Acellular vaccines and resurgence of pertussis. JAMA. 2012;308(20):2149-50. http://dx.doi.org/10.1001/jama.2012.65031
13. de Melker HE, Schellekens JF, Neppelenbroek SE, Mooi FR, Rümke HC, Conyn-van Spaendonck MA. Reemergence of pertussis in the highly vaccinated population of the

Netherlands: observations on surveillance data. Emerg Infect Dis. 2000;6(4):348-57. http://dx.doi.org/10.3201/eid0604.000404

14. Lavine JS, Bjørnstad ON, de Blasio BF, Storsaeter J. Short-lived immunity against pertussis, age-specific routes of transmission, and the utility of a teenage booster vaccine. Vaccine. 2012;30(3):544-51. http://dx.doi.org/10.1016/j.vaccine.2011.11.065
15. Wiese-Posselt M, Hellenbrand W. Changes to the varicella and pertussis immunisation schedule in Germany 2009: background, rationale and implementation. Euro Surveill. 2010;15(16):pii=19548.
16. Amirthalingam G, Gupta S, Campbell H. Pertussis immunisation and control in England and Wales, 1957 to 2012: a historical review. Euro Surveill. 2013;18(38):pii=20587.
17. Riolo MA, King AA, Rohani P. Can vaccine legacy explain the British pertussis resurgence? Vaccine. 2013;31(49):5903-8. http://dx.doi.org/10.1016/j.vaccine.2013.09.020
18. Grgic-Vitek M, Klavs I, Kraigher A. Re-emergence of pertussis in Slovenia: time to change immunization policy. Vaccine. 2008;26(15):1874-8. http://dx.doi.org/10.1016/j.vaccine.2008.01.045
19. Gabutti G, Rota MC. Pertussis: a review of disease epidemiology worldwide and in Italy. Int J Environ Res Public Health. 2012;9(12):4626-38. http://dx.doi.org/10.3390/ijerph9124626
20. Bisgard KM, Rhodes P, Connelly BL, Bi D, Hahn C, Patrick S, et al. Pertussis vaccine effectiveness among children 6 to 59 months of age in the United States, 1998-2001. Pediatrics. 2005;116(2):e285-94. http://dx.doi.org/10.1542/peds.2004-2759
21. Vickers D, Ross AG, Mainar-Jaime RC, Neudorf C, Shah S. Whole-cell and acellular pertussis vaccination programs and rates of pertussis among infants and young children. CMAJ. 2006;175(10):1213-7. http://dx.doi.org/10.1503/cmaj.051637
22. Witt MA, Katz PH, Witt DJ. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. Clin Infect Dis. 2012;54(12):1730-5. http://dx.doi.org/10.1093/cid/cis287
23. World Health Organization (WHO). WHO-recommended standards for surveillance of selected vaccine-preventable diseases. Geneva: WHO; 2003. Available from: http://apps.who.int/iris/bitstream/10665/68334/1/WHO_V-B_03.01_eng.pdf
24. European Centre for Disease Prevention and Control (ECDC). Pertussis. Stockholm: ECDC. [Accessed 19 Dec 2013]. Available from: http://ecdc.europa.eu/en/activities/surveillance/euvac/case_definition/Pages/pertusis.aspx
25. Grupo de Trabajo Tos Ferina 2012 de la Ponencia de Programas y Registro de Vacunaciones. Revisión del programa de vacunación frente a tos ferina en Espa-a. [Review of the vaccination programme against pertussis in Spain]. Madrid: Comisión de Salud Pública del Consejo Interterritorial del Sistema Nacional de Salud. Ministerio de Sanidad, Servicios Sociales e Igualdad; 2013. Spanish. Available from: http://www.msssi.gob.es/profesionales/saludPublica/prevPromocion/vacunaciones/docs/TosFerina.pdf
26. Situación epidemiológica de la tos ferina en Espa-a. [Epidemiological situation of pertussis in Spain]. Madrid: Centro Nacional de Epidemiologia, Insituto de Salud Carlos III, Ministerio de ciencia e innovación; 11 Sep 2012. Spanish.
27. Coberturas de vacunación. Datos estadísticos. [Vaccine coverage. Statistical data]. Updated 30 Apr 2013. Madrid: Ministerio de Sanidad, Servicios Sociales e Igualdad. [Accessed 30 Apr 2013]. Spanish. Available from: https://www.msssi.gob.es/profesionales/saludPublica/prevPromocion/vacunaciones/coberturas.htm
28. CIE-9-MC. Clasificación Internacional de Enfermedades, 9ª Revisión Modificación Clínica. [*International classification of disease*s, 9th revision clinical modification]. 8th ed. Madrid: Ministerio de Sanidad, Política Social e Igualdad, Secretaría General Técnica; 2012. Spanish. Available from http://www.msc.es/estadEstudios/estadisticas/docs/CIE9MC_8ed.pdf
29. Güriş D, Strebel PM, Bardenheier B, Brennan M, Tachdjian R, Finch E, et al. Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 1990-1996. Clin Infect Dis. 1999;28(6):1230-7. http://dx.doi.org/10.1086/514776
30. Wendelboe AM, Van Rie A, Salmaso S, Englund JA. Duration of immunity against pertussis after natural infection or vaccination. Pediatr Infect Dis J. 2005;24(5 Suppl):S58-61. http://dx.doi.org/10.1097/01.inf.0000160914.59160.41
31. Zepp F, Heininger U, Mertsola J, Bernatowska E, Guiso N, Roord J, et al. Rationale for pertussis booster vaccination throughout life in Europe. Lancet Infect Dis. 2011;11(7):557-70. http://dx.doi.org/10.1016/S1473-3099(11)70007-X
32. Wendelboe AM, Njamkepo E, Bourillon A, Floret DD, Gaudelus J, Gerber M, et al. Transmission of Bordetella pertussis to young infants. Pediatr Infect Dis J. 2007;26(4):293-9. http://dx.doi.org/10.1097/01.inf.0000258699.64164.6d
33. Tartof SY, Lewis M, Kenyon C, White K, Osborn A, Liko J, et al. Waning immunity to pertussis following 5 doses of DTaP. Pediatrics. 2013;131(4):e1047-52. http://dx.doi.org/10.1542/peds.2012-1928
34. Urwyler P, Heininger U. Protecting newborns from pertussis – the challenge of complete cocooning. BMC Infect Dis. 2014;14(1)397. [Epub ahead of print]. http://dx.doi.org/10.1186/1471-2334-14-397
35. Amirthalingam G, Andrews N, Campbell H, Ribeiro S, Kara E, Donegan K, et al. Effectiveness of maternal pertussis vaccination in England: an observational study. Lancet. 2014. pii: S0140-6736(14)60686-3.
36. Matlow JN, Pupco A, Bozzo P, Koren G. Tdap vaccination during pregnancy to reduce pertussis infection in young infants. Can Fam Physiscian. 2013;59(5):497-8.
37. Conyn M, van der Maas N, Mooi F. Control of whooping cough in the Netherlands: optimisation of the vaccination policy. Bilthoven: National Institute for Public Health and the Environment (RIVM); 2013. RIVM report 215121002/2012. Synopsis available from: http://www.rivm.nl/en/Documents_and_publications/Scientific/Reports/2013/februari/Control_of_whooping_cough_in_the_Netherlands_Optimisation_of_the_vaccination_policy:181357

Potential association between the recent increase in campylobacteriosis incidence in the Netherlands and proton-pump inhibitor use – an ecological study

M Bouwknegt (martijn.bouwknegt@rivm.nl)[1], W van Pelt[1], M E Kubbinga[1], M Weda[1], A H Havelaar[1,2]
1. National Institute for Public Health and the Environment, Bilthoven, The Netherlands
2. Utrecht University, Utrecht, the Netherlands

Citation style for this article:
Bouwknegt M, van Pelt W, Kubbinga ME, Weda M, Havelaar AH. Potential association between the recent increase in campylobacteriosis incidence in the Netherlands and proton-pump inhibitor use – an ecological study. Euro Surveill. 2014;19(32):pii=20873. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20873

Article submitted on 01 October 2013 / published on 14 August 2014

The Netherlands saw an unexplained increase in campylobacteriosis incidence between 2003 and 2011, following a period of continuous decrease. We conducted an ecological study and found a statistical association between campylobacteriosis incidence and the annual number of prescriptions for proton pump inhibitors (PPIs), controlling for the patient's age, fresh and frozen chicken purchases (with or without correction for campylobacter prevalence in fresh poultry meat). The effect of PPIs was larger in the young than in the elderly. However, the counterfactual population-attributable fraction for PPIs was largest for the elderly (ca 45% in 2011) and increased at population level from 8% in 2004 to 27% in 2011. Using the regression model and updated covariate values, we predicted a trend break for 2012, largely due to a decreased number of PPI prescriptions, that was subsequently confirmed by surveillance data. Although causality was not shown, the biological mechanism, age effect and trend-break prediction suggest a substantial impact of PPI use on campylobacteriosis incidence in the Netherlands. We chose the ecological study design to pilot whether it is worthwhile to further pursue the effect of PPI on campylobacteriosis and other gastrointestinal pathogens in prospective cohort studies. We now provide strong arguments to do so.

