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A case of dengue type 3 virus infection imported from Africa to Italy, October 2009

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In October 2009, a traveller returning from Africa to Italy was hospitalised with symptoms suggestive of a haemorrhagic fever of unknown origin. The patient was immediately placed in a special biocontainment unit until laboratory investigations confirmed the infection to be caused by a dengue serotype 3 virus. This case reasserts the importance of returning travellers as sentinels of unknown outbreaks occurring in other countries, and highlights how the initial symptoms of dengue fever resemble those of other haemorrhagic fevers, hence the importance of prompt isolation of patients until a final diagnosis is reached.

Case description

In October 2009, a Senegalese man in his forties, who had been living in Italy for 20 years, presented to Hospital A in the northern Italian city of Turin three days after returning (via Madrid) from a four-month visit to his home village in Senegal. During his stay, he had never left the village and had not visited any healthcare centres. The initial symptoms of disease, a persistent fever (>38 °C) accompanied by an unremitting headache, started during the flight from Madrid to Turin worsened over the previous two days despite treatment with paracetamol.

On the day after hospitalisation, when laboratory test results showed a platelet count of 5,000 cells/mm³ as well as evidence of altered liver function (aspartate aminotransferases (AST): 3,539 U/L; alanine aminotransferases (ALT): 815 U/L; lactate dehydrogenase (LDH) 3,609 U/L; gamma-glutamyl transferases (gamma-GT): 112 U/L), the patient was first transferred to an infectious diseases hospital in Turin (Hospital B), and two days later to the National Institute for Infectious Diseases in Rome (Hospital C), which is the Italian national reference centre for emerging infections and bioterrorism, on suspicion of a viral haemorrhagic fever. The patient was admitted to a special biocontainment unit during the same night. Over the following

days, the patient's clinical condition improved and he was discharged nine days after admission.

Laboratory investigations

After the patient's admission, series of tests were run in parallel on samples taken at Hospital C and in Turin, before the transfer to Rome. The tests included in the differential diagnosis ruled out malaria (which had already been excluded in Turin), as well as infection with the major agents of viral hepatitis, herpes simplex virus and viral haemorrhagic fever viruses. Serological investigation using an in-house immunofluorescence assay (slides prepared with a mix of uninfected and dengue type 2-infected Vero cells) revealed a high IgG titre against dengue virus soon after the onset of symptoms (in a sample taken on 10 October), with the presence of IgM antibodies at a low titre, and RT-PCR followed by sequencing of the NS5 region confirmed the infectious agent to be a serotype 3 dengue virus. In addition, a 224 bp fragment of the E gene was amplified and sequenced. The phylogenetic analysis of this fragment is shown in the Figure. Our patient was infected with a strain belonging to dengue virus serotype 3, genotype III closely related to other strains found in Africa, but never reported from Senegal before the case described here [1].

The tests carried out the day before the patient left the hospital showed that his IgG titre was still increasing. Overall, the immune response was consistent with a re-infection with a dengue serotype 3, genotype III virus which can lead to a severe form of the disease.

Case management

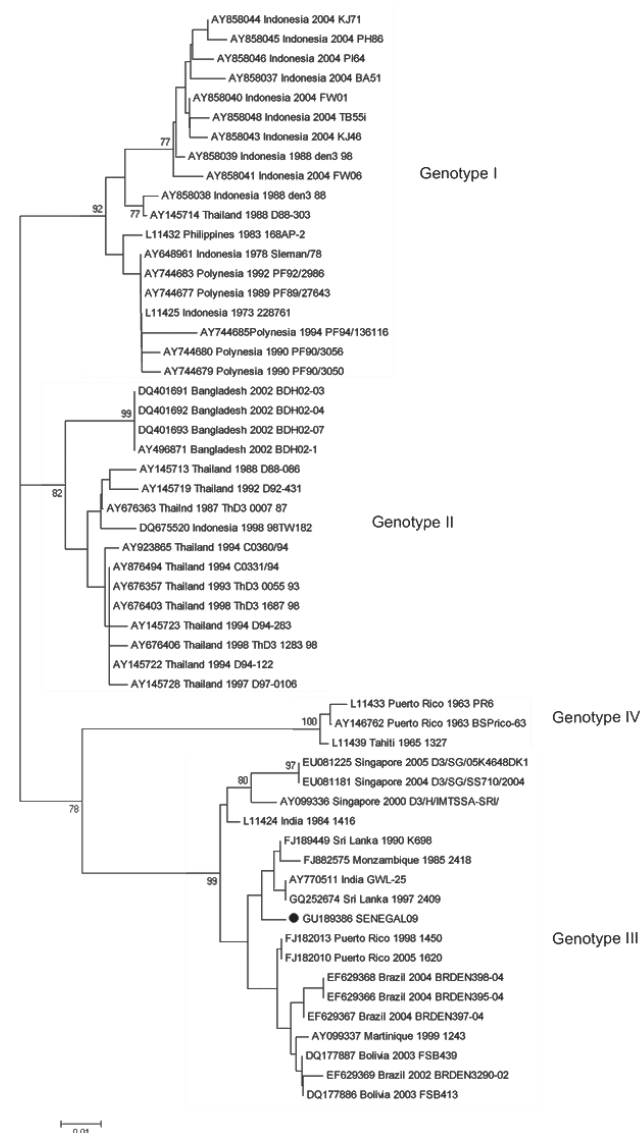
At the time the patient reported to the first hospital in Turin, there was no information on any outbreaks of dengue virus taking place in Senegal [1]. Therefore, the fact that the patient developed symptoms suggestive of a haemorrhagic disease led the clinicians to suspect also other, more dangerous viral infections, namely Lassa virus or Crimean-Congo haemorrhagic fever

virus, both categorised as biosafety level 4 (BSL4) agents [2-3]. As these infections could not be ruled out on clinical and epidemiological criteria, it was decided to transfer the patient to a high-security isolation facility.

