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HIV and AIDS in the European Union, 2011

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In 2011, a total of 28,038 new HIV diagnoses were reported by European Union and European Economic Area countries. The annual rate of HIV diagnoses does not show clear signs of decrease and HIV continues to be concentrated in selected populations such as men who have sex with men and injecting drug users, and a high proportion reported as late presenters. Despite effective and available antiretroviral treatment, the number of AIDS cases increased in a few countries.

HIV diagnoses

Since 2008, the European Centre for Disease Prevention and Control (ECDC) has been coordinating enhanced HIV/AIDS surveillance for the European Union (EU) and European Economic Area (EEA). Data are submitted annually to the European surveillance system (TESSy) in standardised datasets for HIV and AIDS.

In the EU/EEA, 28,038 HIV infections were diagnosed in 2011 and reported by 29 EU/EEA countries, a rate of 6.3 per 100,000 population when adjusted for reporting delay [1]. The overall rate for men was 8.7 per 100,000 population and 2.8 per 100,000 population for women. The highest rates (per 100,000 population) were observed in Estonia (27.3), Latvia (13.4), Belgium (10.7) and the United Kingdom (10.0). The lowest rates were reported by the Czech Republic (1.5) and Slovakia (0.9). Some 11% of HIV infections were reported among young people aged 15–24 years and 25% were female. The overall male-to-female ratio was 3.0 and highest in Slovakia (15.3), Hungary (11.1), Czech Republic (10.8) and Slovenia (6.9) (Figure 1).

Men who have sex with men (MSM) accounted for 39% of new HIV diagnoses (n=10,885) in 2011 in the EU/EEA (38% in 2010 [2]; 35% in 2009 [3]). MSM accounted for more than 50% of the cases in nine countries and more than 30% in another eight countries. Heterosexual transmission accounted for 36% of the HIV infections (n=10,118): more than a third of those cases originated from sub-Saharan Africa countries with a generalised HIV epidemic. More than half of the heterosexually acquired HIV infections in Belgium, Sweden, United Kingdom, Ireland and Norway were reported in persons originating from sub-Saharan Africa. There were 4,384 HIV cases (16%) reported in persons from sub-Saharan Africa in total: they were over-represented in the following transmission modes, as shown in the Table: heterosexual contacts (37%) and mother-to-child transmission (46%). Only 5% (n=1,516) of HIV diagnoses were reported in injecting drug users (IDU). Injecting drug use as predominant mode of transmission was reported in only two countries: Lithuania and Iceland. IDU accounted for 25% or more of the cases in Bulgaria, Greece, Latvia and Romania. Of the remaining 297 cases with reported transmission mode, 222 (1%) were classified as due to mother-to-child transmission and 75 (0.3%) due to transfusion of blood or its products and nosocomial transmission.

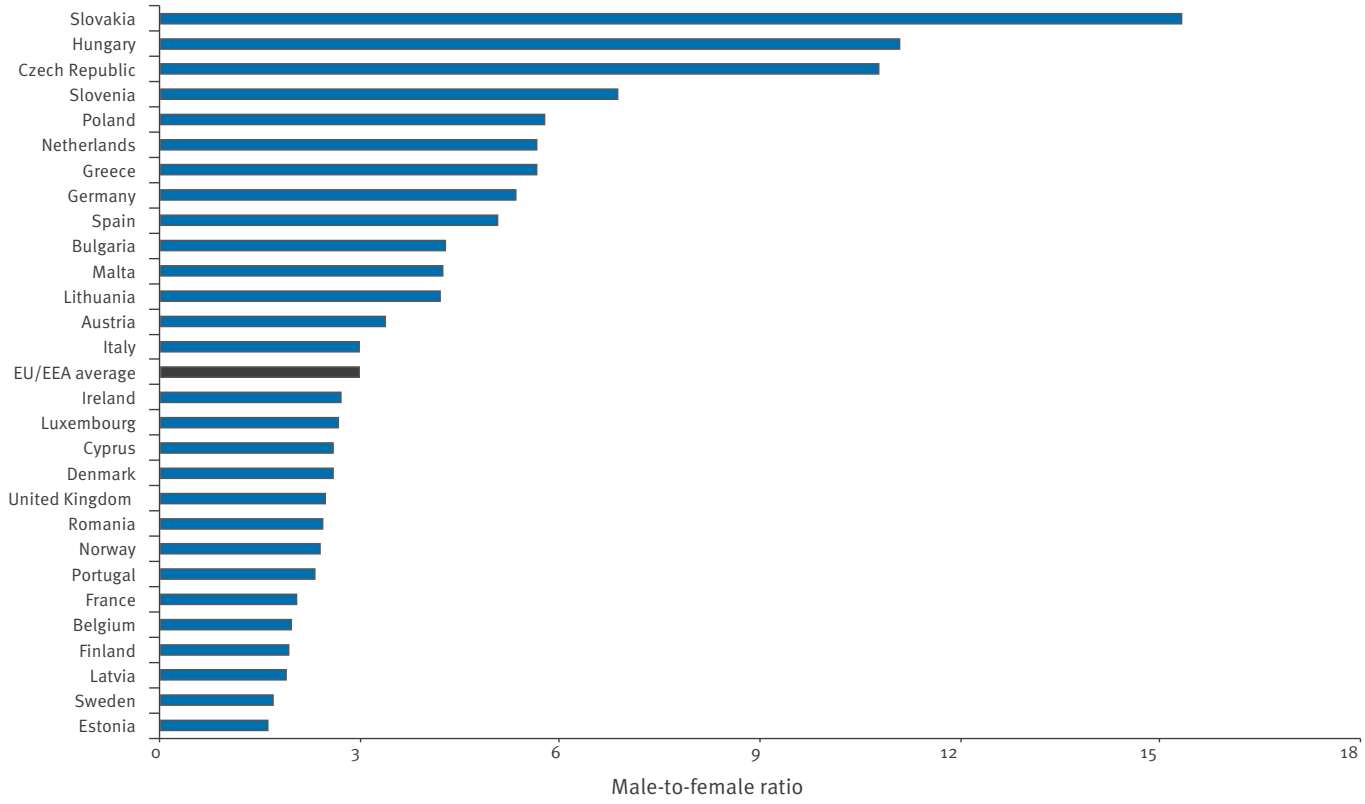
Trends in HIV diagnoses

Among the 29 EU/EEA countries that have consistently reported HIV data since 2004 (no data from Liechtenstein), the rate of HIV cases per 100,000 population has been relatively stable, despite a slight decrease from 6.5 in 2004 to 6.3 in 2011 when adjusted for reporting delay. In recent years, more than 27,000 cases were diagnosed and reported each year, resulting in a cumulative number of over 420,000 cases reported since the beginning of the HIV reporting (Figure 2). Since 2004, the number of national annual HIV diagnoses has tripled in Bulgaria, Iceland and Slovakia and has increased by more than 50% in Cyprus, Czech Republic, Greece, Hungary, Romania and Slovenia.