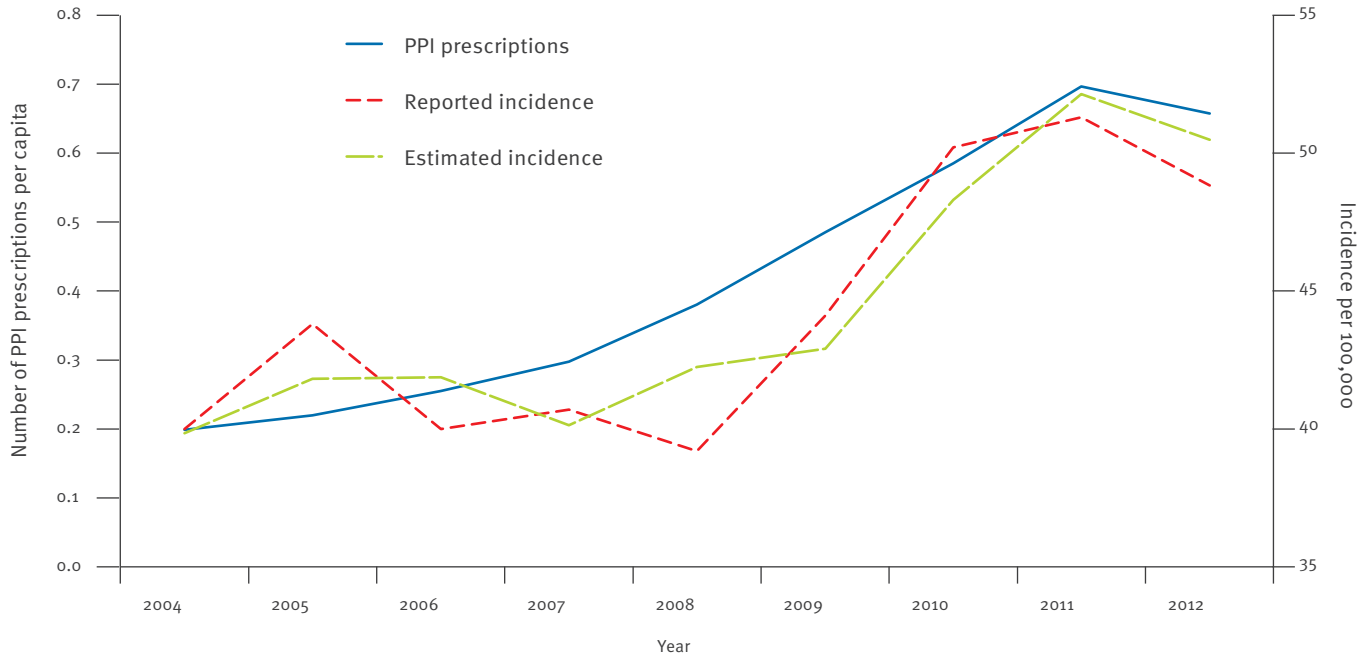
Introduction

Gastric acid is a first barrier against exogenous bacteria. The acid is secreted by parietal cells to maintain a median gastric pH of around 1.4 [1]. This level is generally sufficiently low to inactivate bacteria when the passage time is short (i.e. when the bacterial adaptive acid-tolerance response has not started), whereas pH levels >4 substantially increase their probability of survival [2,3]. Proton pump inhibitors (PPIs) are used to increase gastric pH in patients requiring, for example, treatment of gastro-oesophageal reflux disease and treatment or prevention of gastric and duodenal ulcers (e.g. co-prescribed with analgesics such as nonsteroidal anti-inflammatory drugs). PPI use is therefore hypothesised to facilitate gastrointestinal infections and has been reported repeatedly in case–control studies as a risk factor for *Campylobacter* and *Salmonella* infections with odds ratios between 3.5 and 12, suggesting a substantially increased risk [4]. The estimated attributable fraction for PPI use in campylobacteriosis cases was estimated at 8% in a Dutch case–control study [5].

Several European countries such as the Netherlands, Norway and the United Kingdom, experienced decreasing campylobacteriosis incidence rates from 2001 onwards, but faced a subsequent increase starting between 2003 and 2008. Simultaneously, other European countries, such as Denmark and Iceland, did not observe such an increase. In these countries, measures to reduce exposure of consumers to chicken meat are thought to have contributed to the favourable trends [6,7]. The decreasing incidence in the Netherlands, based on culture-positive campylobacteriosis cases, continued until 2003, followed by an increasing trend until 2011. This increase cannot be explained by improved detection methods and/or changes in testing regime, (data not shown). Furthermore, *Campylobacter* contamination of chicken fillets at retail, a recognised risk factor for *Campylobacter* infection, showed a decreasing trend between 2002 and 2011 in the Netherlands [8]. Anecdotal reports suggested that the use of PPIs in the Netherlands had increased in the years before 2011. We therefore hypothesise that the increase in campylobacteriosis cases in the Netherlands is, at least in part, related to increased PPI use. To study this hypothesis we related national trends in PPI prescriptions to the annual number of reported campylobacteriosis cases between 2004 and 2011, while controlling for age and chicken consumption. We then estimated the proportional incidence that was potentially related to PPI use.

Figure 1

Annual number of prescribed proton pump inhibitors at pharmacies and annual incidence of reported and estimated (with the regression model) campylobacteriosis cases, the Netherlands, 2004–2012



PPI: proton pump inhibitor.

Data and analysis

Data on PPI prescriptions were collected at Anatomical Therapeutic Chemical (ATC) level 5 (according to the World Health Organization (WHO) classification for medicine) from annual reports of the Foundation for Pharmaceutical Statistics (SFK) in the Netherlands (available in Dutch at www.sfk.nl) covering 95% of Dutch pharmacies. These reports cover drugs prescribed by general practitioners and physicians, and exclude over-the-counter sales. Data were available for the two most frequently prescribed PPIs: omeprazole and pantoprazole. For the former, prescription data were available for the whole study period. For the latter, prescription counts were available from 2005 onwards. The number for 2004 was estimated by dividing the available annual sales revenue for the drug (which was available) by the unit price calculated for 2005. Given the similar mode of action of the two drugs, the prescription numbers for both PPIs were aggregated. The age distribution of patients receiving PPI prescriptions was not available from the annual reports. Age-stratified data were available at ATC-4 level from Statistics Netherlands [9] for the period 2006 to 2011, covering more generally use of medicine to suppress peptic ulcers and gastro-oesophageal reflux (ATC-code A02B). These data reflected medicine refunded by 10 (groups of) health insurance companies covering >90% of the Dutch population.

Four age groups were considered: 0–25, 26–50, 51–70 and ≥71 years. Data on national sales of fresh and frozen chicken were provided by the Product Boards for Livestock, Meat and Eggs and used as a proxy for consumption. To indicate exposure, sales of fresh chicken were multiplied with the annual prevalence of campylobacter in fresh chicken meat as estimated from monitoring at retail [8]. Sales data were stratified for the consumers' age based on the population size per age group. The population size per age group per year was obtained from Statistics Netherlands [10]. Annual incidences for reported campylobacteriosis cases, including the cases' age, were obtained for the period 2004 to 2012 from national laboratory surveillance data covering 52% of the Dutch population [11].

Negative binomial regression was used to relate reported campylobacter case numbers per age group (dependent variable) to the independent variables PPI prescriptions, age and chicken consumption (fresh and frozen). The age-stratified population size was used as offset. Data from 2004 to 2011 were used; not all data were available for earlier years. Backward elimination of variables was done until all remaining factors were significantly associated with campylobacteriosis cases at the 95% confidence level. Two-way and three-way interaction terms were subsequently examined for statistical significance at the same level.

The excess incidence due to PPI prescriptions expressed as population-attributable fraction (PAF) was estimated by counterfactual assessment [12]. The regression parameters and their covariance were used

Table

Negative binomial regression analysis for association of factors with the reported campylobacteriosis incidence, the Netherlands, 2004–2011

Variable	Class	IRR		p value
		Most likely	95% CI	
Age (years)	0–25	1.00	-	<0.0001
	26–50	0.65	0.57–0.74	
	51–70	0.59	0.51–0.67	
	≥71	0.56	0.48–0.65	
PPI per capita[a] (per unit increase)	0–25	2.14	0.99–4.64	<0.0001
	26–50	1.81	1.24–2.65	
	51–70	1.69	1.46–1.97	
	≥71	1.33	1.20–1.47	
Frozen chicken purchase	Per 10^6 kg increase	0.95	0.92–0.98	0.0032

CI: confidence interval; PPI: proton pump inhibitor; IRR: incidence rate ratio.

[a] The effect of PPI-prescriptions on the incidence was modified by age (p=0.04).

to estimate the number of campylobacteriosis cases per age group per year, setting the number of PPI prescriptions to zero. The difference was divided by the estimated number of cases based on the collected data to obtain the PAF.

Data for 2012 for the independent variables of the final model (i.e. PPI per capita, chicken purchases and population counts per age group) were used to predict the number of campylobacteriosis cases for 2012 using the regression parameters and their covariance. This number was subsequently compared to the case numbers reported for 2012 to the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu; RIVM) through the laboratory surveillance.

Results

The number of prescribed PPI in the Netherlands has increased since 2004 (Figure 1), especially in the older age groups. Campylobacteriosis incidence has increased since 2003 (Figure 1).

The trend and yearly fluctuations in the number of campylobacteriosis cases were explained statistically by PPI prescriptions (p<0.0001), age (p<0.0001), the interaction between age and PPI prescriptions (p<0.04), and consumption of frozen chicken meat (preventive factor; p=0.003) (Table).

Consumption of fresh chicken adjusted for *Campylobacter* contamination at retail was not significantly associated with the campylobacteriosis incidence (p=0.19). The effect of PPI prescriptions was largest for the younger age groups and gradually decreased for older ages (Figure 2). The estimated counterfactual attributable proportion for PPI prescriptions was 8% (95% confidence interval (CI): 0–16) in 2004 and increased continuously to 27% (95% CI: 30–34) in 2011. The estimated proportion differed by age group: 12% (95% CI: 5–19) for the youngest group, 24% (95% CI: 15–32) for ages 26–50 years, 45% (95% CI: 39–51) for ages 51–70 years and 41% (95% CI: 34–48) for the oldest age group. That the attributable proportion for the elderly was higher despite the lower effect size per PPI prescription compared with the young, results from the larger number of prescriptions in the older groups.

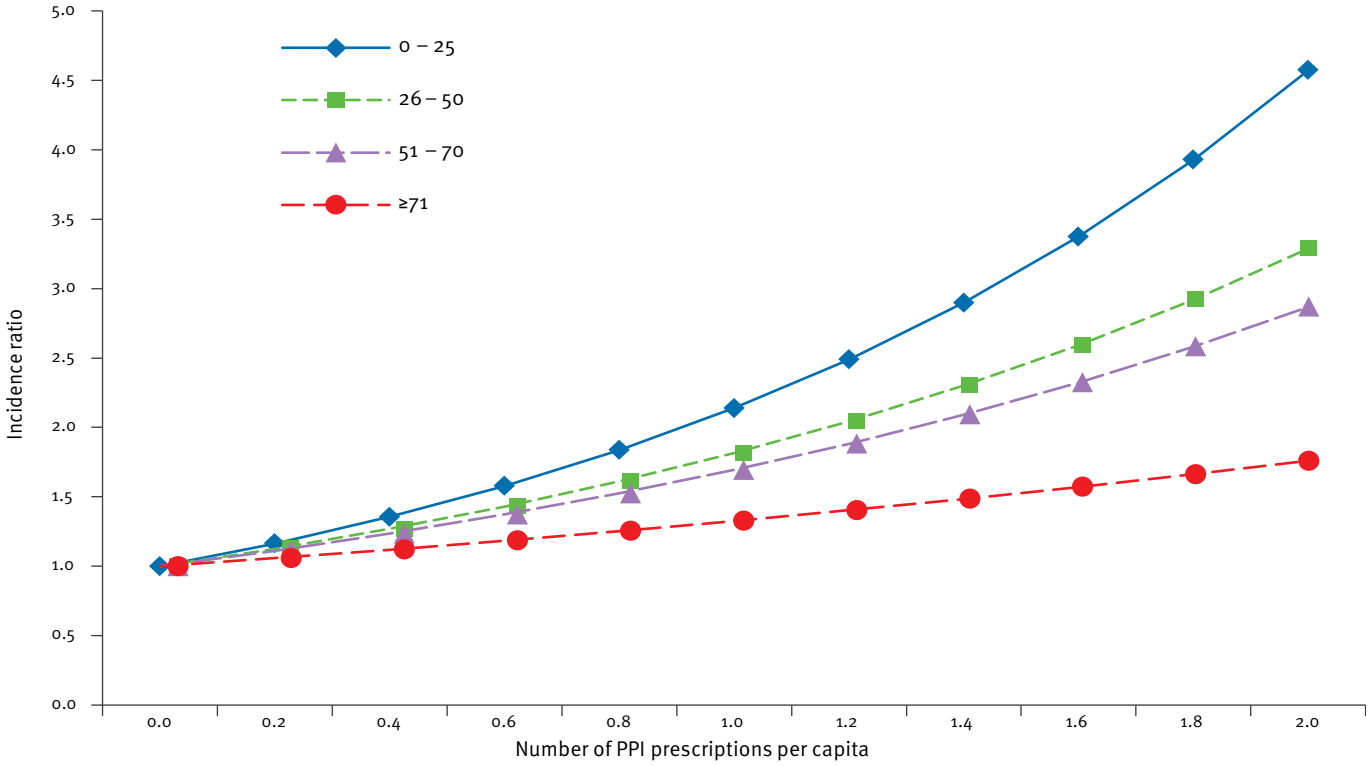
The number of prescribed PPI declined in 2012 compared with 2011, most likely because of changes in refunding policies. In total our model predicted 8,400 (95% CI: 7,600–9,100) cases of campylobacteriosis in 2012, a mean decrease of 230 cases from 2011. In accordance with the prediction, the number of reported campylobacteriosis cases decreased by approximately 320 to 8,200 in 2012.