When contacted by Hospital B in Turin, the National Institute for Infectious Diseases immediately requested the shipping of clinical samples, but because of logistical difficulties the transport could not be organised.

FIGURE

Phylogenetic relationship of the strain isolated in October 2009 in Rome (identified by the black dot) with other dengue serotype 3 virus strains described elsewhere [10]



Sequence identification is as follows: strain name, country and year of isolation. The scale bar indicates nucleotide substitutions per site. Multiple alignment of our sequence and other dengue serotype 3 virus sequences available in GenBank was generated with ClustalW1.7 software included in the Bioedit package. The phylogenetic tree was constructed by using nucleotide alignment, the Kimura-2-parameters algorithm, and the neighbour-joining method implemented in the MEGA 4.1 software. The robustness of branching patterns was tested by 1,000 bootstrap pseudoreplications.

It was then decided by the Italian Ministry of Health, in consultation with the institute, to make use of the procedure already in place in Italy for the transport of highly infectious patients under high isolation conditions, which included the use of military aircraft and equipment of the Italian Air Force and Hospital C (stretcher isolators and a high containment ambulance). On arrival, the patient was immediately transferred to the biocontainment unit and was attended to by specially trained, dedicated staff employing all necessary biosafety precautions.

Conclusions

We have described a case of dengue virus infection imported into Italy from Africa, which to date has been a rare occurrence as most cases seen in European travel clinics have been imported either from Asia or the Americas. Dengue virus has been known to circulate in parts of Africa for decades, and Senegal in particular has experienced several outbreaks, mainly of dengue serotype 2 virus [1]. Following our report [4], which was the first for a Dengue serotype 3 virus in Senegal, the country's health authorities were alerted to a possible epidemic and since then, over 50 cases have been registered in the country [5-6], confirming the importance of returning travellers as sentinels of as yet unreported outbreaks occurring abroad [7].

From a clinical point of view, the patient did not meet the World Health Organization's (WHO) criteria for severe dengue fever [8], but presented a set of warning signs indicative of haemorrhagic fever such as liver enlargement (>2 cm), altered liver enzymes (20-fold) and a rapid decrease in platelet count. Therefore, and because the information available at the time did not allow excluding the involvement of a BSL4 agent, the decision to treat the case as a potential viral haemorrhagic fever was made early in the management process. The recent report of a case of Marburg haemorrhagic fever imported from Uganda into the United States [9] that was diagnosed retrospectively in a serum sample archived six months earlier after it had tested negative for agents of viral haemorrhagic fever, should teach us that in similar cases, patients should immediately be placed in an adequate containment facility until a final diagnosis is reached. Our recommendation is to perform all diagnostic procedures safely at the appropriate biosafety level, and as a general rule any activities involving patient samples at a level lower than BSL3 should be kept to a minimum. It remains a problem that the transport of samples is still an unsolved issue in Italy as well as in Europe and worldwide. The timely shipping of biological samples in advance of the patient's arrival would have shortened the time to reach a diagnosis and avoided the extra costs of unnecessary biosafety measures.

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Recent expansion of dengue virus serotype 3 in West Africa

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Due to non-existing or limited surveillance in Africa, little is known about the epidemiology of dengue illness in the continent. Serological and virological data obtained from returning European travellers is a key complement to this often flawed information. In the past years, dengue 3 virus has emerged in West Africa and has been detected in travellers returning to Europe. The first dengue epidemic in Cape Verde with more than 17,000 cases from September to December 2009 demonstrated that dengue virus is still expanding worldwide to new territories.

Introduction

Dengue virus is widely distributed in tropical and subtropical countries and is transmitted by day-biting mosquitoes of the genus *Aedes*. It often goes unrecognised in African countries, where the lack of surveillance systems, or their poor implementation, is the cause of missing information on dengue virus activity [1].

Laboratory-based surveillance of dengue virus infection in febrile travellers could provide useful information about the different dengue virus serotypes circulating worldwide and in particular those circulating in areas where limited surveillance is available. To this end, the European Network for Imported Viral Disease - Collaborative Laboratory Response Network (ENIVD-CLRN network) provides outbreak support, in particular related to laboratory diagnostics, to assist European Union (EU) Member States, candidate countries and members of the European Economic Area and European Free Trade Association (EEA/EFTA) in detecting, investigating and responding to outbreak-prone diseases, imported, rare or unknown infectious agents, or outbreaks related to the intentional release of pathogens.

A large outbreak of dengue illness with more than 17,000 cases occurred in the Cape Verde archipelago at the end of 2009 [2]. It was the first time that dengue virus was detected in the archipelago. Concomitant

detection of dengue virus in Senegal and identification of several imported cases among travellers returning from West Africa were reported. This article provides a brief review of historic reports of dengue virus in Africa focused on West Africa and summarises the recent outbreaks and the links to imported cases of dengue virus infection in Europe.

Dengue virus in West Africa

The burden of dengue virus infection in Africa has not been estimated yet. Outbreaks of dengue fever and dengue haemorrhagic fever are poorly documented, however, we cannot conclude that mild and severe dengue infection is infrequent in African countries. The circulation of different dengue virus serotypes is also poorly documented. Nevertheless some information is provided in publications on outbreaks and serosurvey studies in Africa and reports involving dengue virus infection in travellers.