Since 2004, 25 EU/EEA countries have consistently reported HIV cases by transmission mode (Estonia, Poland, Italy and Spain were excluded; no data from Liechtenstein). The overall number of reported cases among MSM increased by 22% between 2004 and 2011; an increase of more than 100% was observed in Cyprus, Hungary, Czech Republic, Ireland, Latvia, Slovakia and Slovenia. The total number of annually reported heterosexually acquired cases ranged from 4,300 to 5,300 during 2004 to 2011. The number of cases originating from sub-Saharan African countries decreased by 54% (6,874 in 2004 to 3,159 in 2011). In most countries, the numbers reported among IDU were low or decreasing. However, in 2011, a substantial increase was reported: Greece reported 245 cases as compared with 22 in 2010, and Romania 108 cases versus nine in 2010. In several other countries, Bulgaria, Latvia and Iceland,

Figure 1

Male-to-female ratio in HIV infections by country, European Union/European Economic Area countries, 2011 (n=27,963)[a]



EEA: European Economic Area; EU: European Union.

[a] Information on sex or country not available for 75 persons.

Source: [1].

Table

Reported HIV diagnoses, including those originating from sub-Saharan African countries with a generalised HIV epidemic, by transmission mode, European Union/European Economic Area countries, 2011 (n=28,038)

Reported HIV diagnoses[a]	Mode of transmission						Total
	Heterosexual contacts	Injecting drug use	Sex among MSM	MTCT	Nosocomial and transfusion	Unknown	
HIV infections originating from countries of sub-Saharan Africa (%)	3,744 (37.0%)	27 (1.8%)	143 (1.3%)	103 (46.4%)	19 (25.3%)	348 (6.6%)	4,384 (15.6%)
Total number of cases	10,118	1,516	10,855	222	75	5,252	28,038

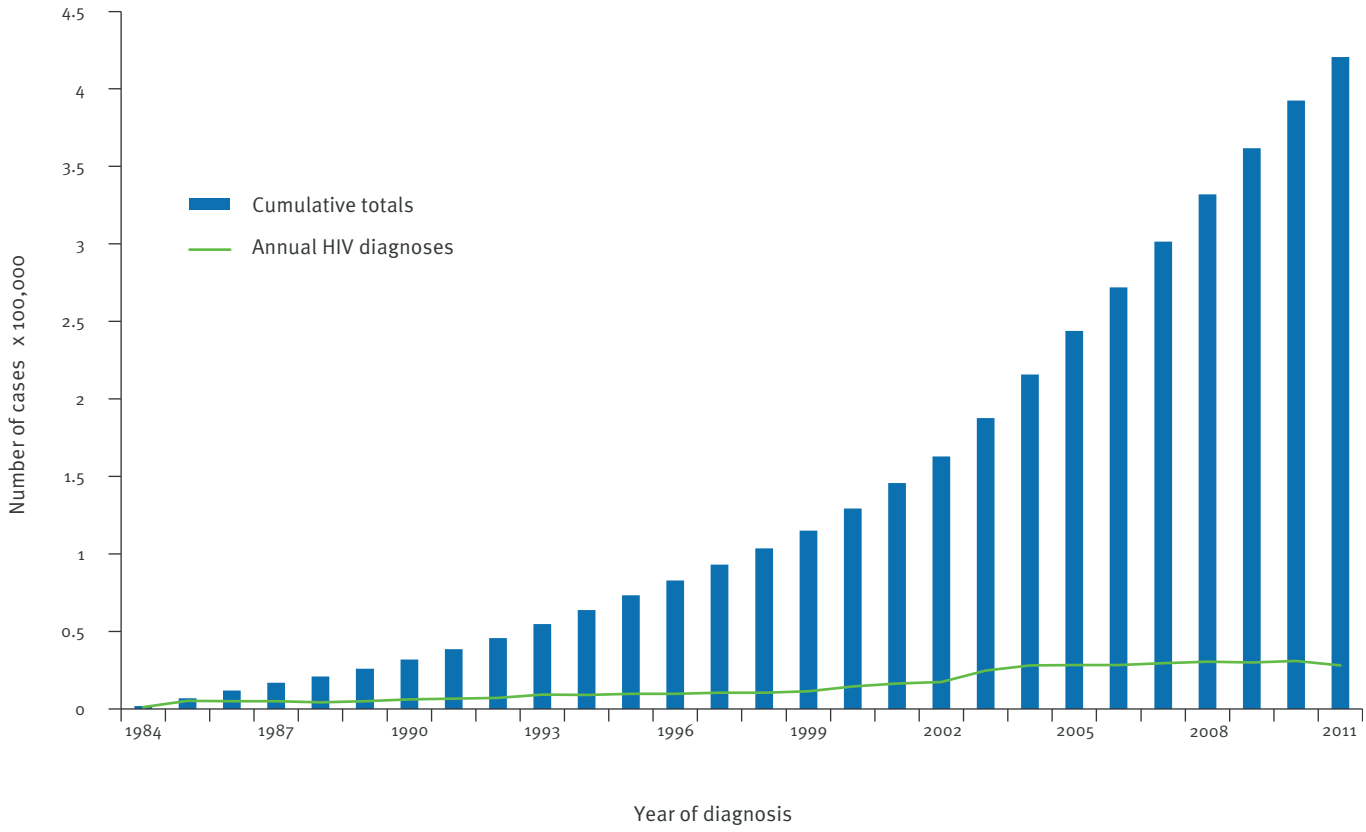
MSM: men who have sex with men; MTCT: mother-to-child transmission.

[a] In many European countries, migrants from countries with generalised HIV epidemics comprise a high proportion of reported cases. As a proxy, HIV diagnoses in persons originating from sub-Saharan Africa are chosen.

Source: [1].

Figure 2

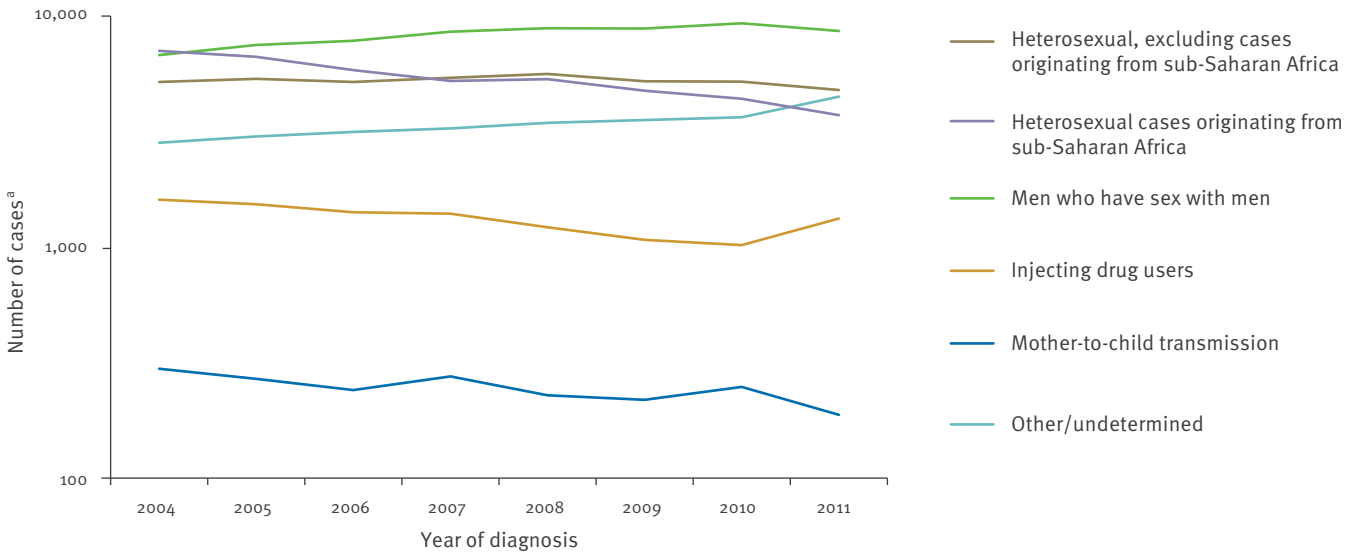
HIV diagnoses reported annually and cumulative totals, European Union/European Economic Area countries, 1984–2011



Source: [1].