Discussion

The current study was set up as an ecological study to generate hypotheses based on data that were available at an aggregate level. Ecological associations often fail to reflect the biological effect on the individual, and the aggregation of data undermines the control of confounding [13]. Proper data to analyse causal associations were, however, not available. Collecting such data would have required extensive time and funding, which we did not have at the time of our study. We therefore chose the approach of an ecological study to examine whether it is worthwhile to pursue studies on the effect of PPI on campylobacteriosis further. As such, we are unable to conclude on causality between PPI prescriptions and *Campylobacter* infections. Nevertheless, a number of arguments favour a causal association. Firstly, the biological rationale for the observed effect is plausible: gastric acid secretion is impaired structurally by PPI use, leading to an increased gastric pH that favours pathogen survival. Secondly, the age modification of the PPI effect can be explained biologically. Gastric acid secretion in the elderly appears to be impaired compared with younger individuals due to e.g. atrophic gastritis [14,15], resulting in a slower return to baseline levels after pH-level disruption [16], and probably in an increased probability of pathogen passage to the intestines [17]. The effect of PPI prescriptions on this probability may therefore be smaller than in younger age groups. Thirdly, the model predictions for 2012, based on covariate data for 2012, suggested a trend break, which was confirmed by surveillance data. Lastly, the current study estimated that 8% of the reported campylobacteriosis cases in 2004 were caused by PPI prescriptions. Our estimate for 2004 is similar to the estimated population-attributable fraction for PPI use of 8% in an independent case–control study on *Campylobacter* in the Netherlands that was based on data collected in 2003 at the individual level [5].

Figure 2

Effect of the number of prescribed proton pump inhibitors per capita per age group on the campylobacteriosis incidence, the Netherlands, 2004–2011



PPI: proton pump inhibitor.

The findings in the current study are similar to those obtained by Strachan et al. [18] for Scotland. These authors found a consistent association between reported campylobacteriosis cases and PPI use between 1990 and 2011. The attributable fraction was largest for those aged over 65 years, reaching more than 30% in the period 2007 to 2011, which is comparable to our average estimate of ca 35% in that period. Brophy et al. [19] recently showed an increased risk of PPI use for acquiring campylobacteriosis (hazard ratio 1.5) at individual level. However, they also showed that this risk was low compared to the demographic profile of individuals observed in the cohort. The authors concluded that there was no evidence that PPI use led to an increase in diagnosed infections, but rather that these other factors did. The cohort in Brophy et al. [19] comprised mainly persons from our two oldest age groups (average age: 58 years), the age groups for which we estimated the effect of PPI use to be smaller compared with younger ages. Mimicking their calculation methodology by dividing our counterfactual incidence rate (i.e. no PPI prescription) among those older than 50 years by the reported rate, we obtained similar hazard ratios ranging from 1.3 to 1.7 depending on the year. In our study, it was the combination of the volume of PPI prescriptions per age group and the increased risk, albeit smaller in the elderly, that resulted in the estimated impact of PPI use on campylobacteriosis incidence at population level. In addition, the sudden trend break in 2012 at population level in the Netherlands is not easily explained completely by a change in factors related to the demographic profile of those being prescribed PPIs, as these factors are generally not as dynamic as drug use. Thus, our data do not conflict with the results from Brophy et al. [19], but support the hypothesis that PPI use results in an increased campylobacteriosis risk at population level.

The change in purchases of fresh chicken fillet during the study period, corrected or not for the prevalence of *Campylobacter* contamination in retail stores, was not associated with reported campylobacteriosis case numbers. However, changes in the consumption of frozen chicken were related to the increasing number of campylobacteriosis cases. No statistical correlation existed between purchases of fresh vs frozen meat at population level (data not shown), suggesting that an increase in purchases of frozen chicken was not associated with a decrease in purchase of fresh chicken fillet. Freezing of chicken fillet has been suggested to reduce contamination levels, and hence the risk of infection, in other studies [20], and this effect may also have influenced our findings. Alternatively, increases in the consumption of frozen chicken may represent a

reduction in other risk-increasing food-related exposures, as suggested previously [5]. The fact that the consumption of fresh chicken fillet was not associated with the incidence of campylobacteriosis may be due to several factors modulate the representativeness of chicken fillet purchases as proxy for chicken fillet as risk factor. These include variations in the numbers of *Campylobacter* on individual fillets or changes in antigenic types that limit the protective effects of acquired immunity. Chicken fillet consumption was associated with increased campylobacteriosis risk in a previous study in the Netherlands [5].

Annual reports of the SFK are based on records for PPIs prescribed by general practitioners and physicians and dispensed by pharmacies. PPIs that are obtained over the counter in the Netherlands are not included in these reports. If the proportion obtained over the counter was negligible, or the ratio was similar in all study years, then the effect of disregarding the use of non-prescribed PPIs on our estimates was probably minimal. This proportion may, however, be sensitive to changes in refund policies of health insurance companies. For instance, refund policies changed in 2012, which is likely to have caused an abrupt decrease in PPIs prescribed at pharmacies. This may have led to an increase in over-the-counter sales, but data to examine such changes were unavailable.

In conclusion, we found a potential association between increasing PPI prescriptions and increasing campylobacteriosis incidence. The effect of PPI prescriptions on incidence was age-dependent and largest for the youngest age group, but the oldest age group contributed most to the overall incidence because they had the largest share of PPI prescriptions. In addition to the beneficial health effects of PPIs, this ecological study suggests a substantial impact of PPI use on the campylobacteriosis incidence in the Netherlands. Comparison with other countries with a different history of PPI prescriptions and campylobacteriosis trends may add to the understanding of the role of these drugs in the incidence at population level. Furthermore, a risk-benefit analysis, based on a prospective, individual-based study design, could provide insight in the net health benefit of PPI use and could inform a review of current prescription guidelines. Such a study should also include gastrointestinal pathogens other than *Campylobacter* spp. for which a similar effect can be expected [4]. While waiting for such results to become available, however, critical evaluation of current prescription policies is indicated. Furthermore, our study suggests that PPI users should be added to the susceptible groups targeted for specific food safety information and stresses the need for effective food safety management in the light of an increasing number of vulnerable consumers.

Acknowledgements

The authors thank Riny Janssen (RIVM) for stimulating discussions on this topic.

Conflict of interest

None declared.

Authors' contributions

WvP, MB, and AHH conceived the study and evaluated study results. MB conducted the data search and data analysis. WvP provided surveillance data. MK and MW provided data and evaluated the study results. MB drafted the first version of the manuscript and all authors contributed to its finalisation.

References

1. Schubert ML. Gastric secretion. Curr Opin Gastroenterol. 2008;24(6):659-64. http://dx.doi.org/10.1097/MOG.0b013e328311a65f
2. de Jonge R, Ritmeester WS, van Leusden FM. Adaptive responses of Salmonella enterica serovar Typhimurium DT104 and other S. Typhimurium strains and Escherichia coli O157 to low pH environments. J Appl Microbiol. 2003;94(4):625-32. http://dx.doi.org/10.1046/j.1365-2672.2003.01875.x
3. de Jonge R, Takumi K, Ritmeester WS, van Leusden FM. The adaptive response of Escherichia coli O157 in an environment with changing pH. J Appl Microbiol. 2003;94(4):555-60. http://dx.doi.org/10.1046/j.1365-2672.2003.01865.x
4. Bavishi C, Dupont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. Aliment Pharmacol Ther. 2011;34(11-12):1269-81. http://dx.doi.org/10.1111/j.1365-2036.2011.04874.x
5. Doorduyn Y, Van Den Brandhof WE, Van Duynhoven YT, Breukink BJ, Wagenaar JA, Van Pelt W. Risk factors for indigenous Campylobacter jejuni and Campylobacter coli infections in The Netherlands: a case-control study. Epidemiol Inf. 2010;138(10):1391-404. http://dx.doi.org/10.1017/S095026881000052X
6. Boysen L, Nauta M, Duarte AS, Rosenquist H. Human risk from thermotolerant Campylobacter on broiler meat in Denmark. Int J Food Microbiol. 2013;162(2):129-34. http://dx.doi.org/10.1016/j.ijfoodmicro.2013.01.009
7. Tustin J, Laberge K, Michel P, Reiersen J, Dadadottir S, Briem H, et al. A national epidemic of campylobacteriosis in Iceland, lessons learned. Zoonoses Public Health. 2011;58(6):440-7. http://dx.doi.org/10.1111/j.1863-2378.2010.01387.x
8. Maassen C, De Jong A, Stenvers O, Valkenburgh S, Friesema I, Heimeriks K, et al. Staat van zoönosen 2011. [State of zoonotic diseases 2011]. Bilthoven: Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment; RIVM); 2012. Dutch. Available from: http://www.rivm.nl/bibliotheek/rapporten/330291008.pdf
9. Personen met verstrekte geneesmiddelen; leeftijd en geslacht. [Individuals with medicines supplied; age and sex]. StatLine database. The Hague: Statistics Netherlands (CBS). [Accessed: 10 Jan 2013]. Available from: http://statline.cbs.nl/StatWeb/publication/?DM=SLNL&PA=81071NED&D1=0&D2=0&D3=a&D4=6&D5=a&HDR=T,G4,G1&STB=G3,G2&VW=T
10. Bevolking; geslacht, leeftijd en burgerlijke staat, 1 januari. [Population; sex, age and marital status, 1 January]. StatLine database. The Hague: Statistics Netherlands (CBS). [Accessed: 10 Jan 2013]. Available from: http://statline.cbs.nl/StatWeb/publication/?DM=SLNL&PA=7461BEV&D1=0&D2=a&D3=0-100&D4=54-62&HDR=T,G3&STB=G1,G2&VW=T
11. van Pelt W, de Wit MA, Wannet WJ, Ligtvoet EJ, Widdowson MA, van Duynhoven YT. Laboratory surveillance of bacterial gastroenteric pathogens in The Netherlands, 1991-2001. Epidemiol Inf. 2003;130(3):431-41.
12. Mathers CD, Vos T, Lopez AD, Salomon J, Ezzati M, editors. National burden of disease studies: a practical guide. Edition 2.0. Global Program on Evidence for Health Policy. Geneva: World Health Organization; 2001. Available from: http://www.who.int/healthinfo/nationalburdenofdiseasemanual.pdf
13. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2008.