A retrospective serological study in 1956 [3] suggests that dengue virus caused an epidemic in Durban, South Africa in 1927. This report is the first documented dengue virus epidemic in Africa. It was not until the end of the 1960s, however, that the virus responsible for dengue fever outbreaks in Africa could be isolated. The first dengue virus (DENV) isolate was DENV-1, detected in Nigeria in 1964 [4]. Since then DENV-1, 2 and 4 have been circulating in West Africa although the main serotype reported has been DENV-2 [1]. Viral isolates have been predominantly detected in wild-caught mosquitoes (*Aedes luteocephalus*, *Ae. taylori* and/or *Ae. furcifer*) involved in sylvatic transmission cycles in Senegal and Nigeria, and from a few cases in humans who were in contact with forest cycles [5,6]. DENV-2 from sylvatic cycles have also been isolated in Côte d'Ivoire, Burkina Faso and Guinea [5,7,8]. More recently in 2005, DENV-2 was identified in a traveller returning from Ghana [9]. The last detection of DENV-4 in West Africa was in the 1980s in two inhabitants of Dakar, Senegal [10].

The first description of DENV-3 activity in Africa was related to outbreaks detected during 1984 and 1985 in Pemba, Mozambique, with two deaths due to dengue haemorrhagic fever [11]. DENV-3 was then detected in 1993 in Somalia and areas around the Persian Gulf [12]. Phylogenetic studies suggested that these outbreaks were caused by a virus imported from the Indian sub-continent [13]. DENV-3 circulation in West Africa was first identified in a traveller returning to Spain from Cameroon in 2006 and subsequently in a traveller returning to Spain from Senegal in 2007 (C. Domingo *et al.*, unpublished results). However the first article on DENV-3 in West Africa was published in 2008, when DENV-3 was detected co-circulating with yellow fever in Côte d'Ivoire [14].

Dengue virus importation from Africa into Europe

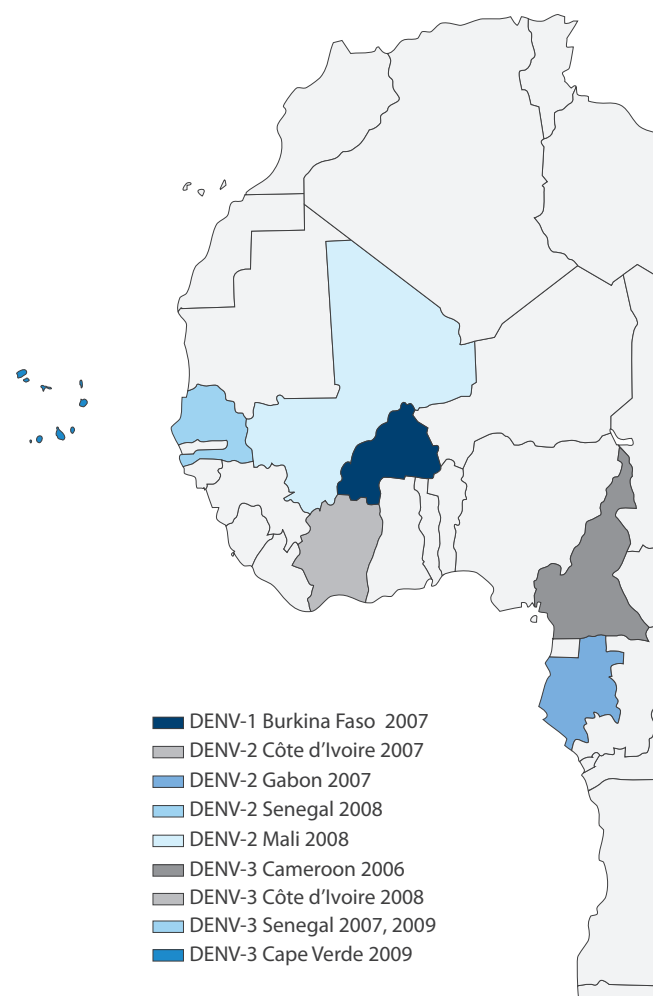
Reports on the importation of dengue virus to Europe have been increasing since the 1990s. Some of these are publications or reports from single countries or networks and show that the frequency of travel-acquired

dengue virus infections in Africa is low compared to south-east Asia and the Americas [15,16]. This distribution is due to two main factors: worldwide dengue virus activity and the popularity of certain countries as tourist destinations. In a study by the European Network on Imported Infectious Disease Surveillance (TropNetEurop) covering 481 European travellers between 1999 and 2002, 8% of dengue fever cases were imported from Africa [17], a proportion similar to that found in other European studies. In France, between 2002 and 2005, 14% of imported dengue fever cases originated in Africa [18]. Ten per cent of the cases in Austrian and Finnish travellers were also acquired in Africa, as analysed over a 15-year (1990-2005) and 10-year (1999-2009) period, respectively [19,20]. In 2008, dengue virus cases imported from Africa reported by TropNetEurop dropped to 4% [21].

Dengue virus importation from West Africa (2006-2008)

In recent years, dengue fever has been documented in travellers returning from several West African countries, caused in particular by DENV-3 which was recently identified in that region (Figure 1).

FIGURE 1
West African countries where dengue serotypes have been identified in recent years (2006-2009)



DENV: dengue virus.

Sources: ENIVD-CLRN, INVS (France), WHO, ECDC.

From January 2006 to August 2008, 19 imported cases of dengue virus infection were reported in travellers from West Africa in France: 11 cases from Côte d'Ivoire (one in 2006, three in 2007 and seven in 2008), four cases in Burkina Faso (one in 2006 and three in 2007), two cases in Benin in 2006, one case in Senegal in 2007 and one case in Mali in 2008 [22]. Dengue serotypes detected during this period were DENV-1 in Burkina Faso [22], DENV-2 in Côte d'Ivoire [22] and DENV-2 in a simultaneous outbreak of chikungunya and dengue viruses in Gabon [23], all in 2007. In 2008, DENV-2 serotype was identified in Mali [14] and in Senegal [24]. Moreover, DENV-3 was detected in a Japanese tourist and in a French expatriate returning from Côte d'Ivoire between May and July 2008 [25]. DENV-3 activity was also detected in East Africa in 2008 in a Finnish traveller returning from Eritrea (O. Vapalahti, personal communication).