Figure 3

Reported HIV diagnoses by transmission mode and year of diagnosis, adjusted for reporting delay, European Union/European Economic Area countries, 2004–2011



[a] Semi-logarithmic scale.

Source: [1].

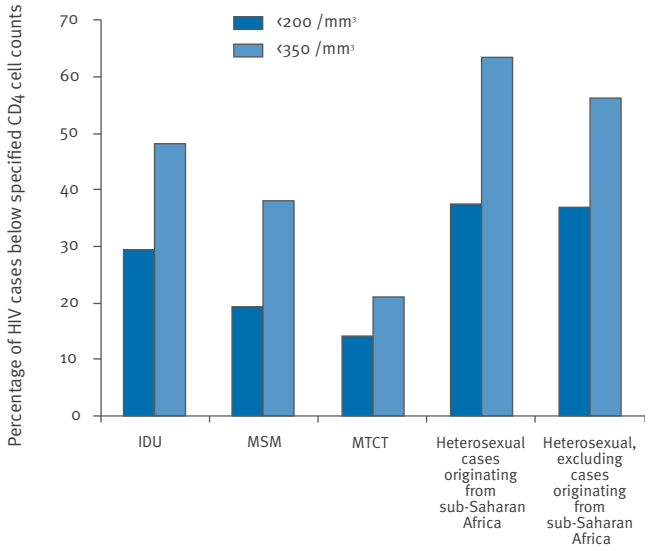
a more gradual increase in HIV cases in IDU was noted over the last two to three years. The number of cases transmitted from mother to child decreased by 36%, from 295 in 2004 to 188 in 2011. The number of cases with unknown transmission mode increased by 30%, from 2,773 in 2004 to 3,611 in 2011 (Figure 3). It has to be acknowledged that there are reporting delays for a number of countries, which limit the interpretation of the trends in the most recent years. Reporting delay affects all transmission modes consistently and adjusting for delay results in an increase of 4–10% in the number of reported cases for 2011.

AIDS diagnoses

In 2011, a total of 4,424 AIDS diagnoses were reported by 28 EU/EEA countries (no data from Sweden or Liechtenstein), a rate of 0.9 per 100,000 population. The highest rates (per 100,000 population) were reported by Estonia (2.8), Latvia (4.8), Portugal (2.8) and Spain (1.8). In these EU/EEA countries, an overall 33% decline was observed, from 9,195 cases (1.9 per 100,000 population) in 2004 to 4,424 (0.9 per 100,000 population) in 2011, although there is a considerable reporting delay and under-reporting for the most recent years. The number of AIDS diagnoses decreased in the majority of the countries, but since 2004, an increase of more than 20% was observed in Bulgaria (81%), Czech Republic (77%), Estonia (31%), Hungary (39%) and Slovenia (50%).

Figure 4

Percentage of HIV cases with a CD4 cell count <350/mm³ and <200/mm³ by transmission mode, European Union/European Economic Area countries, 2011 (n=15,625)



IDU: injecting drug users; MSM: men who have sex with men; MTCT: mother-to-child transmission.

Source: [1].

Late presenters

Late presenters are defined as persons with a CD4 cell count less than 350/mm³ at the time of HIV diagnosis. CD4 cell counts were available for 15,625 HIV diagnoses (56%) in adults and adolescents reported in 2011. Cell counts were available for more than half of the HIV cases reported in 19 EU/EEA countries. Among those, 49% were reported with a CD4 cell count ‹350/mm³, including 29% of cases with a CD4 cell count ‹200/mm³, categorised as advanced HIV infection. By transmission mode, the highest proportion of cases with CD4 ‹350/mm³ was observed among heterosexually acquired cases, especially among those originating from sub-Saharan Africa (63%). The lowest proportion of cases with CD4 ‹350/mm³, as well as those with CD4 ‹200/mm³, was observed among cases due to mother-to-child transmission (21% and 14%, respectively) and MSM (38% and 19%, respectively) (Figure 4).

Conclusions

HIV surveillance data show that the population of people living with HIV in the EU/EEA is increasing due to effective and widely available treatment and due to the number of new HIV diagnoses reported annually. HIV continues to be highly concentrated in specific populations, such as MSM, IDU and persons originating from sub-Saharan African countries. Although there is an apparent decline in the number of HIV diagnoses among IDU, substantial numbers of HIV diagnoses were reported in the Baltic States and recent outbreaks of HIV infection in Greece and Romania signal the potential for rapid spread of HIV among IDU [4-8].

Interventions to control the HIV epidemic need to be adapted to the national epidemiological situation. In the EU/EEA, the prevention and control of HIV infection among MSM is the cornerstone of the HIV response. In addition, as a third of the heterosexual HIV cases were reported in people from sub-Saharan Africa with a generalised HIV epidemic, countries need to ensure that treatment and care are accessible to these migrant populations. The observed increase in the number of HIV cases among IDU in a number of countries demonstrates the importance of maintaining or scaling up of harm reduction interventions in the EU/EEA.

It is of concern that half of the HIV cases with information on CD4 cell counts are diagnosed with a low count, when individuals are already eligible for treatment. It is of equal concern that the number of AIDS diagnoses is increasing in a number of countries despite the widespread availability of effective antiretroviral therapy. Delayed start of lifesaving HIV treatment decreases the clinical benefits, as well as the preventive value of the treatment in terms of further HIV transmission. HIV counselling and testing need to be promoted to ensure earlier diagnosis, access and adherence to treatment that will in turn result in the reduction of transmission. Equal access to HIV treatment and care for all should be ensured by countries, to sustain high quality of life

of citizens and to reach the (inter)national commitments even in times of economic austerity.

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Sequencing and phylogenetic characterisation of a fatal Crimean – Congo haemorrhagic fever case imported into the United Kingdom, October 2012

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A patient with fever, and haemorrhagic symptoms was admitted to a hospital in Glasgow on 2 October 2012. Since he had returned from Afghanistan, serum samples were sent for diagnosis at the Rare and Imported Pathogens Laboratory, where a real-time reverse transcriptase-PCR diagnosis of Crimean – Congo haemorrhagic fever was made within 3hrs after receipt of the sample. Hereafter the patient was transferred to a high-security infectious diseases unit in London but died on 6 October.