14. Feldman M, Cryer B, McArthur KE, Huet BA, Lee E. Effects of aging and gastritis on gastric acid and pepsin secretion in humans: A prospective study. Gastroenterol. 1996;110(4):1043-52. http://dx.doi.org/10.1053/gast.1996.v110.pm8612992

15. Hurwitz A, Brady DA, Schaal SE, Samloff IM, Dedon J, Ruhl CE. Gastric acidity in older adults. JAMA. 1997;278(8):659-62. http://dx.doi.org/10.1001/jama.1997.03550080069041

16. Russell TL, Berardi RR, Barnett JL, Dermentzoglou LC, Jarvenpaa KM, Schmaltz SP, et al. Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women. Pharm Res. 1993;10(2):187-96. http://dx.doi.org/10.1023/A:1018970323716

17. Takumi K, de Jonge R, Havelaar A. Modelling inactivation of Escherichia coli by low pH: application to passage through the stomach of young and elderly people. J Appl Microbiol. 2000;89(6):935-43. http://dx.doi.org/10.1046/j.1365-2672.2000.01193.x

18. Strachan NJ, Rotariu O, Macrae M, Sheppard SK, Smith-Palmer A, Cowden J, et al. Operationalising factors that explain the emergence of infectious diseases: a case study of the human campylobacteriosis epidemic. PloS One. 2013;8(11):e79331. http://dx.doi.org/10.1371/journal.pone.0079331

19. Brophy S, Jones KH, Rahman MA, Zhou SM, John A, Atkinson MD, et al. Incidence of campylobacter and salmonella infections following first prescription for PPI: A cohort study using routine data. Am J Gastroenterol. 2013;108(7):1094-100. http://dx.doi.org/10.1038/ajg.2013.30

20. Rosenquist H, Sommer HM, Nielsen NL, Christensen BB. The effect of slaughter operations on the contamination of chicken carcasses with thermotolerant Campylobacter. Int J Food Microbiol. 2006;108(2):226-32. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.12.007

The hanta hunting study: underdiagnosis of Puumala hantavirus infections in symptomatic non-travelling leptospirosis-suspected patients in the Netherlands, in 2010 and April to November 2011

M Goeijenbier (m.goeijenbier@erasmusmc.nl)[1], R A Hartskeerl[2], J Reimerink[3], J Verner-Carlsson[4], J F Wagenaar[2], M G Goris[2], B E Martina[1], Å Lundkvist[4,5], M Koopmans[1,3], A D Osterhaus[1], E C van Gorp[1,6], C B Reusken[1,3,6]

1. Erasmus MC, Department of Virology, Rotterdam, the Netherlands
2. Royal Tropical Institute (KIT), KIT Biomedical Research, Amsterdam, the Netherlands
3. Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
4. The Public Health Agency of Sweden, Solna, Sweden
5. Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden
6. These authors share senior authorship

Citation style for this article:
Goeijenbier M, Hartskeerl RA, Reimerink J, Verner-Carlsson J, Wagenaar JF, Goris MG, Martina BE, Lundkvist Å, Koopmans M, Osterhaus AD, van Gorp EC, Reusken CB. The hanta hunting study: underdiagnosis of Puumala hantavirus infections in symptomatic non-travelling leptospirosis-suspected patients in the Netherlands, in 2010 and April to November 2011 . Euro Surveill. 2014;19(32):pii=20878. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20878

Article submitted on 27 September 2013 / published on 14 August 2014

Leptospirosis and haemorrhagic fever with renal syndrome (HFRS) are hard to distinguish clinically since these two important rodent-borne zoonoses share hallmark symptoms such as renal failure and haemorrhage. Leptospirosis is caused by infection with a spirochete while HFRS is the result of an infection with certain hantaviruses. Both diseases are relatively rare in the Netherlands. Increased incidence of HFRS has been observed since 2007 in countries that border the Netherlands. Since a similar rise in incidence has not been registered in the Netherlands, we hypothesise that due to overlapping clinical manifestations, hantavirus infections may be confused with leptospirosis, leading to underdiagnosis. Therefore, we tested a cohort of non-travelling Dutch patients with symptoms compatible with leptospirosis, but with a negative diagnosis, during 2010 and from April to November 2011. Sera were screened with pan-hantavirus IgG and IgM enzyme-linked immunosorbent assays (ELISAs). Sera with IgM reactivity were tested by immunofluorescence assay (IFA). ELISA (IgM positive) and IFA results were confirmed using focus reduction neutralisation tests (FRNTs). We found hantavirus-specific IgG and/or IgM antibodies in 4.3% (11/255) of samples taken in 2010 and in 4.1% (6/146) of the samples during the 2011 period. After FRNT confirmation, seven patients were classed as having acute Puumala virus infections. A review of hantavirus diagnostic requests revealed that at least three of the seven confirmed acute cases as well as seven probable acute cases of hantavirus infection were missed in the Netherlands during the study period.

Introduction

Hantaviruses, negative-stranded RNA viruses belonging to the *Bunyaviridae* family, can cause severe disease in humans. Depending on the type of hantavirus, either haemorrhagic fever with renal syndrome (HFRS) or hantavirus cardiopulmonary syndrome may occur after inhalation of virus-containing aerosols [1]. HFRS is characterised by acute renal failure, fever (above 38.5 °C) and potentially accompanied by severe bleeding complications [2]; it is a notifiable disease in the Netherlands. HFRS cases are found in large parts of Europe and Asia [3]. Pathogenic hantaviruses are rodent-borne and each of these viruses are spread by a specific rodent species. For the HFRS-causing hantaviruses, these include *Apodemus*, *Myodes*, *Rattus* and possibly *Microtus* species [4,5]. The causative agent of HFRS known to be endemic in the Netherlands is Puumala virus (PUUV), which is spread by its chronically infected reservoir, the bank vole (*Myodes glareolus*) [6]. Symptomatic cases of PUUV infection may develop mild HFRS, often referred to as nephropathia epidemica. Recent reports describe PUUV infections with a broader clinical spectrum, ranging from mild febrile cases, without renal impairment or haemorrhage, to severe respiratory manifestations without any signs of renal involvement [7,8]. Historically, the occurrence of PUUV infection in the Netherlands has been restricted to the eastern and southern parts of the country, with an incidence of 25–30 cases reported per year (approximately 0.04–0.18 cases per 100,000 population) [9]. Since 2007, several studies have described an increase in the number of human PUUV infections in neighbouring countries at the eastern (Germany) and southern borders (Belgium) [10-12]. To date, a similar

increase in the number of human PUUV infections has not been observed in the Netherlands.

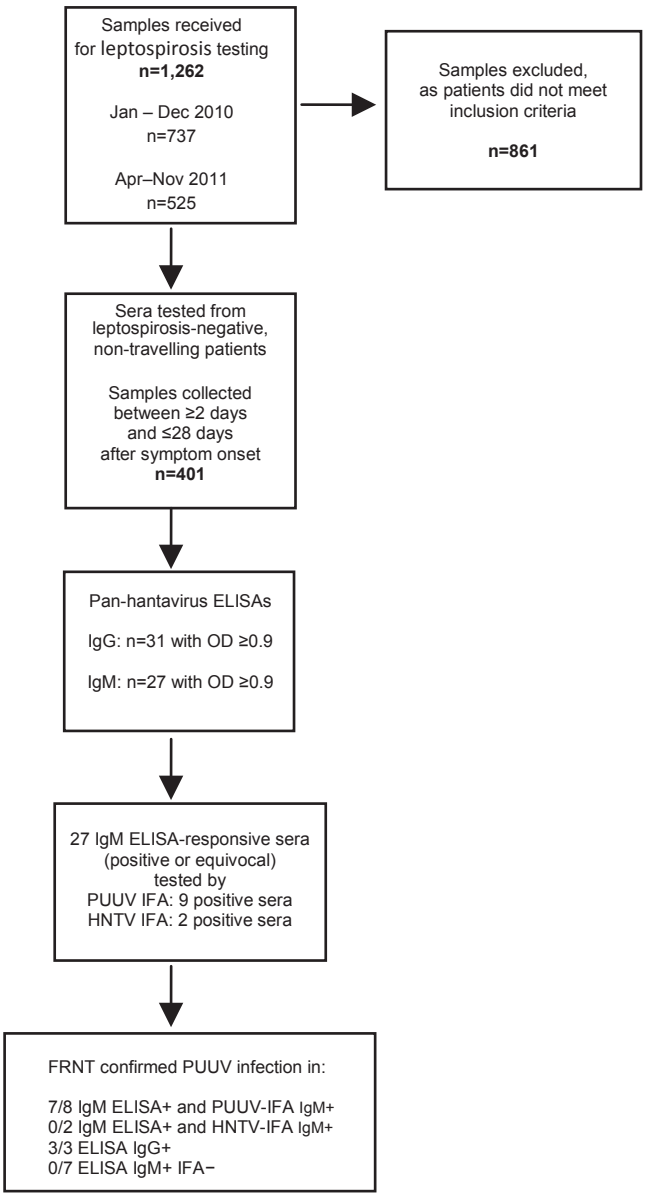
In the early 1990s, Groen et al. tested 8,892 sera obtained in the Netherlands from different risk groups, such as renal disease patients, and subjects from suspected occupational risk groups, such as forestry workers and military personnel, for the presence of hantavirus antibodies [13]. The highest prevalence (up to 6%) was seen in participants with known occupational risk factors associated with increased rodent exposure [13,14]. Data (which are as yet unpublished but a summary of the main results is available) from a large serum bank study in the Netherlands that started in 2006 showed a hantavirus seroprevalence, in a cross-sectional population based study, of 1.7% [15]. Given that 70–80% of PUUV infections are asymptomatic and that only 5–10% of symptomatic patients will probably seek medical attention [16], the 25–30 cases reported every year in the Netherlands (with a population of 16.8 million [17]) are indicative of potential underdiagnosis of hantavirus infections [4,5]. In 2011, we described a case report of a patient with nephropathia epidemica diagnosed outside the area known to be endemic for hantavirus circulation in the Netherlands, Overijssel [18]. Although the patient had visited a known PUUV-endemic area, this information in the patient history did not result in rapid diagnosis of the cause of the disease, illustrating unawareness of hantavirus infections.

Leptospira, a genus of helical-shaped bacteria, forms another important group of causative agents of rodent-borne haemorrhagic fever in the Netherlands [19]. Pathogenic *Leptospira* cause leptospirosis, which shares many clinical manifestations with HFRS, such as renal failure, thrombocytopenia and potential bleeding complications [3]. Interestingly, two studies, from Italy and Sri Lanka, showed an increased hantavirus seroprevalence in patients suspected of having leptospirosis. Compared with control groups consisting of office personnel or healthy blood donors, the number of confirmed cases of hantavirus infection was significantly higher among those who were clinically suspected, by a clinician, of having leptospirosis [20,21]. Groen et al. reported a hantavirus seroprevalence of about 1% in patients suspected of having acute leptospirosis in the Netherlands in samples collected between 1972 and 1994 [13]. The actual prevalence of confirmed acute leptospirosis was slightly higher (3%) [13].