Recent DENV-3 activity in West Africa

In the beginning of October 2009, a case of dengue fever was reported in a Senegalese returning to Italy after a holiday in his home country. DENV-3 infection was diagnosed at the National Institute for Infectious Diseases in Rome [26]. At the same time France reported DENV-3 in travellers returning from Senegal (C. Renaudat, personal communication). Also, ProMED posted several archives describing dengue virus outbreaks in Senegal: in the Kedoungo region [27] and Dakar [28]. The Pasteur Institute in Dakar identified DENV-3 in febrile patients (A. Sall, personal communication).

Meanwhile, an unprecedented outbreak has been detected in the Cape Verde archipelago in the beginning of September 2009 (week 40) [29]. This is the first

report of dengue virus activity in that country (Figure 1). The highest number of cases were reported during week 45 (5,512 cases), decreasing in week 47 to 1,447 cases and finally five cases in week 53. A total of 17,224 cases including six deaths were reported from 18 of the 22 municipalities in Cape Verde by the end of 2009. The municipality of Praia on Santiago island, notified the highest number of cases (13,000 cases) followed by Sao Felipe in Fogo Island (3,000 cases) [2]. The first samples tested at the Pasteur Institute in Dakar confirmed DENV-3 circulation [29].

Recently imported DENV-3 cases in Europe and the identified outbreaks in West Africa suggest that this serotype is spreading in the region.

We used a phylogenetic approach in order to determine genotype association among the recent DENV-3 circulating in the region, using sequences provided by ENIVD-CLRN laboratories. DENV-3 viruses are divided into four geographically different genotypes (I, II, III, and IV) [13]. The emergence of a virulent lineage of genotype III in Sri Lanka at the end of 1980s was largely associated with a high incidence of the disease and the emergence of Dengue haemorrhagic fever in Asia and

the Americas. All DENV-3 detected in East Africa from 1984 to 1993 [13] belonged to this lineage of genotype III (Figure 2).

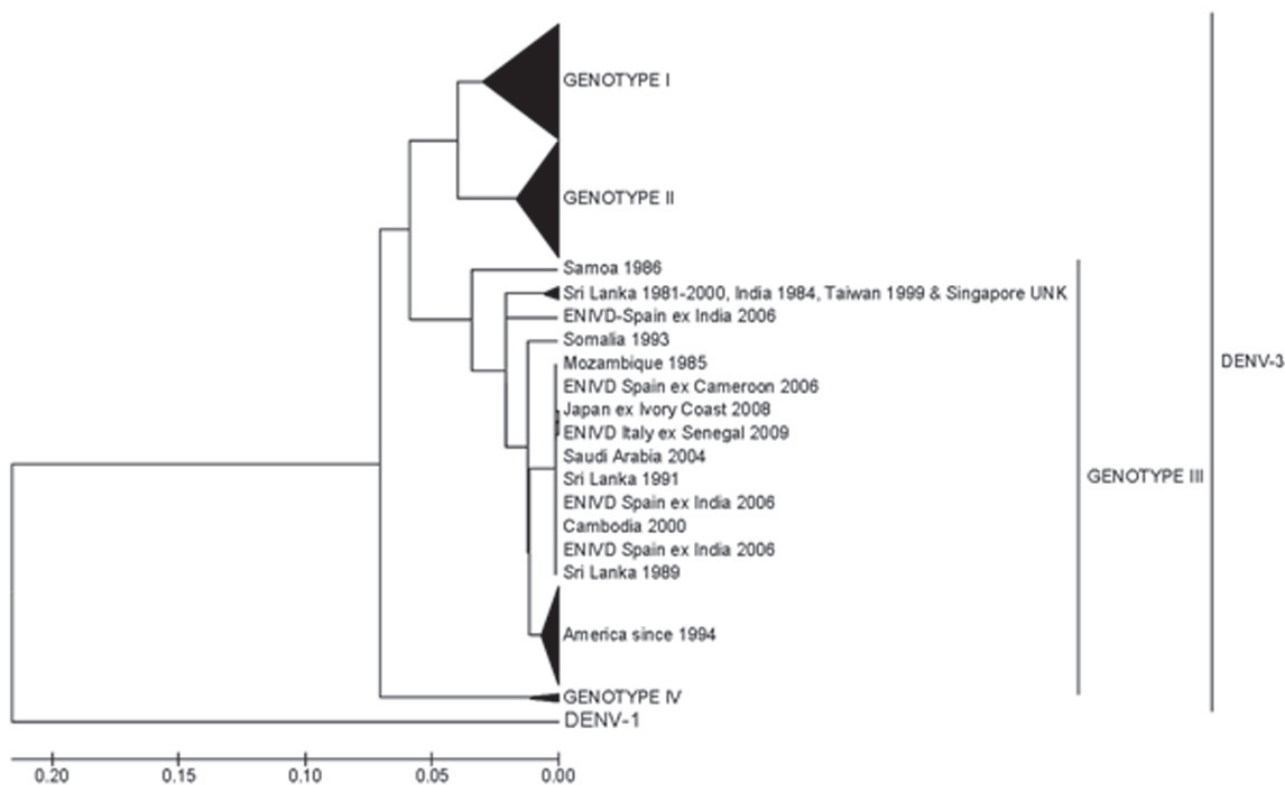
Also, isolates from geographically distant outbreaks, strains ENIVD Spain ex Cameroon 2006, Japan ex Ivory Coast 2008 and Saudi Arabia 2004 (Figure 2), and from Eritrea in 2008 (not shown in Figure 2; E. Huhtamo *et al*, unpublished results) belong to DENV-3 genotype III. The DENV-3 strain that circulated in Senegal in 2009 and was isolated from a traveller in Italy [26], also belonged to genotype III and was closely related to the DENV-3 strain circulating in Côte d'Ivoire in 2008. Therefore, the DENV-3 that has emerged in the Cape Verde archipelago is likely to have been introduced from a West African country due to the geographical proximity with strong trade and travel activities.