Case report

A 38 year-old male resident of the United Kingdom (UK), returned to Glasgow on Tuesday 2 October 2012 after having visited Afghanistan. He arrived in Glasgow from Kabul, Afghanistan via Dubai and was symptomatic from approximately 28 September with fever, diarrhoea, abdominal pain, haemoptysis and haematemesis. He attended the Emergency Department of a local hospital within three hours of his arrival from where he was transferred to a negative pressure room at Gartnavel General hospital, Glasgow. The patient had alanine aminotransferase (ALT) levels of ›1,000 IU/L (norm: 20-50 IU/L) and a platelet count of $6x10^9$/L (norm: 150-400$x10^9$/L). No other indices were available. On 3 October 2012, a serum sample was couriered to the Health Protection Agency's (HPA) Microbiology Services Division (MSD), Porton Down which tested PCR-positive for Crimean – Congo haemorrhagic fever (CCHF) in an assay developed in-house following collaborative work between HPA and Central Asian colleagues. The patient was given intravenous ribavirin and stabilised overnight before being transferred under high security precautions by air to the high-security infectious diseases unit at the Royal Free Hospital, London on 5 October in a specialist isolation facility with the support of the Scottish Ambulance Service and the Royal Air Force (RAF). The patient died on 6 October, despite intensive treatment.

Laboratory investigations

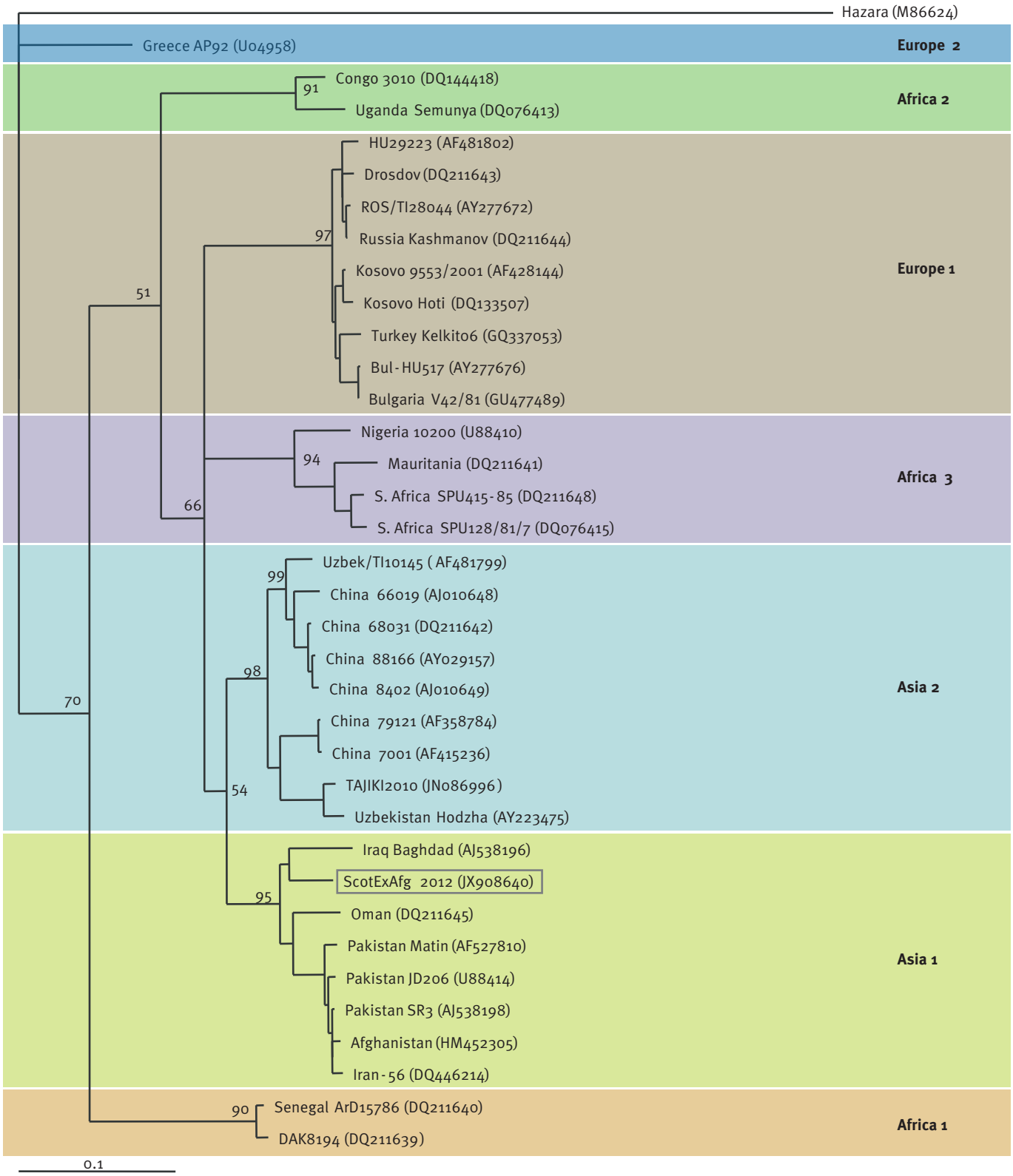
The patient's serum sample arrived at MSD on the night of 3 October and was processed for RNA extraction immediately using an EZ1 DSP Virus kit (Qiagen) for PCR by on-call staff. Given the patient's travel history and symptoms, serum RNA was tested using an in-house CCHF real-time reverse transcriptase (RT) PCR assay [1]. This assay enabled rapid and specific detection of the CCHF small genomic segment (S segment) within one hour of extraction, permitting prompt communication of diagnosis of CCHF to clinicians. The viral load in this sample was estimated to be $6.0x10^7$ copies (Segment)/mL. Additional characterisation of the virus was carried out by standard Sanger sequencing on a 3130xl sequencer (Life Technologies) using our CCHF primer collection. This provided full length sequence of the S segment within 48hrs, contributing additional information on the CCHF virus infection. Sequence characterisation facilitated a detailed phylogenetic analysis with other CCHF viruses, placing the S segment within the Asia 1 group as was expected given the geographic location of the infection (Figure). The sequence was released to Genbank on 8 October (GenBank accession number: JX908640).