In the Netherlands, a relatively low number of leptospirosis cases are registered annually, as are HFRS cases, with a reported incidence of 0.25 leptospirosis cases/100,000 population [19]. To investigate the putative underdiagnosis of hantavirus infection in symptomatic patients, we tested a cohort of leptospirosis-suspected, but confirmed-negative, patients with no travel history for the previous three months, for the presence of hantavirus-specific antibodies, using a two-step strategy: pan-hantavirus enzyme-linked

Figure 1

Design, inclusion criteria and confirmatory steps of the hanta hunting study, the Netherlands, 2010 and April–November 2011



\+ Positive test result

– Negative test result

OD: optical density (an OD of >1.10 was regarded as positive, between ≥0.90 and ≤1.10 as equivocal and <0.90 as negative); ELISA: enzyme-linked immunosorbent assay; FRNT: focus reduction neutralisation test; HNTV: Hantaan virus; IFA: immunofluorescence assay; PUUV: Puumala virus.

The rationale for the chosen period was an overall increase in hantavirus activity in countries neighbouring the Netherlands (Germany and Belgium) (January–December 2010) [4] and the known season of PUUV activity in northern and western Europe (April–November 2011) [26].

immunosorbent assays (ELISAs) followed by two separate immunofluorescence assays (IFA): one to detect PUUV serogroup antibodies and one to detect Hantaan virus (HNTV) serogroup antibodies (HNTV was used as it belongs to the same serogroup as Seoul hantavirus (SEOV). Recent evidence indicates the circulation of SEOV in Europe [22-24], spread by *Rattus norvegicus*, which is also a well-known carrier of *Leptospira* [3]). To confirm the ELISA and IFA results, focus reduction neutralisation tests (FRNTs) were used, the gold standard technique in hantavirus serology. All IFA IgM-positive sera were tested in the FRNT with PUUV virus. In addition, because recent evidence indicates the circulation of SEOV in Europe, we included SEOV, as well as Dobrava virus (DOBV), in the FRNT, although not the main aim of this study, to gain insight into the potential introduction of these viruses in the Netherlands.

Methods

Serum bank

Sera from non-travelling Dutch patients with a negative leptospirosis diagnosis – based on a microscopic agglutination test (MAT), ELISA and culture performed at the National Leptospirosis Reference laboratory (NRL) at the Royal Tropical Institute (KIT) in Amsterdam – were included in our study. A sample was deemed to be negative for leptospirosis if the patient did not meet the case definition for leptospirosis – i.e. the in-house ELISA for leptospirosis was below the cut-off titre of 1:80 and the MAT showed no relevant titre of *Leptospira*-specfic antibodies (<1:160) [25]. The study cohort was taken from submissions of sera to the NRL in 2010, as a large increase in the number of PUUV infections in Germany and Belgium were observed that year [4]. In addition, we also included sera from patients meeting the above inclusion criteria that were received by the NRL during April to November 2011, the season for PUUV activity in northern and western Europe [26] (Figure 1).

As we were interested in patients who had pan-hantavirus IgM antibodies, we selected patients whose samples had been collected at least two days after symptom onset, up to four weeks (28 days) after symptom onset. All samples were heat inactivated (30 minutes at 56 °C) and stored at –20 °C until testing. Requests for leptospirosis testing were accompanied by a standardised form with information about place of residence, travel history, presenting symptoms and occupation: these data were reviewed.

Hantavirus underdiagnosis was assessed by checking if testing for hantavirus was requested at either of the hantavirus diagnostic laboratories in the Netherlands (Erasmus MC in Rotterdam and RIVM in Bilthoven) for any of the pan-hantavirus ELISA-responsive sera (equivocal or positive result in an IgG or IgM ELISA).

Patients whose sera were responsive in any of the diagnostic tests were ranked by likelihood of hantavirus infection. Patients whose sera were positive in the IgM ELISA, IFA and FRNT were considered a confirmed case of acute hantavirus infection. If only the IgM ELISA was positive (or equivocal) and IFA was positive, the patient was considered a probable acute case. If only the IgG ELISA was positive and therefore IFA was not performed, but the FRNT was positive for PUUV, the patient was also considered a probable acute case. If only the IgM ELISA was positive or equivocal, the patient was considered not a case of hantavirus infection.

Enzyme-linked immunosorbent assay

Sera were screened using pan-hantavirus IgG and IgM DxSelect ELISAs (Focus Diagnostics). These ELISAs are used for testing a broad range of hantaviruses, although there are variations in sensitivity and specificity per specific hantavirus. According to the material supplied by the manufacturer, the IgM test has an overall sensitivity of 95.1% (83.5–99.4%) and a specificity of 94.1% (83.8–98.8%). The IgG test has comparable performance characteristics, with an overall specificity of 95% (91.4–100%) and a sensitivity of 95% (75–98%); both tests are compared with a reference ELISA by external investigators. For the Netherlands, the performance of these ELISAs in detecting antibodies to PUUV and, potentially, SEOV, is of importance. Data supplied by the manufacturer showed a sensitivity of 70% (45.7–88.1%) in the IgM ELISA and 95% (83.2–100%) in the IgG ELISA for PUUV-specific antibodies, as tested by FRNT. For SEOV FRNT-positive samples, the sensitivity was 50% (11.8–88.2%) in the IgM ELISA and 95% (54.1–100%) in the IgG ELISA.

An optical density (OD) of >1.10 was regarded as positive, between ≥0.90 and ≤1.10 as equivocal and <0.90 as negative.

Immunofluorescence assay

ELISA IgM-positive or equivocal sera were tested in IFA by using commercial slides with PUUV- and HNTV-infected cells (PROGEN Biotechnik). Only IgM-reactive samples were chosen as these are indicative of a recent infection, possibly related to the clinical symptoms that were the basis of the initial request for leptospirosis testing. IFA was used because of its higher reported specificity and the possibility of being able to distinguish between PUUV- or HTNV-like serotype infections (manufacturer's insert, PROGEN Biotechnik). Before testing, the sera were incubated with liver acetone powder from calves (Sigma-Aldrich, Germany) to reduce background fluorescence. For the IgM test, the sera were pretreated with GullSORB (Meridian Bioscience Inc., United States) to reduce isotype competition. Sera were serially diluted twofold starting at 1:32 and incubated on the slides for 1 hour at 37 °C. After this step, the wells were incubated with either a fluorescein isothiocyanate (FITC)-labelled goat anti-human IgG or IgM conjugate. Fluorescence was scored under an immunofluorescence microscope. The cut-off titre for a positive result was defined as the sample dilution for which specific fluorescence was greater

Table 1

Serological test results from samples selected for focus reduction neutralisation test confirmation of hantavirus infection, the Netherlands, 2010 and April–November 2011 (n=22)

Sample number	Date of sampling	ELISA[a]		IFA[b]				FRNT[c]			Acute hantavirus infection case status
		IgM	IgG	PUUV IgM	PUUV IgG	HNTV IgM	HNTV IgG	PUUV	SEOV	DOBV	
PUUV IFA IgM positive											
4	May 2010	POS	POS	1:128	1:128	NEG	1:128	POS	NEG	NEG	Confirmed
7	Jul 2010	POS	POS	1:128	1:128	1:128	NEG	POS	NEG	NEG	Confirmed
10	Aug 2010	POS	POS	1:128	1:128	NEG	1:128	POS	NEG	NEG	Confirmed
11	Aug 2010	POS	POS	1:128	1:128	NEG	NEG	POS	NEG	NEG	Confirmed.
12	Aug 2010	POS	Equi	1:128	1:128	NEG	NEG	POS	NEG	NEG	Confirmed.
14	Aug 2010	POS	NEG	1:128	1:128	NEG	NEG	NEG	NEG	NEG	Probable
19	Jul 2011	POS	POS	1:128	1:128	1:128	1:128	POS	NEG	NEG	Confirmed
21	Sep 2011	POS	NEG	1:128	1:128	NEG	NEG	NS	NS	NS	Probable
22	Sep 2011	POS	Equi	1:128	1:128	1:128	1:128	POS	NEG	NEG	Confirmed
HNTV IFA IgM positive and ELISA IgM reactive											
6	Jun 2010	POS	POS	NEG	NEG	1:512	1:512	NEG	NEG	NEG	Probable
5	Jun 2010	Equi	NEG	NEG	NEG	1:128	NEG	NEG	NEG	NEG	Probable
ELISA IgM reactive and PUUV or HNTV IFA negative											
2	Feb 2010	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Not a case
3	Feb 2010	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Not a case
13	Aug 2010	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Not a case
15	Oct 2010	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Not a case
17	May 2011	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Not a case
18	Jul 2011	POS	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Not a case
20	Aug 2011	POS	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	Not a case
9	Jul 2010	Equi	POS	NEG	NEG	NEG	NEG	NS	NS	NS	Not a case
Not tested by IFA (as ELISA IgM negative), but ELISA IgG positive											
1	Feb 2010	NEG	POS	NT	NT	NT	NT	POS	NEG	NEG	Probable[d]
8	Jul 2010	NEG	POS	NT	NT	NT	NT	POS	NEG	NEG	Probable[d]
16	Apr 2011	NEG	POS	NT	NT	NT	NT	POS	NEG	NEG	Probable[d]

DOBV: Dobrava virus; ELISA: enzyme-linked immunosorbent assay; Equi: equivocal; FRNT: focus reduction neutralisation test; HTNV G: Hantaan virus; IFA: immunofluorescence assay; NEG: negative; NS: insufficient amount of serum; NT: not tested in IFA (samples not responsive in the IgM ELISA were not tested by IFA); POS: positive; PUUV: Puumala virus; SEOV: Seoul virus.

[a] ELISA results were scored as positive if OD >1.10,equivocal if between ≥0.90 and ≤1.10 and negative if <0.90.

[b] The IFA was scored positive when the reactive titres were >1:64.

[c] The FRNT was scored positive with a cut-off of 80% virus neutralisation.

[d] IgM antibodies were not detected in these samples, but as hantavirus infection was confirmed by FRNT, the patients were still scored as probable acute hantavirus cases.

than the sample dilution for which specific fluorescence was just identifiable: in this study, it was >1:64.

Focus reduction neutralisation test

All samples positive in the pan-hantavirus IgM ELISA and PUUV IgM IFA were selected for FRNT confirmation (samples 4, 7, 10, 11, 12, 14, 19, 21 and 22). We also selected two samples positive or equivocal in the pan-hantavirus IgM ELISA and positive in the IgM HNTV IFA for FRNT (samples 5 and 6). In addition, eight samples that were positive in the IgM ELISA but negative in the IFAs were also selected for FRNT (samples 2, 3, 9, 13, 15, 17, 18 and 20). As a fourth category, three samples that had not been included in the IFA analysis (as they were ELISA IgM negative), but that tested positive in the IgG ELISA (samples 1,8 and 16), were selected for FRNT confirmation.