Future outlook

The recent DENV-3 expansion in West Africa was first detected in European travellers returning from this area, which triggered the alert for active surveillance in the exporting countries.

FIGURE 2

Phylogenetic tree of DENV-3 sequences



This tree is based on a 139 nt fragment of the E gene. Sequence identification is as follows: country of origin, year of identification. The sequences from imported cases are labelled as ENIVD/country of detection/country of exportation/year of detection. Phylogenetic analysis was conducted using MEGA 4.0 (Tamura, Dudley, Nei, and Kumar 2007). Genetic distance was calculated with the Tamura Nei algorithm. Phylogenetic tree was constructed using Neighbor-Joining model and the resultant tree was tested by Bootstrap (1,000 Replicates). Only bootstrap probabilities over 60% are shown.

However, although most of the African countries are prepared for surveillance of yellow fever and human immunodeficiency virus infections, most of them lack specific methods for dengue virus diagnostics and require new diagnostic tools. As a recent example of such technology transfer, the Pasteur Institute laboratories in Paris and Dakar have implemented differential diagnostics for dengue at the Pasteur Institute in Abidjan [14]. This model of cooperation is required also in other African countries.

As long as active dengue virus surveillance is poorly implemented in Africa, the study of febrile travellers returning to Europe could help to detect viral activity on the African continent.

As part of the ENIVD-CLRN, a collaborative study on imported chikungunya and dengue virus infections in European travellers will start in 2010. The aim of the study is to complete the global map of chikungunya and dengue virus circulation, including the global distribution of viral genotypes of those viruses. It will permit clinicians to compare the clinical symptoms, signs and analytical data of imported cases in Europe. The surveillance of travellers returning to Europe will continue to improve our knowledge about dengue virus distribution in Africa.

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Salmonella meningitis and septicaemia in a non-immunocompromised adult, associated with a cluster of Salmonella Enteritidis PT 14b, Ireland, November 2009

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We report a fatal case of meningitis caused by *Salmonella* Enteritidis phage type 14b in a middle-aged man who had no history or findings to suggest he was immunocompromised. To our knowledge, this is the first reported case of *Salmonella* meningitis in an adult in Ireland, and the first case of meningitis in an adult caused by phage type 14b. This case was associated with a nationwide cluster of salmonellosis which is still under investigation at the time of writing.

Introduction

Salmonella infection is recognised as a common cause of gastroenteritis which can result in large outbreaks [1]. Acute bacterial meningitis is a rare manifestation of *Salmonella* infection, and when it does occur, it is most commonly a disease of infants [2,3]. In adults, *Salmonella* meningitis, although rare, is most commonly seen in patients with impaired immunity, particularly in infection with human immunodeficiency virus (HIV) [4,5].

In this report, we describe a case of a non-immunocompromised adult with *Salmonella* Enteritidis meningitis and severe sepsis, with a rapid onset and a fatal outcome, that occurred in Dublin in November 2009.

This case is one of a cluster of at least 15 cases of *Salmonella* Enteritidis phage type 14b infection in Ireland that started in October 2009 [6; Health Protection Surveillance Centre, personal communication]. This variant has also been implicated in over 443 cases, including 14 outbreaks, in the United Kingdom since August 2009 [7,8].

Case description

The patient was a man in his late 40s who had a stable chronic mental illness and lived in a community psychiatric hostel in an urban area, supervised by 24-hour nursing staff. His regular medications included clozapine, amisulpride and valproate. Regular blood tests

for potential side effects such as agranulocytosis did not show any abnormalities. He was a regular smoker. There was no abuse of alcohol or intravenous drugs and no known risk factors for HIV infection.

The patient woke in the early morning hours with a headache, which was relieved by treatment with 1 g paracetamol. After a quiet night he was found agitated and feverish six hours later. He was seen by a doctor and, due to rapid deterioration, was transferred by ambulance to the nearby acute hospital as an emergency with a seven-hour history of headache and a two-hour history of fever (38.5 °C), rigors, inability to stand and progressive reduction in level of consciousness. He was admitted to the intensive care unit on the same day.

On admission, a lumbar puncture revealed turbid cerebrospinal fluid, with a milky-brown colour. Three samples of cerebrospinal fluid (CSF) were taken at the time of admission. All of them showed a white blood cell count of >5,000 and a red blood cell count of zero. Microscopy of the CSF, performed urgently, showed abundant Gram-negative bacilli. A blood sample taken on admission showed a leukocyte count of $8.97 \times 10^9/L$, with a neutrophil count of $8.13 \times 10^9/L$. Plasma urea, electrolytes, liver enzymes, total protein and albumin concentration were all within the normal range at that time.

A progressive neutrophilia was documented, with a count of $17.73 \times 10^9/L$ on day 2, rising to $31.61 \times 10^9/L$ on day 4 of hospitalisation. The patient developed acute renal failure on day 2 of hospitalisation.

The patient received one dose of 2 g cefotaxime and 2.4 g benzylpenicillin in the emergency department. After Gram-negative organisms were identified in the CSF samples taken on admission, this treatment was discontinued and the patient received 2 g meropenem

three times a day and 1 g vancomycin twice a day for the following three days. On day 4 of hospitalisation, cultures from the CSF samples and a series of three blood samples taken on the day of admission grew *Salmonella* sensitive to cefotaxime, and the patient's treatment was changed to 1 g cefotaxime every four hours.