Control measures

In the absence of a timely diagnosis, CCHF virus has a propensity for nosocomial transmission [2, 3, 4], thus rapid diagnosis is important for clinicians so that prompt implementation of barrier nursing techniques and patient isolation can be established. Once confirmatory diagnosis of CCHF was made in this case, the decision to transfer the patient to the high security infectious disease unit at the Royal Free was taken in line with national protocols [5] for the management of viral haemorrhagic fever cases in the UK. The patient was transported to the hospital in London via the ambulance service and the RAF's air transport isolator [6]. Confirmatory diagnosis also initiated the follow-up

Figure

Maximum likelihood phylogenetic tree compiled from full length S segments of Crimean–Congo haemorrhagic fever (CCHF) virus



The tree was constructed to determine the similarity and origin of the strain affecting the patient. It is rooted with the closely related Hazara virus. The genomic S segment of SCT ex Afg (GenBank accession number: JX908640) clusters with the Asia 1 group, showing close similarity to other strains from the Middle East.

of potential patient contacts by the National Health Service (NHS), Greater Glasgow and Clyde, together with colleagues from Health Protection Scotland. All potential contacts including the airline crew, cleaning staff and passengers who had sat closest to the patient were interviewed. Individuals who had direct contact with the patient were risk-assessed and, as a precaution, three people that had contact with tissue or blood from the patient were followed up with daily temperature and symptom monitoring for 14 days after contact. Efforts were also made to inform other potential contacts including passengers on the flight from Kabul to Dubai. Nevertheless, no secondary transmission from the original case has been observed as of 28 November.

Discussion

While CCHF is an uncommon disease, importations from countries where the virus is endemic and where European countries have links such as Afghanistan, Pakistan and India; are possible. CCHF poses important challenges to patient management and infection control; however, the attentiveness of medical staff, laboratory workers and public health officials to global virological and zoonotic developments can ultimately help deal with such problems.

Rapid and sensitive detection of CCHF virus using a PCR approach offers an important advantage to infection control and patient management. This is significant during the early stages of disease when high titres of infectious virus are present in blood, bodily secretions and fluids such as vomit [7].

The estimated viral load in the sample collected approximately on the fifth day of disease, of 6.0×10^7 copies per mL seems to be consistent with other reports of poor outcome [8]. However, care should be made in drawing comparisons with other studies as only one sample was obtained from this case and it was not possible to retrieve immunological data on the IgM and IgG responses.

Incidents of nosocomial transmission are common in the absence of a clear CCHF diagnosis, and direct contact with bodily secretions [9,10], including infection via small-particle aerosol or droplets through the eye mucosa have been reported [11]. In addition, interventions to gastrointestinal bleeding, and emergency operations on patients who have yet to be diagnosed with CCHF offer the most hazardous route for acquiring the disease in a hospital setting [12-14]. Rapid diagnosis of CCHF in this particular case and similar situations is therefore important in preventing nosocomial transmission. CCHF viruses are highly diverse and it is important that assays based on rapid technology such as PCR are able to detect all possible CCHF genetic variations. To achieve this it has been fundamental to our work on CCHF to maintain ongoing research programmes that are linked to virus endemic areas of the world such as Tajikistan and Pakistan [15,16]. As part of these programmes, our assay has been tested and validated with circulating strains of virus. In particular collaborative work in Tajikistan and Pakistan has enabled the assay to be fine-tuned, so that there is confidence in the ability to detect strains from the region.

Interestingly, when compared to other CCHF virus sequences, the SCT ex Afghanistan S sequence obtained from the patient in this report, shows strong similarity to CCHF viruses from the Middle East which cluster together in the Asia 1 group (Figure). Such information alludes to the source of virus and it is noteworthy that the patient is believed to have acquired the virus, through direct contact with infected blood or other tissues, during the slaughtering of an animal while in the village of Aibak in Samangan Province, approximately 250 km northwest of Kabul.

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Azole-resistant *Aspergillus fumigatus* due to TR46/Y121F/T289A mutation emerging in Belgium, July 2012

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A new azole resistance mechanism in *Aspergillus fumigatus* consisting of a TR46/Y121F/T289A alteration in the cyp51A gene was recently described in the Netherlands. Strains containing these mutations are associated with invasive infection and therapy failure. This communication describes the first case of fatal invasive aspergillosis caused by TR46/Y121F/T289A outside the Netherlands, in the neighboring country of Belgium, suggesting geographical spread. TR46/Y121F/T289A leads to a recognisable phenotypic susceptibility pattern which should trigger cyp51A genotyping to monitor further spread.

Case report

A 57-year-old male, diagnosed with stage IIIA multiple myeloma (IgG kappa) in 2009, received a fully matched, unrelated haematopoietic stem cell transplantation following reduced-intensity conditioning (fludarabine-melphalan-ATG) in May 2012. Prior treatment regimens included multiple lines of chemotherapy, autologous transplantation, proteasome inhibitors (bortezomib), immunomodulatory agents (lenalidomide) and high-dose corticosteroids. At the time of transplantation, the patient had achieved a very good partial response (<10% residual monoclonal paraprotein). The post-transplantation course was complicated by grade III hyperacute graft-versus-host disease (GVHD), involving mainly the skin and the gastro-intestinal tract. Methylprednisolone was started at 2 mg/kg and slowly tapered over the following weeks. However, high-dose corticotherapy needed to be re-installed in June 2012 because of a relapse of grade III acute GVHD. The patient was receiving fluconazole 400 mg daily since May 2012 as prophylaxis, but was never exposed to mold-active azoles.

One month later, in July 2012, the patient presented with dyspnea, pleuritic-type chest pain and fever, up to 39.9°C. Thoracic computed tomography (CT)-scan imaging showed multiple ill-defined lesions surrounded by ground glass opacities, suggestive of angio-invasive pulmonary mold infection. Serum galactomannan testing was repeatedly positive (maximum index 5.2; norm <0.5). Galactomannan detection in broncho-alveolar lavage (BAL) fluid tested positive as well (index 5.8), and *Aspergillus fumigatus* was cultured from BAL fluid. A diagnosis of probable pulmonary invasive aspergillosis (IA) was made following revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [1]; the patient agreed to participate in a double-blinded phase III clinical trial comparing two azoles with anti-*Aspergillus* activity. However, serum galactomannan levels did not decrease while he received azole therapy and the clinical condition of the patient deteriorated rapidly.

Meanwhile, the isolate was tested for azole susceptibility, following Clinical and Laboratory Standards Institute (CSLI) protocols and showed an azole-resistant phenotype, with high-grade resistance to voriconazole (minimal inhibitory concentration (MIC) >16 mg/L) and less pronounced resistance to itraconazole (MIC =4 mg/L) and posaconazole (MIC =1 mg/L). Formal clinical breakpoints have not been established by CLSI for *Aspergillus* susceptibility testing. Based on the epidemiological cut-off values (wild-type distributions), resistance to itraconazole is defined as an MIC >1 mg/L, to voriconazole as >1 mg/L and to posaconazole as >0.5 mg/L [2]. An excellent essential agreement (EA) between CLSI and EUCAST methods was described in susceptibility testing of *A. fumigatus* to these azoles [3] and EUCAST already established breakpoints for itraconazole and posaconazole (itraconazole ≤1 mg/L is considered susceptible and >2 mg/L resistant; posaconazole ≤0.12 is considered susceptible and >0.25 resistant) [4]. A recent report, using an in vitro dynamic model of pulmonary IA that enabled simulation of human voriconazole pharmacokinetics, proposed CLSI breakpoints for voriconazole as ≤ 0.5 mg/L for susceptible and >1 mg/L for resistant [5].