FRNTs for DOBV strain Slovenia, SEOV strain 80-39 and PUUV strain Kazaan were carried out as described elsewhere [27]. Diluted sera were mixed with an equal volume of diluted virus containing 30–70 focus-forming units/100 µl. The serum end-concentration was 1:40. The mixture was incubated at 37 °C for 1 hour and subsequently inoculated into wells of six-well tissue culture plates containing confluent Vero E6 cell

monolayers. The wells were overlaid with a mixture of agarose and tissue culture medium and incubated for 7–13 days. The agarose was removed from the wells and the cells were fixed. For PUUV-infected cells, polyclonal macaque serum [28] was used as the primary antibody and the monoclonal antibody 1C12 for DOBV- and SEOV-infected cells as described elsewhere [29]. This step was followed by adding peroxidase-labelled goat-anti-human IgG for the macaque serum and goat-anti-mouse IgG for the 1C12 monoclonal antibody to the cells, to indicate virus-infected cells. Tetramethylbenzidine was used as substrate and foci were counted. An 80% reduction in the number of foci, compared with the virus control, was used as the criterion for virus neutralisation titres.

Review of hantavirus diagnostic requests carried out during suspicion of leptospirosis at the time of sampling

All samples responsive in the IgM and/or IgG pan-hantavirus ELISA were checked for patient-specific characteristics (sex and date of birth). The combination of sex and date of birth was checked in the databases of the hantavirus diagnostic laboratories in the Netherlands. If the sample combination matched the information from the database and the diagnostic request was made in 2010 or 2011, the patient was scored as having been adequately diagnosed for hantavirus disease during the onset of their symptoms. If the sex and date of birth combination could not be found in the databases, but the patient's sample was reactive in any of our tests, the case was scored as a missed probable or confirmed hantavirus case, as described in the serum bank section above.

Ethical issues

This study was exempted from ethical review of human subject research by the Medical Ethical Review Committee of the Erasmus MC Medical Centre, University of Rotterdam. All data have been anonymised and are not attributable to individual patients.

Results

Enzyme-linked immunosorbent assay and immunofluorescence assay serology

Of the 1,262 samples received for leptospirosis diagnostic testing during January– December 2010 and April–November 2011, 861 were excluded, as the patients did not meet the inclusion criteria of our study.

All selected and available sera (n=401) were tested by pan-hantavirus ELISAs. Overall, the IgG ELISA resulted in 18 positive and 13 equivocal samples. The IgM testing resulted in 17 positive and 10 equivocal samples. A total of 11 samples reacted in both the IgG and IgM ELISAs, bringing the total number of samples that responded in both ELISAs to 47, i.e. 11.7% of the 401 samples (4.3% (11/255) of samples taken in 2010 and 4.1% (6/146) of the samples taken in 2011).

Subsequently, the 27 samples with a positive or equivocal response in the IgM ELISA were tested using both PUUV and HTNV IFAs. In total, nine of the 27 IgM ELISA-responsive samples tested positive for both PUUV IgM and IgG by IFA.

Interestingly, two serum samples were positive in the HTNV IFA, but negative in the PUUV IFA, despite repeated PUUV testing. One of the HTNV-positive samples tested positive for both HNTV IgG and IgM with titres of 1:512; the other sample was positive only for HNTV IgM, with a titre of 1:128.

Confirmation by focus reduction neutralisation test

FRNT was performed on eight of the nine sera with a positive IgM response in the PUUV IFA (there was an insufficient amount of serum in the ninth sample). It confirmed that seven of the eight samples tested were from patients with recent PUUV infections (samples 4, 7, 10, 11, 12, 19 and 22) (Table 1).

We also tested the two sera with an HNTV IgM-positive IFA and a positive or equivocal ELISA IgM response (samples 5 and 6): both sera were negative by FRNT.

To test if cases had been missed due to lack of sensitivity of the IFAs, we selected eight samples, of which seven samples had enough serum left for FRNT, with only IgM reactivity in the ELISAs and a negative PUUV or HNTV IFA (samples 2, 3, 13, 15, 17, 18 and 20): all seven samples were negative by FRNT.

We also selected three samples that had not been included in the IFA analysis (as they were ELISA IgM negative), but that tested positive in the IgG ELISA (Cases 1,8 and 16). These samples were tested by FRNT because there was a high degree of suspicion of PUUV infection based on the application form for leptospirosis diagnostic request sent by the clinician (e.g. recorded renal failure and possible rodent exposure). All three patients had a long duration of their complaints (more than three weeks since symptom onset, making it possible that hantavirus disease, without the detection of IgM antibodies, was the cause of their symptoms. All three were positive in the PUUV FRNT.

Thus in total, FRNT for PUUV, SEOV and DOBV was performed on 20 of the 22 selected samples, due to an insufficient amount of serum in two samples. Of the 20 serum samples tested, 10 were confirmed as PUUV positive, seven of which were considered due to a recent infection, based on the presence of IgM antibodies.

Patient characteristics and registered clinical signs and symptoms in confirmed cases

Because of the retrospective nature of our study, we could confirm if patients had been adequately tested for hantavirus infection during their disease course or if the patient was a missed case of PUUV infection. Of the 27 samples with at least an equivocal response in

Table 2

Information on cases of hantavirus infection undiagnosed at time of sampling during suspicion of leptospirosis, the Netherlands, 2010 and April–November 2011

Sample number	Date of request for leptospirosis testing	Sex	Age group in years	State	Information at time of request for leptospirosis testing	Retroactive hantavirus diagnostic test results			Acute hantavirus infection case status
						ELISAa	IFAb	FRNTc	
16	Apr 2011	M	20–24	Overijssel	Acute kidney failure and hepatitis	IgG +	NT	PUUV +	Probable
1	Feb 2010	M	20–24	Overijssel	Prolonged severe disease	IgG +	NT	PUUV +	Probable
8	Jul 2010	M	10–14	Groningen	Contact with soil water and potential rodent exposure	IgM Equi IgG +	PUUV – HNTV –	PUUV +	Probable
21	Sep 2011	F	25–30	Overijssel	Icteric; non-responsive to antibiotics	IgM +	PUUV IgM +	NS	Probable
14	Aug 2010	F	20–24	Zuid-Holland	No additional information	IgM +	PUUV IgM +	All –	Probable
5	Jun 2010	M	20–24	Limburg	No additional information	IgM +	HNTV IgM +	All –	Probable
6	Jun 2010	M	25–30	Gelderland	Emergency hospital admission, to an intensive-care unit	IgM + IgG +	HNTV IgM + HNTV IgG +	All –	Probable
22	Sep 2011	M	45–49	Gelderland	Clinical picture not understood	IgM + IgG +	PUUV IgM + PUUV IgG +	PUUV +	Confirmed
19	Jul 2011	M	50–54	Groningen	Extreme tiredness, fever and diarrhoea	IgM + IgG +	PUUV IgM + PUUV IgG +	PUUV +	Confirmed
7	Jul 2010	M	35–39	Noord-Brabant	Severe disease with high fever and emergency hospital admission	IgM + IgG +	PUUV IgM + PUUV IgG +	PUUV +	Confirmed

\+ Positive test result

– Negative test result

DOBV: Dobrava virus; ELISA: Enzyme-linked immunosorbent assay; EQUI: equivocal; F: female; FRNT: focus reduction neutralisation test; HNTV: Hantaan virus; IFA: immunofluorescence assay; M: male; NS: insufficient amount of serum; NT: not tested in IFA (samples not responsive in the IgM ELISA were not tested by IFA); PUUV: Puumala virus; SEOV: Seoul virus.

[a] ELISAs for pan-hantavirus IgM and IgG antibodies were used.

[b] For IFA, both PUUV and HNTV sergroups were used.

[c] For FRNT, PUUV, SEOV and DOBV were used.

the IgM ELISA in our study, which would necessitate further testing of a follow-up serum sample, four were adequately tested by routine serology for hantavirus infection at diagnostic centres at the time of sampling during suspicion of leptospirosis (samples 4, 10, 11 and 12).

The two samples that were responsive in the ELISAs and HNTV IFA, but not in FRNT (samples 5 and 6), were not tested for hantavirus antibodies by ELISA or IFA at the time the patients were sampled.

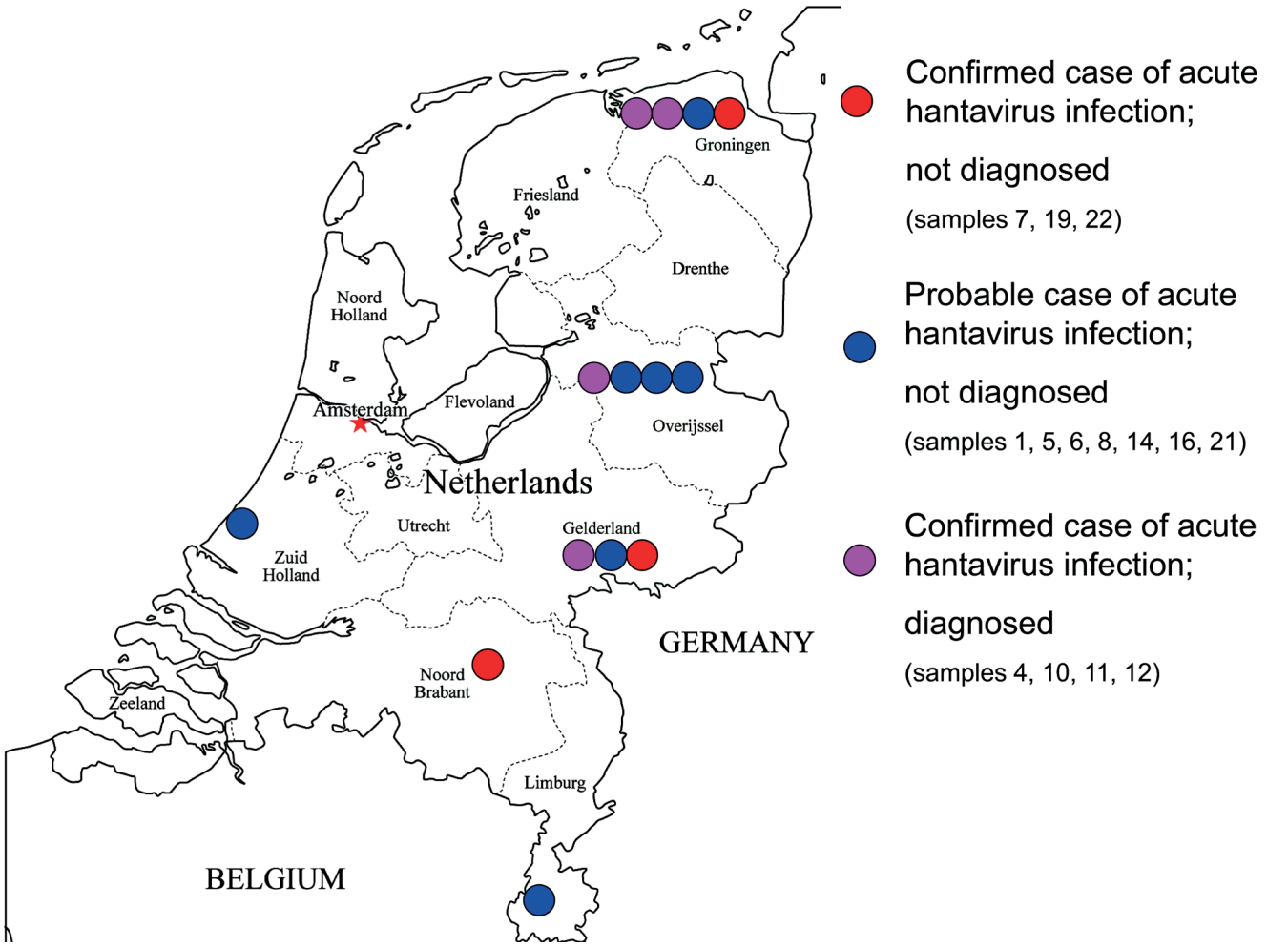
All available information from retroactively determined probable or confirmed cases of hantavirus infection that were not tested for hantavirus infection during suspicion of leptospirosis at the time of sampling is shown in Table 2. In general, most of the cases were in the eastern parts of the Netherlands. Newly recognised areas with confirmed cases were in the northern province of Groningen and the western province of Zuid-Holland. The missed confirmed and probable cases of hantavirus infection are shown in Figure 2 according to the location of sampling, ranked by likelihood of hantavirus infection, with the highest level of evidence being that of a sample with a positive response in the IgM ELISA confirmed by IFA (IgM and IgG) tests and a positive FRNT result.