In view of known association between *Salmonella* meningitis in adults and immunodeficiency, and despite the absence of risk factors, the patient was tested on day 5 for HIV, hepatitis B virus and hepatitis C virus infections, all of which were negative. Nor were there any incidental clinical signs, radiographic or laboratory findings to suggest underlying malignancy or opportunistic infection.

Despite treatment with appropriate antibiotics and interventions to support failing organ systems, he deteriorated and died five days after admission. The final *post mortem* report was not available at the time of writing this report.

Environmental investigation

In view of the fatal outcome, and despite the fact that no staff member or resident at the hostel had a history of gastroenteritis, stool samples from staff and fellow residents were obtained as part of the public health investigation. In addition to testing samples of food, eggshells and water from the hostel, other food premises where the patient was known or thought to have eaten were inspected, foods sampled and distribution chains traced. Particular attention was paid to foods containing chicken, eggs or egg products of any kind. None of the dozens of stool or food samples grew *Salmonella*.

The *Salmonella* species involved was nalidixic acid-resistant *Salmonella* Enteritidis phage type 14b. The same phage type has been identified in a cluster of cases notified to Irish departments of public health since November 2009, all of whom had gastro-enteritis alone and made a full recovery [6]. As with the above case, meticulous tracing of relevant foods through the distribution chains was and is being conducted for all cases, but to date the source of infection has not been identified.

Discussion

Human *Salmonella* infection is categorised into four manifestations: enteric infections, sepsis, non-enteric focal infections (including meningitis) and a chronic carrier state [3]. Bacterial meningitis is characterised by acute onset of fever, headache and one of the following signs: neck stiffness, altered consciousness or other meningeal signs [9].

The first case of *Salmonella* meningitis in the literature was reported in 1907 by Ghon [10]. In a study of 7,779 infections identified at the New York *Salmonella* Centre, meningitis accounted for only 0.8% [3]. In

adults, *Salmonella* meningitis is most commonly seen in patients with intercurrent illness [11,12], including particularly immunosuppression associated with HIV [4,5].

Irish legislation requires doctors to send notifications of infectious diseases, including *Salmonella* infections, to medical officers of health in regional public health departments. Notification of bacterial meningitis from any cause must also be given. These data are collated at the national level by the Health Protection Surveillance Centre (HPSC). In a review of national data for the ten-year period from 2000 to 2009, the number of notifications of bacterial meningitis in Ireland was 1,229 and the number of notifications of *Salmonella* infection was 4,395, including 88 cases of typhoid and/or paratyphoid. The data from this period include only one case of *Salmonella* meningitis, a three-week-old baby with S. Dublin (medical officer of health, personal communication). Before this period, Foley *et al.* published in 1980 one series of three cases of childhood *Salmonella* meningitis in Ireland; all were infants [13].

Of all *Salmonella* infections listed in 2008 in the database of enteric infections collected by Enter-net, the European surveillance network for human gastrointestinal infections, *Salmonella* Enteritidis was by far the most common serotype, while S. Typhi, Oranienburg, Paratyphi and Berta were not listed among the top ten [14]. However, the European literature includes only two previous case reports of adults with meningitis due to *Salmonella* Enteritidis, one of whom was immunocompromised [15,16]. Other cases of *Salmonella* meningitis in adults previously reported in the literature have involved a diversity of serotypes, including at least 19 cases of S. Typhi [17-22], two cases of S. Typhimurium [18,11], and one case each of S. Oranienburg [23], S. Virchow [24], S. Paratyphi [21] and S. Berta [25].

The case described here is the only notified case of *Salmonella* meningitis in an adult in Ireland in the last ten years, and the first published adult case in Ireland. Kauffman *et al.* reported that *Salmonella* meningitis, arising in association with a variety of serotypes, may present without preceding symptoms of gastroenteritis [11], as was also true in our case.

There were no clinical signs, laboratory or radiographic findings to suggest a compromised immune system in this case. While agranulocytosis is a recognised adverse effect of clozapine, the fact that the patient's white blood cell count was monitored regularly and that he developed a marked neutrophilia indicates that this was not a factor.

Conclusion

We describe the first reported case of *Salmonella* meningitis in an adult in Ireland, who was not immunocompromised. The association of this fatal case with a phage type that has also been implicated in a large

number of sporadic cases and several recent outbreaks in the United Kingdom indicates the need for continuing vigilance in terms of surveillance and investigation to reduce the risk of further such infections with *Salmonella* Enteritidis PT 14b.

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Dispensing of antibiotics without prescription in Greece, 2008: another link in the antibiotic resistance chain

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Antibiotic resistance has been associated with the use of antibiotics. The dispensing of antimicrobials without prescription is a potential source of inappropriate antibiotic use. In our study, antibiotics were requested without prescription from pharmacies in the metropolitan area of Athens in Greece in 2008. Twenty-one collaborators visited 174 pharmacies and asked for either amoxicillin/clavulanate acid or ciprofloxacin without providing a prescription or any other justification for the request. In Greece additional restrictions for fluoroquinolone prescriptions were implemented in 2003 after which a separate specific prescription form needs to be filled in by the prescriber, justifying the choice of any fluoroquinolone. Amoxicillin/clavulanate acid was dispensed in all cases. Furthermore, despite the regulation restricting the prescription of ciprofloxacin, this drug was dispensed by 53% of the pharmacies. It appears that the implementation of measures to restrict the use of certain antibiotics (e.g. ciprofloxacin that was studied in our case) was effective in reducing, although not eliminating, inappropriate dispensing. Overall, dispensing of antimicrobials without prescription is a widespread practice in the studied area and is contributing to the overuse of antibiotics.