Given this new finding of azole resistance and the rapid clinical decline, the investigators decided to withdraw the patient from the clinical study. Nine days after the start of azole therapy, liposomal amphotericin B was started at a dose of 3 mg/kg. Nevertheless, the patient

developed widespread IA with eye and brain involvement. A brain magnetic resonance imaging (MRI) scan taken 15 days after the initial diagnosis of invasive aspergillosis showed multiple nodular non-contrast-enhancing lesions suggestive of cerebral aspergillosis; this was confirmed by positive galactomannan testing in cerebrospinal fluid (index 4.8). The patient died 19 days after his first presentation with dyspnea. Azole resistance in the strain affecting the patient was shown to be due to cyp51A mutation TR46/Y121F/T289A.

Characterisation of the *Aspergillus* isolate derived from the patient

The *Aspergillus* isolate, cultured from BAL fluid, was identified as *Aspergillus fumigatus* complex based on microscopic and macroscopic characteristics. This identification was confirmed to the species level with beta-tubulin sequencing, as described previously [6]. The isolate was tested for susceptibility with broth microdilution following the CLSI M38-A2 protocol [7]. Genotypic identification of the resistance mechanism was performed by sequencing of the cyp51A gene, as described previously [8].

Discussion and conclusion

Invasive aspergillosis is an important infectious complication in haematologic patients [9], but also in other groups of immunocompromised and intensive care patients [10]. Triazoles are the mainstay of therapy, with voriconazole the first-line therapy for IA [11]. However, reports of azole resistance have emerged, not only after long-term azole exposure [12], but also after short-term exposure and in azole-naïve patients [13]. In the Netherlands, over 90% of the resistant clinical strains were attributable to the same resistance mechanism [13]. Therefore, an environmental route of resistance development is assumed and this is suspected to be related to the selective pressure of azole fungicides in the environment [14]. This predominant resistance mechanism is mediated by a tandem repeat of 34 bases (TR34) in the promoter region of the cyp51a gene and a substitution at position 98 (TR34/L98H), which encodes a residue of the azole target, sterol 14-alpha-demethylase. This resistance mechanism, conferring pan-azole resistance, has to date spread across Europe as well as to China and India [8,12,13,15-17]. Because of the widespread and abundant consumption of azole fungicides in agriculture, there is a risk that other resistance mechanisms might emerge in the environment as well [14]. Recently, in October 2011, a new resistance mechanism due to a 46 base tandem repeat (TR46) in the promoter of the cyp51a gene and two point mutations (TR46/Y121F/T289A) was described in persons with IA who failed therapy in the Netherlands [18].

To our knowledge, the case described in this report is the first case of azole resistance in *A. fumigatus* due to TR46/Y121F/T289A outside the Netherlands. The patient lived about 60 kilometres from the Dutch border and did not have any recent travel history to the Netherlands. TR46/Y121F/T289A-bearing strains have also been found throughout the environment in Belgium, among azole-resistant *A. fumigatus* isolates cultured from outdoor air sampling, which was performed from June to September 2012 (data not shown). This case confirms the geographic spread of this new resistance mechanism, possibly following the same path as TR34/L98H, which now causes therapy-resistant infections across Europe and even outside Europe [8,12,13,15-17]. The phenotype of the TR46/Y121F/T289A strains consists of a very high MIC to voriconazole (>16 mg/L), and an itraconazole MIC which is often multiple dilutions lower. In contrast, in TR34/L98H mutated strains, itraconazole MICs are typically higher than voriconazole MICs. This finding (MIC voriconazole >16 mg/L and voriconazole MIC ≥MIC itraconazole) should raise awareness of this new TR46/Y121F/T289A resistance mechanism in other centres and countries.

Susceptibility testing should not delay initiation of therapy. Culture has a low sensitivity and takes about 48h to become positive; susceptibility testing takes at least another 48h. Resistance is therefore often a late finding in the management of the individual patient. Molecular techniques are a promising tool to rapidly provide information about resistance genotype, but clinicians should be aware that they are often designed to detect known resistance mechanisms and can therefore miss new mutations. On the other hand, not all mutations necessarily lead to a resistant phenotype [19]. Surveillance programs are crucial to monitor the local epidemiology of azole resistance, to correctly assess the risk of resistance associated with current treatment strategies. Susceptibility testing in individual patients with invasive aspergillosis should not be delayed until treatment failure because of the life-threatening character of this disease which is illustrated by this case.

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Conflicts of interest

Potential conflicts of interest are listed as follows. JM has served as consultant to Schering-Plough, Gilead Sciences, Merck, Sharp & Dohme, Pfizer Inc., Bio-Rad, Fujisawa healthcare, Inc., Astellas, Nextar and Zeneus (Cephalon). JM has received research funding from Bio-Rad, Merck, Sharp & Dohme, and Pfizer Inc. JM has been on the speaker's bureau for Schering-Plough, Gilead Sciences, Merck, Sharp & Dohme, Pfizer Inc., Bio-Rad, Fujisawa healthcare, Inc, Astellas and Zeneus (Cephalon). KL has received research grants from Gilead Sciences, Pfizer Inc. and Merck, Sharp & Dohme and served on the speakers' bureau of Pfizer Inc. and Merck, Sharp & Dohme. HS has served as consultant to Bristol Meyer Squibb.

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Probable autochthonous introduced malaria cases in Italy in 2009–2011 and the risk of local vector-borne transmission

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We describe two cases of probable autochthonous introduced *Plasmodium vivax* malaria that occurred in 2009 and 2011 in two sites of South-Central Italy. Although the sources of the infections were not detected, local transmission could not be disproved and therefore the cases were classified as autochthonous. Sporadic *P. vivax* cases transmitted by indigenous vectors may be considered possible in some areas of the country where vector abundance and environmental conditions are favourable to malaria transmission.

Two probable autochthonous introduced cases of *Plasmodium vivax* malaria were notified from two sites in South-Central Italy in 2009 and 2011. We report on the possible risk of local vector-borne malaria transmission in areas in Italy where the vector abundance and environmental conditions are favourable to malaria transmission.

Although soon after the World War II malaria was eliminated from Mediterranean countries, the rise in the average temperature of the earth [1], environmental modifications, the increase of international travel, and socio-economic constraints recorded in the last decades [2], have raised the concern about the possible re-emergence of malaria in some of these countries, such as Italy, where malaria had been endemic before [3].

However, the presence of competent vectors and of reservoirs of the parasites, i.e. humans carrying gametocytes in their blood may play a major role in malaria re-emergence. The recent outbreaks of malaria in Greece, although limited in size, are paradigmatic of this possible public health threat [4].

In Italy, since the five-year eradication campaign in 1947-1951, one single confirmed case of autochthonous introduced *P. vivax* malaria (transmitted by an indigenous vector) [5], has been recorded in Grosseto, Tuscany Region in 1997 [6]. Hereby, we report two probable but not proved autochthonous introduced [5] malaria cases that occurred in Italy in the last three years, namely between 2009 and 2011.