Discussion

In the samples tested, a positive response of hantavirus IgM antibodies in the ELISA was observed in 4.3% (11/255) of samples taken in 2010 and 4.1% (6/146) of the samples taken in 2011. When including samples with an equivocal ELISA result, the overall percentage with an IgM response was 6.7% (27/401). Confirmation with IFA IgM resulted in a 2.7% (11/401) seropositivity in the cohort. Of these 11 samples, seven were confirmed by FRNT, corresponding to an overall seropositivity in the cohort of almost 2%. However, this percentage could very well be an underestimation. For instance,

Figure 2

Distribution of probable and confirmed cases of hantavirus infection in the Netherlands, 2010 and April–November 2011 (n=14)



The star marks the capital, Amsterdam.

This figure shows the probable and confirmed cases of acute hantavirus infection in the Netherlands and if they were adequately diagnosed during their illness or if these were 'missed' cases. The cases are ranked by likelihood of hantavirus infection. A confirmed case of acute hantavirus infection being that of a positive IgG and IgM response in both pan-hantavirus enzyme-linked immunosorbent assay (ELISA) and immunofluoresence assay (IFA) and a positive result in the focus reduction neutralisation test (FRNT). A probable case is strongly suggestive of an acute case of hantavirus based on ELISA, IFA or FRNT results, but either the FRNT was negative or we were unable to show the presence of IgM antibodies.

one sample could not be confirmed by FRNT due to a lack of available serum after ELISA and IFA screening. It is conceivable that in our cohort of symptomatic patients, of the seven confirmed hantavirus diagnoses, at least three cases were not adequately diagnosed at the time of disease (Cases 7, 19 and 22). The other four confirmed cases were found in the databases of the hantavirus diagnostic centres in the Netherlands, and thus were adequately diagnosed at the time of disease. We also identified seven probable cases of acute hantavirus infection: we consider that hantavirus infection was a highly plausible explanation for their symptoms, but either FRNT confirmation was not performed due to the lack of serum or the presence of IgM antibodies (confirming acute infection) could not be proved by ELISA and IFA.

All samples confirmed by FRNT (n=10) only showed PUUV-neutralising activity. The vector of this hantavirus is *Myodes glareoulus* (bank vole), a small, reddish rodent that inhabits large parts of the Netherlands, solely in grasslands and forests [9]. Case 6, with high OD values in IgM and IgG ELISA screening and a positive HNTV IFA, did not neutralise PUUV, DOBV or SEOV.

This study revealed a high seroprevalence of about 2% of hantavirus antibodies in a cohort of leptospirosis-suspected patients who tested negative for leptospirosis. Leptospirosis in the Netherlands may be either endemic or imported [19]. In our cohort, travel history was well documented and hence we consider it quite certain that the patients we studied contracted hantaviruses in the Netherlands.

This cohort also gave us the opportunity to study the circulation of hantaviruses other than PUUV in The Netherlands. It is important to monitor this, since evidence is mounting of an increase in the number of SEOV infections in Europe and worldwide, with a recent case reported in the United Kingdom [30]. However, in the samples tested from the Netherlands, the SEOV FRNT was negative. Results were also not indicative for infections with DOBV, which is vectored by the yellow-necked mouse (*Apodemus flavicollis*). It is possible that the ELISA and IFA results in samples 5 and 6 were false positives. The specificity of the ELISA and IFA is below 100%, resulting in a (small) chance of cross-reactivity. Test specifications of the ELISA test showed no known cross-reactive pathogens, but this presumption is based on results from very small serum cohorts (manufacturer's insert). Although SEOV and DOBV have been excluded as the causative agents in our study, the remaining hantaviruses in the HNTV serogroup are vectored by reservoir species not known to be present in the Netherlands. Thus, while we cannot rule out the possibility that other hantaviruses from the HNTV serogroup caused the disease in patients from whom samples 5 and 6 were obtained, with no travel history, this remains highly unlikely.

Our results show quite a large discrepancy between the initial ELISA screening, followed by IFA analysis and eventual gold standard FRNT confirmation. Samples that tested positive only in the IgM ELISA (n=7) were not confirmed positive by FRNT. In most cases (7/9), a positive response in the IgM pan-hantavirus ELISA in combination with a positive result in the PUUV IgM IFA was later confirmed by FRNT (sample 14 tested negative in the FRNT and sample 21 could not be tested). Therefore, we underline the importance of FRNT validation in epidemiological studies before drawing any major conclusions, particularly since hantavirus serology is highly prone to giving false-positive results [31].

Acute leptospirosis and HFRS share many clinical manifestations and certain epidemiological features. Exposure to rodents is a known risk factor for both diseases. Hallmark symptoms in both HFRS and leptospirosis include kidney failure. Two of the four cases who were diagnosed at the time of their disease course had documented kidney disorders (data not shown). However, of the 10 cases who were not diagnosed, only one (sample 16) had documented kidney failure. Of the other nine undiagnosed cases, one was described as having 'high fever' (sample 7), two as having 'severe disease' (samples 1 and 7) and one as 'clinical picture not understood' (sample 22): the fact that these cases were undiagnosed in the Netherlands during their disease course could be due to a potential lack of typical presenting symptoms for hantavirus disease in the Netherlands, meaning the absence of kidney failure. Hepatic involvement, often present in leptospirosis – one of the classic triads in Weil's disease [3] – could lead a clinician to think of leptospirosis, while not considering PUUV infection: this would have applied to the patient with hepatitis (sample 16), a probable case of acute hantavirus infection in our study. Atypical presentation of HFRS, as seen in some of the cases listed in Table 2 (samples 21 and 19), has been the subject of several recent case reports [7,32,33]. However, unawareness and/or lack of clinicians' knowledge of how to recognise hantavirus disease could also be a reason for underdiagnosis.

Cases may also be underdiagnosed if the patients are outside the hantavirus-endemic area in the Netherlands. Such cases might not be identified due to the low, but clinically important number of infections, resulting in lack of awareness of the clinicians in these areas. If we compare the distribution of the previously undiagnosed cases in our cohort with the earlier serological data, for instance, data published by Groen et al. [14], 7 of 10 cases of hantavirus infection not tested at the time of sampling for hantavirus disease (listed in Table 2) were from outside the known endemic area. Our conclusions regarding underdiagnosis are supported by a recent study showing 1.7% hantavirus seroprevalence in the Dutch population, which should lead to more symptomatic cases than the 25–30 cases reported annually [15].

In this relatively small cohort with specific clinical indications for leptospirosis diagnostics, we have shown the presence of undiagnosed hantavirus cases. Leptospirosis itself is potentially an often-missed diagnosis in the Netherlands, due to unawareness [19]. It is conceivable that physicians, who do not include leptospirosis in their differential diagnosis, are even less aware of the possibility of hantavirus infections. Vice versa, it cannot be excluded that clinicians who are aware of hantavirus infections might miss potential leptospirosis. This hypothesis could be validated by performing larger-scale serological studies with broader cohorts, comprising patients who are suspected of having leptospirosis or hantavirus infection.

On the basis of the results in this paper, we feel it is important to increase awareness of hantavirus infection in the Netherlands. The increased incidence of hantavirus infections in Europe in recent years makes this even more important. This increase is affected by a multitude of factors. Some, such as changes in landscape architecture (e.g. (de)forestation, fragmentation of land by motorways, railways and agriculture and available burrow space) and increased food availability for the rodent reservoirs, are beneficial for the spread of hantaviruses [12]. The introduction or discovery of new hantavirus strains in Europe has been documented [23] and presents another major concern, necessitating epidemiological monitoring of vectors and patients. We advise that hantavirus and leptospirosis diagnostics should be considered for every patient with an undifferentiated fever in any area with potential rodent-borne infections, including typing of the causative agents if the results are positive.

By decreasing the unnecessary use of antibiotics [34] and providing clinicians with an accurate prediction of the disease course and a choice of adequate biomarkers of disease severity [3], the identification of hantavirus infections might have a limited, but important, clinical importance. Furthermore, adequately diagnosing and typing hantavirus infections is of major public health importance in order to correctly identify and educate risk groups and to design tailor-made prevention programmes, such as rodent-control programmes and changes to landscape architecture.

Acknowledgements

The authors would like to acknowledge Sjoerd Kuiling for the help with IFA testing.

This study was partially funded by European Union grant FP7-261504 EDENext and is catalogued by the EDENext Steering Committee as EDENext225 (http://www.edenext.eu).

Conflict of interest

None declared.

Authors' contributions

Marco Goeijenbier, Rudy Hartskeerl, Jiri Wagenaar, Marga Goris, Byron Martina, Eric van Gorp and Chantal Reusken designed the experiment. Marco Goeijenbier, Marga Goris, Johan Reimerink, Jenny Verner-Carlsson and Chantal Reusken did all the laboratory work and experiments. Ake Lundkvist, Eric van Gorp, Byron Martina, Chantal Reusken, Johan Reimerink, Rudy Hartskeerl, Albert Osterhaus and Marion Koopmans assisted with interpretation of the data. All authors contributed to the writing of the manuscript.