Introduction

Antibiotic resistance is a major public health threat worldwide. There is plenty of evidence that the use of antibiotics is associated with the emergence of resistance [1]. Rates of antibiotic consumption correlate closely with rates of antimicrobial resistance at national level, including both the hospital environment and outpatient settings [2,3]. Variation in resistance correlates with variability in antibiotic use across Europe [4,5]. This difference reflects socio-economic, healthcare system, legislative and cultural differences among European countries [6]. To a large extent, this variation is a result of the over- and misuse of antibiotics, especially for upper respiratory tract infections, many of which are of viral cause. Excessive use is partly attributed to inappropriate prescriptions by physicians. However, self-medication with antibiotics is also a known practice in various European countries,

especially in the southern and eastern parts [6,7], and this may exacerbate the problem [6,7]. The two main sources of self-medication include the use of left-over antibiotics from previous courses of treatment and the acquisition from pharmacies without prescription [8].

In Greece, both the use of antibiotics and resistance rates are high, including resistance to penicillin and macrolides among community-acquired isolates of *Streptococcus pneumoniae* [9] and *Streptococcus pyogenes* [10]. Since 1950, national law forbids the dispensing of antibiotics 'over the counter' without a prescription. Additionally, since 2003 and according to an official decision by the Ministry of Health, a separate specific prescription form needs to be filled in by the prescriber, justifying the choice of any fluoroquinolone or third generation cephalosporin prescribed orally, before the drug is dispensed. This measure was implemented in an effort to preserve the efficacy of these classes of antimicrobials. However, there is no control of the actual practice and in effect the law is not implemented. It is generally accepted by the public that antibiotics can be acquired from pharmacies without prescription, either by direct purchase or by retrospectively providing a prescription. The present study was undertaken in order to quantify the extent of this practice.

Methods

Pharmacies in the greater Athens metropolitan area, were visited from April to May 2008 by 21 voluntary collaborators, who asked for either ciprofloxacin or amoxicillin/clavulanate acid without providing a prescription or any other relevant justification. Amoxicillin/clavulanate acid was chosen for our study because it is one of the most frequently used antibiotics and ciprofloxacin because of the additional restrictions in dispensing for fluoroquinolones in Greece. The collaborators were advised to neither insist in case the antibiotic was refused, nor to simulate any specific disease or symptom, in order not to influence the pharmacist in the decision. Collaborators included physicians, nurses and laboratory technicians, but the identity or occupation was not revealed during the visit. For the

purposes of the study, the greater Athens area was divided in four main regions of comparable size – centre, northern suburbs, western suburbs and southern suburbs with respective populations of 745,000, 594,000, 469,000 and 784,000 people, according to a 2001 census [11]. The total number of pharmacies in the greater Athens area was 3,426 in 2006 according to the most recent available data from the national statistical service of Greece [11]. The number of pharmacies visited in each area was chosen to roughly reflect the population. Each collaborator was assigned a specific area and the pharmacies visited were chosen randomly by the collaborator. All pharmacies visited were privately owned small enterprises. During the visit there was no way of differentiating between pharmacy employees and pharmacist, and the voluntary collaborators did not attempt to do this as it might have compromised the aim of the study. The collaborators purchased the antibiotic and the number of pharmacies that dispensed the antibiotics was recorded. In addition the reason for refusing sale of the requested drugs and any comments made by the pharmacist, were recorded. The difference between dispensing the non-restricted (amoxicillin/clavulanate acid) and the restricted (ciprofloxacin) antibiotic was compared using the Fisher's exact test.

Results

One hundred and seventy-four visits in different pharmacies were performed, covering 5% of the 3,426 pharmacies in the greater Athens area. Ciprofloxacin was requested during 102 visits and it was dispensed by 54 pharmacies (53%). Amoxicillin/clavulanate acid was requested during 72 visits and it was dispensed in all cases (100%). The difference between restricted and non-restricted antibiotics was significant ($p < 0.001$). Antibiotic dispensing in different areas of Athens is shown in the Table. Dispensing practices did not differ significantly in the various areas.

In 107 (85%) of the 126 visits where the antibiotic was sold without prescription, no comment was made by the pharmacist and no reason for the intended antibiotic use was requested. In all 48 cases where dispensing of ciprofloxacin was refused, the reason offered by the pharmacist was the requirement of the special prescription form for fluoroquinolones. In one case the

pharmacist refused ciprofloxacin and offered amoxicillin/clavulanate acid as an alternative. In three (6%) of the cases where ciprofloxacin was sold, the pharmacist informed the collaborator that “normally a physician's prescription is required”. The same occurred only once when amoxicillin/clavulanate acid was requested. During another visit the pharmacy employee initially refused to dispense ciprofloxacin but the drug was consequently dispensed by the pharmacist. In only three cases of amoxicillin/clavulanate acid dispensing the collaborator was informed by the pharmacist about adverse events - mainly allergies and diarrhoea - or asked whether such events had occurred in the past when the buyer had used antibiotics. In three other cases information on dosage was provided by the pharmacist.

Discussion

The results of this study indicate that antibiotics can be very easily bought in Greek pharmacies without prescription. No pharmacist refused to dispense amoxicillin/clavulanate acid without prescription, and none asked for any justification for the purchase. Even ciprofloxacin, that is supposed to be restricted and for which a special prescription form needs to be filled in, in order for it to be dispensed, was freely dispensed in more than half of the pharmacies visited.

It appears that the implementation of measures to restrict the use of certain antibiotics (e.g. ciprofloxacin that was studied in our case) was effective in reducing, although not eliminating, inappropriate dispensing. It is notable that the only reason offered by the pharmacists for not selling ciprofloxacin, was the requirement of the special fluoroquinolone prescription form, implemented in Greece. The latter finding may well justify extending this measure to all classes of antibiotics, in addition to implementing measures aimed at curbing antibiotic dispensing without prescription.

The present results complement those of another Greek study, that included visits in pharmacies in north-western Greece by actors simulating rhinosinusitis and asking for antibiotics [12]. In that study 69 to 86% percent of the pharmacists offered an antibiotic. In our study no justification for the request of antibiotics was given by the researchers and specific antibiotics were requested.