Case reports

Case 1

A 41-year-old Caucasian man living in the outskirts of Rome was admitted to the intensive care unit of the local Hospital for Infectious Diseases on 8 August 2009 with high remittent fever, peaking every 48 hours and classical paroxysms with alternate cold, hot and sweating stages. Clinical suspicion of malaria was confirmed by microscopic (blood smear observation, thick and thin films) and molecular (nested PCR analysis) [7] diagnosis as *P. vivax* malaria. The patient had no history of recent travel in malaria-endemic areas; he only reported a one-week holiday in 2003 in Santo Domingo, Dominican Republic (where foci of malaria still exist in limited areas) and in 2004 in Sharm el Sheikh, Egypt, but he didn't undertake any malaria prophylactic treatment on these occasions. He had no history of blood transfusions, tissue/organ transplantation, intravenous drug use, and no event of high fever in the previous six years. In July 2009, the month before the onset of symptoms, he had spent two weeks in two different holiday farms, one in Terracina, between 4 and 5 July, and the other in Pontinia, between 25 and 26 July. Both sites are located in the former 'Pontine marshes', a rural coastal plain of Central Italy, where malaria was hyperendemic until 1946. Although long term *P. vivax* relapses have been reported [8] and could not be entirely ruled out in this case, the short stay in Santo Domingo and the lack of a history of febrile attacks shortly after the travel made unlikely the hypothesis of a travel-acquired infection and oriented towards the hypothesis of an autochthonous case. After the detection of this case, the search for potential sources of infection (i.e. gametocyte carriers), was directed to the identification of migrants from endemic countries. A large number of regular migrants from India and Sri Lanka, employed in buffalo rearing and

horticulture in green houses, in a site which was very close to both holiday farms but no malaria case was identified among these people in 2009, through the regular surveillance system.

Considering a minimum of 10–12 days for the development of the sporogonic cycle of *P. vivax* into the insect and another 8–10 days for the onset of symptoms, malaria infection was probably acquired in the first half of July. Due to the late notification of the case to our reference centre at Istituto Superiore di Sanità, the entomological investigation started one month after malaria diagnosis. One *Anopheles maculpennis* s.s. engorged female was collected from one of the animal shelters in the farms. Potential breeding sites suitable for anopheline larvae (few small canals for water supply in agriculture) were investigated but no mosquitoes or larvae were found. The entomological investigation conducted around the house of the patient in Rome also gave negative results (no adult of anopheline mosquitoes or suitable larval breeding sites were found).

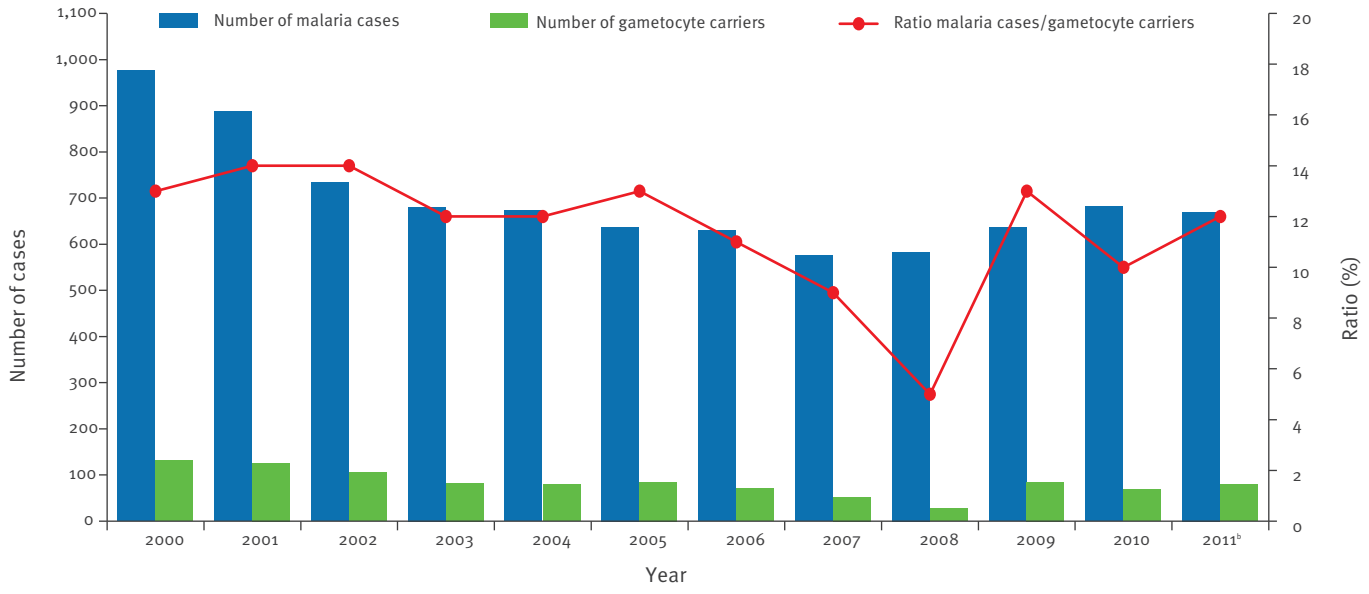
Case 2

A 39-year-old Caucasian man resident in the midtown of Rende, a residential district close to the town of Cosenza, in Calabria Region, southern Italy, was hospitalised on 15 September 2011 with the same symptoms as Case 1 (high remittent fever, peaking every 48 hours and classical paroxysms with alternate cold, hot and sweating stages), although the onset of symptoms had been on 1 September. Microscopic and molecular diagnosis of *P. vivax* malaria was performed as reported above. The patient did not report any blood transfusions, tissue/organ transplantation and intravenous drug use, and no remarkable febrile attacks during the previous six years. In his travel history he reported no recent travel in malaria-endemic areas, but a holiday in the Island of Santorini, Greece, in 2003 and a cruise along the coasts of the Mediterranean Sea in 2005. In August 2011, he spent the weekends in the village of Scalea, located along the Tyrrhenian coast, where there are some historically productive breeding sites where both *An. labranchiae* and *An. superpictus* occur [9]. Two investigations were carried out around the summer house in Scalea and in the area of the habitual residence in Rende to identify the source of infection but no gametocyte carriers were found. Three reception centres for African and Asian refugees, located in Cosenza Province, were not considered in our investigation because they were more than 10 km away from both houses of the index case and because the refugee people were under medical control (i.e. they were screened for infectious diseases upon arrival in Italy and had easy access to medical care when sick).