References

1. Watson DC, Sargianou M, Papa A, Chra P, Starakis I, Panos G. Epidemiology of Hantavirus infections in humans: a comprehensive, global overview. Crit Rev Microbiol. 2014;40(3):261-72. http://dx.doi.org/10.3109/1040841X.2013.783555
2. Bi Z, Formenty PB, Roth CE. Hantavirus infection: a review and global update. J Infect Dev Ctries. 2008;2(1):3-23. http://dx.doi.org/10.3855/jidc.317
3. Goeijenbier M, Wagenaar J, Goris M, Martina B, Henttonen H, Vaheri A, et al. Rodent-borne hemorrhagic fevers: under-recognized, widely spread and preventable - epidemiology, diagnostics and treatment. Crit Rev Microbiol. 2013;39(1):26-42. http://dx.doi.org/10.3109/1040841X.2012.686481
4. Heyman P, Ceianu CS, Christova I, Tordo N, Beersma M, João Alves M, et al. A five-year perspective on the situation of haemorrhagic fever with renal syndrome and status of the hantavirus reservoirs in Europe, 2005-2010. Euro Surveill. 2011;16(36):pii=19961.
5. Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Mäkelä S, et al. Uncovering the mysteries of hantavirus infections. Nat Rev Microbiol. 2013;11(8):539-50. http://dx.doi.org/10.1038/nrmicro3066
6. Gerding MN, Groen J, Jordans JG, Osterhaus AD. Hantavirus nephropathy in The Netherlands: clinical, histopathological and epidemiological findings. Neth J Med. 1995;47(3):106-12. http://dx.doi.org/10.1016/0300-2977(95)00054-Q
7. Gizzi M, Delaere B, Weynand B, Clement J, Maes P, Vergote V, et al. Another case of "European hantavirus pulmonary syndrome" with severe lung, prior to kidney, involvement, and diagnosed by viral inclusions in lung macrophages. Eur J Clin Microbiol Infect Dis. 2013;32(10):1341-5. http://dx.doi.org/10.1007/s10096-013-1885-x
8. Christova I, Younan R, Taseva E, Gladnishka T, Trifonova I, Ivanova V, et al. Hemorrhagic fever with renal syndrome and Crimean-Congo hemorrhagic fever as causes of acute undifferentiated febrile illness in Bulgaria. Vector Borne Zoonotic Dis. 2013;13(3):188-92. http://dx.doi.org/10.1089/vbz.2011.0938
9. Reusken CB. Hantavirus infections in the Netherlands, a risk profile. Bilthoven: National Institute for Public Health and the Environment (RIVM); 2009 RIVM Letter report 330361001/2009. Available from: http://www.rivm.nl/dsresource?objectid=rivmp:13325&type=org&disposition=inline&ns_nc=1
10. Heyman P, Cochez C, Ducoffre G, Mailles A, Zeller H, Abu Abu Sin M, et al. Haemorrhagic Fever with Renal Syndrome: an analysis of the outbreaks in Belgium, France, Germany, the Netherlands and Luxembourg in 2005. Euro Surveill. 2007;12(5):pii=712.
11. Boone I, Wagner-Wiening C, Reil D, Jacob J, Rosenfeld UM, Ulrich RG, et al. Rise in the number of notified human hantavirus infections since October 2011 in Baden-Wurttemberg, Germany. Euro Surveill. 2012;17(21):pii=20180.
12. Reusken C, Heyman P. Factors driving hantavirus emergence in Europe. Curr Opin Virol. 2013;3(1):92-9. http://dx.doi.org/10.1016/j.coviro.2013.01.002
13. Groen J, Gerding MN, Jordans JG, Clement JP, Nieuwenhuijs JH, Osterhaus AD. Hantavirus infections in The Netherlands: epidemiology and disease. Epidemiol Infect. 1995;114(2):373-83. http://dx.doi.org/10.1017/S0950268800058003
14. Gerding MN, Groen J, Jordans JG,Osterhaus AD. Hantavirus nephropathy in The Netherlands: clinical, histopathological and epidemiological findings. Neth J Med. 1995;47(3):106-12. http://dx.doi.org/10.1016/0300-2977(95)00054-Q
15. Van der Klis F, Mollema L, Van Weert Y. RIVM report: Pienter 2 study; a summary of the main findings. Bilthoven: National Institute for Public Health and the Environment (RIVM); 2012. RIVM Letter report. Available on request.
16. Vaheri A, Henttonen H, Voutilainen L, Mustonen J, Sironen T, Vapalahti O. Hantavirus infections in Europe and their impact on public health. Rev Med Virol. 2013;23(1):35-49. http://dx.doi.org/10.1002/rmv.1722
17. Centraal Bureau voor de Statistiek (CBS). Bevolkingsteller. [Population counter]. The Hague: CBS. [Accessed 22 Feb 2014]. Dutch. Available from: http://www.cbs.nl/nl-NL/menu/themas/bevolking/cijfers/extra/bevolkingsteller.htm
18. Goeijenbier M, Nur E, Goris M, Wagenaar JF, Grunberg K, Nurmohamed SA, et al. An unusual cause of a usual presentation. Hantavirus infection. Neth J Med. 2011;69(6):285-9.
19. Goris MG, Boer KR, Duarte TA, Kliffen SJ, Hartskeerl RA. Human leptospirosis trends, the Netherlands, 1925-2008. Emerg Infect Dis. 2013;19(3):371-8. http://dx.doi.org/10.3201/eid1903.111260
20. Gamage CD, Yasuda SP, Nishio S, Kularatne SA, Weerakoon K, Rajapakse J, et al. Serological evidence of Thailand virus-related hantavirus infection among suspected leptospirosis patients in Kandy, Sri Lanka. Jpn J Infect Dis. 2011;64(1):72-5.
21. Nuti M, Amaddeo D, Autorino GL, Crovatto M, Crucil C, Ghionni A, et al. Seroprevalence of antibodies to hantaviruses and leptospires in selected Italian population groups. Eur J Epidemiol. 1992;8(1):98-102.
22. Jameson LJ, Taori SK, Atkinson B, Levick P, Featherstone CA, van der Burgt G, et al. Pet rats as a source of hantavirus in England and Wales, 2013. Euro Surveill. 2013;18(9):pii=20415.
23. Jameson LJ, Logue CH, Atkinson B, Baker N, Galbraith SE, Carroll MW, et al. The continued emergence of hantaviruses: isolation of a Seoul virus implicated in human disease, United Kingdom, October 2012. Euro Surveill. 2013;18(1):pii=20344.
24. Macé G, Feyeux C, Mollard N, Chantegret C, Audia S, Rebibou JM, et al. Severe Seoul hantavirus infection in a pregnant woman, France, October 2012. Euro Surveill. 2013;18(17):pii=20464.
25. Goris MG, Leeflang MM, Boer KR, Goeijenbier M, van Gorp EC, Wagenaar JF, et al. Establishment of valid laboratory case definition for human leptospirosis. J Bacteriol Parasitol. 2012;3:132.
26. Tersago K, Verhagen R, Servais A, Heyman P, Ducoffre G, Leirs H. Hantavirus disease (nephropathia epidemica) in Belgium: effects of tree seed production and climate. Epidemiol Infect. 2009;137(2):250-6. http://dx.doi.org/10.1017/S0950268808000940
27. Lundkvist A, Hukic M, Hörling J, Gilljam M, Nichol S, Niklasson B. Puumala and Dobrava viruses cause hemorrhagic fever with renal syndrome in Bosnia-Herzegovina: evidence of

highly cross-neutralizing antibody responses in early patient sera. J Med Virol. 1997;53(1):51-9. http://dx.doi.org/10.1002/(SICI)1096-9071(199709)53:1<51::AID-JMV9>3.3.CO;2-J http://dx.doi.org/10.1002/(SICI)1096-9071(199709)53:1<51::AID-JMV9>3.0.CO;2-P

28. Sironen T, Klingström J, Vaheri A, Andersson LC, Lundkvist A, Plyusnin A. Pathology of Puumala hantavirus infection in macaques. PLoS One. 2008;3(8):e3035. http://dx.doi.org/10.1371/journal.pone.0003035
29. Lundkvist A, Fatouros A, Niklasson B. Antigenic variation of European haemorrhagic fever with renal syndrome virus strains characterized using bank vole monoclonal antibodies. J Gen Virol. 1991;72 (Pt 9):2097-103. http://dx.doi.org/10.1099/0022-1317-72-9-2097
30. Jameson LJ, Logue CH, Atkinson B, Baker N, Galbraith SE, Carroll MW, et al. The continued emergence of hantaviruses: isolation of a Seoul virus implicated in human disease, United Kingdom, October 2012. Euro Surveill. 2013;18(1):pii=20344.
31. Tischler ND, Rosemblatt M, Valenzuela PD. Characterization of cross-reactive and serotype-specific epitopes on the nucleocapsid proteins of hantaviruses. Virus Res. 2008;135(1):1-9. http://dx.doi.org/10.1016/j.virusres.2008.01.013
32. Eckerle I, Jakob E, Hofmann J, Schmidt-Bacher A, Ettinger J, Schnitzler P. Atypical severe Puumala hantavirus infection and virus sequence analysis of the patient and regional reservoir host. Zoonoses Public Health. 2012;59 Suppl 2:110-5. http://dx.doi.org/10.1111/j.1863-2378.2011.01452.x
33. Kramski M, Achazi K, Klempa B, Krüger DH. Nephropathia epidemica with a 6-week incubation period after occupational exposure to Puumala hantavirus. J Clin Virol. 2009;44(1):99-101. http://dx.doi.org/10.1016/j.jcv.2008.10.005
34. Brorstad A, Oscarsson KB, Ahlm C. Early diagnosis of hantavirus infection by family doctors can reduce inappropriate antibiotic use and hospitalization. Scand J Prim Health Care. 2010;28(3):179-84. http://dx.doi.org/10.3109/02813432.2010.506058

WHO publishes handbook on the analysis of TB surveillance data

Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

Citation style for this article:
Eurosurveillance editorial team. WHO publishes handbook on the analysis of TB surveillance data. Euro Surveill. 2014;19(32):pii=20876. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20876

Article published on 14 August 2014

The World Health Organization (WHO) published a handbook on the analysis of tuberculosis (TB) surveillance data with the title 'Understanding and using tuberculosis data' on 13 August 2014 [1].

The handbook which is available for free, has been developed by the WHO Global Task Force on TB Impact Measurement, as part of its mission to help strengthen TB surveillance through the use of data and subsequent improvement of its quality.

Country health information and registration systems often provide a rich source of data on the burden of disease caused by TB and the effectiveness of programmatic efforts to reduce this burden. However, the available data are often underused, or not used at all. At least in part, this may be a result of the lack of clear guidance on recommended approaches to the analysis of such data.

The handbook is designed to address this gap through detailed practical examples of the analysis of TB surveillance data, in particular TB notification data, data from surveillance of anti-TB drug resistance, and mortality data compiled in national vital registration systems. It starts from the most basic kinds of analyses, and progresses to the description of more challenging topics such as the estimation of disease burden using multiple sources of evidence, including data from special surveys.

Analysis of these data can help programme managers and other staff to track the level of and trends in TB disease burden, detect outbreaks of disease and identify ways to improve existing TB prevention, diagnostic and treatment services.

References

1. World Health Organization (WHO). Understanding and using tuberculosis data. Geneva: WHO; Aug 2014. Available from: http://www.who.int/tb/publications/understanding_and_using_tb_data/en/