In contrast to previous studies, where actors simulating patients made visits to the pharmacies [12-14], antibiotics were asked for and purchased without any justification whereas in the present study the collaborators avoided simulating specific symptoms or diseases. Although this is not the only way that antibiotics are requested at the pharmacy, it indicates the ease of obtaining antibiotics without prescription, as if antibiotics were everyday commodities.

The amount of antibiotics dispensed without prescription in Greece is not known. From a large public

TABLE

Dispensing of ciprofloxacin without prescription in pharmacies by areas in Athens, Greece, April-May 2008 (n=54)

	Number of pharmacies visited	Number of pharmacies dispensing ciprofloxacin (%)
Western suburbs	26	14 (54)
Northern suburbs	22	11 (50)
City centre	26	12 (46)
Southern suburbs	28	17 (61)
Total	102	54 (53)

survey with personal interviews conducted in 2007 that included over 1,000 individuals in Greece (Giamarellou, personal communication), 25% of the sample used antibiotics in the last three months, the greater majority for upper respiratory tract infections. Twelve per cent of the sample admitted purchasing antibiotics without prescription. A questionnaire survey of 173 adults and 150 parents carried out in the urban area of Patras, Greece in 2003, revealed that 74.6% of the adults consumed non-prescribed antibiotics and 22.7% of parents administered non-prescribed antibiotics to their children [15]. Furthermore, in the same study 55% of the participants admitted to using left-over antibiotics. Availability of antibiotics 'over the counter' is considered by the authors as one of the main reasons accounting for these observations. Self-medication by 'over the counter' antibiotics appears to be an extensive problem in Greece and this practice may contribute significantly to excess antibiotic use and increased antibiotic resistance.

Similar problems have been described in other European countries. In Spain 31% of antibiotics consumed were found to be dispensed by community pharmacies without prescription, although it is not legal [16]. Even after an extensive institutional campaign launched by the Ministry of Health in 2007 aiming at reducing this practice, antibiotics were dispensed without a prescription by 79.7% of the pharmacies studied in Catalonia just one year after the campaign [13]. In Malta 19% of the participants in a study admitted to self-medication with antibiotics, which were provided without prescription by local pharmacies in 85% of cases [17]. In the United Kingdom a survey on antibiotic use included in the 2003 Office for National Statistics Omnibus Household Survey revealed that 4.8% of adults surveyed had at least once taken an antibiotic without advice from a doctor, dentist or nurse [18]. This phenomenon is not restricted to Europe. One of the largest questionnaire studies on antibiotic use carried out in 2001 in nine countries (United Kingdom, France, Belgium, Italy, Spain, Turkey, Thailand, Morocco and Colombia) indicated that antibiotics could be obtained without prescription in all countries [19]. In Brazil 58-74% of the pharmacies studied dispensed antibiotics 'over the counter' [14]. Non-prescribed antimicrobial drug use has also been described in latino communities in the United States [20].

Antibiotics are generally considered safe and 'over the counter' use has been advocated, for certain specific indications [21]. However, in contrast to all other groups of medicines, in addition to possible adverse effects to the consumer, antibiotics have an effect on the community, namely the emergence of resistance. In this study, information about possible adverse events to the individual was only provided by the pharmacy in a minority of cases. Important questions relevant to antibiotic resistance, including previous use of antibiotics, were not asked by the pharmacists. In addition no comment was made in most of the cases of the indication of

the antibiotic dispensed, risking administration of an ineffective antibiotic and inadequate treatment. These observations indicate that the dispensing of antimicrobials at the pharmacy without prescription, at least as practiced in Greece and witnessed by the present study, may be unsafe.

The dispensing of antibiotics without prescription is not the only way of antibiotic overuse and misuse. Leftovers from previous treatment courses due to non-completion of the previous treatment or the dispensing of excess medication, acquisition of antibiotics over the internet and inappropriate prescription by physicians for viral upper respiratory infections are further contributing factors [8]. These causes need to be addressed in order to ensure that this valuable class of medications is safeguarded.

Possible ways of intervention would include educating the public and pharmacists as well as stricter implementation of regulations already in force, especially regarding antibiotics. According to Greek legislation antibiotics are prescription-only medicines, however this law is not applied as there is neither control nor penalty for those not abiding by it. The European Union (EU) supports and encourages policies on the prudent use of antimicrobials in order to control antimicrobial resistance and recommends control measures to support the prudent use of antimicrobials, e.g. by "restricting systemic antimicrobial agents to prescription-only use" [22]. It is the responsibility of EU Member States to ensure the implementation of these strategies on a national level in order to avoid or limit microbial resistance. Eliminating the dispensing of antibiotics without prescription could be a valuable measure towards this goal.

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New WHO influenza vaccine recommendations for use in the 2010-11 influenza season in the northern hemisphere

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The World Health Organization (WHO) today, 18 February 2010, published recommendations for the composition of influenza virus vaccines for the forthcoming season in the northern hemisphere (November 2010 to April 2011) [1]. They foresee a trivalent vaccine including also a 2009 pandemic influenza A(H1N1) strain:

- an A/California/7/2009 (H1N1)-like virus;
- an A/Perth/16/2009 (H3N2)-like virus*;
- a B/Brisbane/60/2008-like virus.

* A/Wisconsin/15/2009 is an A/Perth/16/2009 (H3N2)-like virus and is a 2010 southern hemisphere vaccine virus.

Each year, representatives of the WHO Collaborating Centres on Influenza meet to analyse the data and make recommendations for the following year's vaccine strains. The recommendations are used by pharmaceutical manufacturers to update the composition of the vaccine, so that vaccine strains are matched to circulating strains the following year. WHO also provides the manufacturers with prototype strains for the seasonal vaccine.

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