The infection was probably acquired in mid-August, and the entomological investigation conducted on 27 and 28 September (a couple of weeks after malaria diagnosis) in the town of Scalea, revealed the presence of a possible anopheline breeding site in a canal that

Figure 1

Annual imported malaria cases and gametocyte carriers[a], Italy, 2000–2011



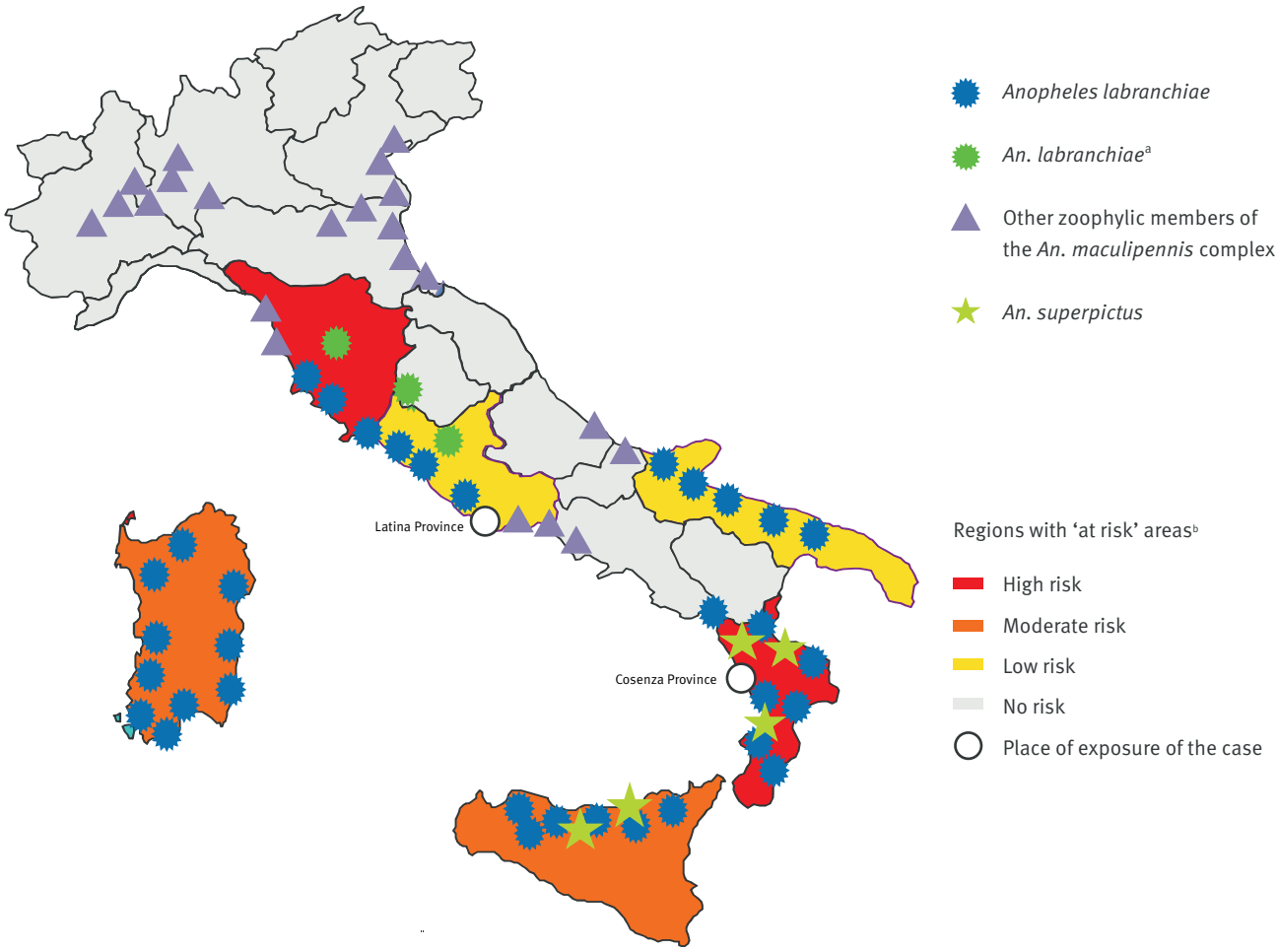
[a] Gametocyte carriers have blood smears positive for malaria parasites, showing, among the other haematic forms of the *Plasmodium*, the gametocytes, the only form able to infect the anopheline vector (in Italy they are all imported cases that may act as reservoirs of the disease).

[b] Data for 2011 are provisional.

Source: Istituto Superiore di Sanità and Ministry of Health, Italy.

Figure 2

Distribution of the potential malaria vectors and regions considered at risk of malaria reintroduction, Italy, 2005-2011



[a] First detected in 2010–2011 in northern-central Italy.

[b] Areas with presence of foci and seasonal abundance of the potential vector and with seasonal climatic conditions favourable to malaria transmission.

crossed the plain area of the town before flowing into the sea, close to the house of the patient. The canal was almost dry, with few residual pools of water along the edges, due to the presence of heavy vegetation. No anopheline larvae were found in those pools. Among the animal shelters inspected, only one resulted positive for anopheline mosquitoes: two engorged females of *An. labranchiae* were found in a cow shed of a farm located about 600 m from the summer residence of the patient. In Rende, only a single animal shelter was detected in the surrounding of the habitual residence: it was a small goat shelter located in a public garden about 200 m away from the case's house, where 26 *Anopheles* mosquitoes belonging to the *An. maculipennis* complex were found, 21 of which were identified as *An. labranchiae* by ITS2 sequence analysis [10]. One *An. maculipennis* s.s. IV instar larva was found in one of the two streams which were considered suitable breeding sites, located at 150 m and 600 m from the case's house, respectively.

Discussion

During the last 15 years (1997-2011), a total of 17 possible autochthonous cases of malaria occurred in Italy. Only one of them was likely transmitted by local vectors, thus fitting the criteria for an introduced case [5]. Other 14 cases were attributed to either iatrogenic transmission (nosocomial infection (n=7), post-transfusion (n=3), and post-transplant (n=1)) or bites from infected mosquitoes imported with baggage (n=3) [11]. The remaining two cases, discussed in this report, were classified as autochthonous [5]. In fact, although they can potentially be considered cases of introduced malaria transmitted by indigenous vectors, the source of infection remains undefined.

In Italy, as well as in other Mediterranean countries recently investigated for the risk of malaria reintroduction, the return to a situation of endemic malaria is unlikely, but the occurrence of sporadic, isolated cases of introduced *P. vivax* malaria may still be considered

possible [12,13]. Even if the vulnerability of the Italian territory during the summer months seemed to be very low between 2000 and 2011 as shown in Figure 1, recent entomological surveys carried out in areas historically considered 'at risk' for malaria showed a remarkable presence (or even high abundance in some places) of the main indigenous vector *An. labranchiae*, and confirmed its ability to bite humans both in the presence and in the absence of alternative hosts, indoors as well as outdoors [12-14].

A map displaying the current distribution of the potential malaria vectors and the regions of the country considered 'at risk', based on our own recent field collected data (2005-2011), is showed in Figure 2.

It should also be stressed that several direct or indirect effects of human activities in rural areas may quickly modify the distribution and the abundance of the vectors and may promote the contacts between mosquitoes and gametocyte carriers; these factors, together with the presence of a large non-immune human population, may have an impact on malaria reintroduction in a newly vulnerable area [14].

In the light of the recent malaria outbreaks that occurred in Greece, the two cases reported here stress the importance of vigilance for this disease and the need to improve existing epidemiological and entomological surveillance systems for malaria.

Finally, it should be mentioned that the occurrence of cases of autochthonous transmission of exotic vector-borne infections in Europe may concern infections other than malaria. The recent outbreaks that occurred in some European countries such as Italy (West Nile and Chikungunya viruses) France, Croatia, and Portugal (Dengue virus) [15-20], and the widespread presence and abundance of the potential vectors of these diseases in our country, represent a clear threat to the public health which needs adequate countermeasures.

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