indeed represent antibodies that can specifically neutralize the H9N2 virus but not H3N2, H1N1 or H6N1 influenza viruses used as controls. Our result is not unexpected, given that the H5N1 virus is highly pathogenic and asymptomatic infection is unlikely. However, infection with the avian H9N2 virus causes only mild symptoms. It is possible that the G1-like H9N2 virus may be prevalent in the poultry in the area from where those sera were collected. While only 11 human cases have been identified up to date, the avian H9N2 virus was not considered to infect humans regularly. Because we found a similar rate of positivity for antibodies against the seasonal H1N1 virus (Table 1), the detection of antibodies specific to the H9N2 virus suggests that natural infection with this virus in the general population may occur at higher levels than previously thought. While we have just experienced the swine-origin H1N1 pandemic and continue to closely watch the activity of the avian H5N1 virus, the potential for the H9N2 virus or one of its derivatives to cause a pandemic should be further assessed.

Acknowledgements

We thank Feng-Cai Zhu from Jiangsu CDC and Zhong-Ze Wang from Dongtai CDC in Jiangsu, China for their help in this study. This work was supported by grants from the National Natural Science Foundation of China (30901077), the Key Project of the National 863 Program of China (2010AA022801), the Natural Science Foundation of Fujian Province in China (2010J05083), the Areas of Excellence Scheme of the University Grants Committee (AoE/M-12/06), the National Institutes of Health (NIAID contract HHSN2662007 00005C) and the Research Fund for the Control of Infectious Diseases, Hong Kong SAR, China.

Transparency Declaration

All authors declare no conflicts of interest.

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Imported cases of dengue virus infection: Emilia-Romagna, Italy, 2010

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Abstract

Dengue is a significant mosquito-borne infection in humans, and its worldwide prevalence is rapidly increasing. In 2010, 83 serum samples from febrile travellers returning from dengue-endemic countries to a region in north-eastern Italy, densely infested with *Aedes albopictus*, were analysed for dengue virus (DENV). DENV RNA was detected in 20.5% of patients. By RT-PCR, DENV serotypes 1 and 3 were the most common. DENV must be identified early in symptomatic travellers returning from high-risk countries, to prevent outbreaks where potential vectors exist.

Keywords: Dengue virus, imported cases, Italy, laboratory diagnosis, surveillance, vector-borne

Original Submission: 21 January 2011; **Revised Submission:** 25 March 2011; **Accepted:** 27 March 2011

Editor: T. A. Zupanc
Article published online: 5 April 2011

Clin Microbiol Infect 2011; **17:** 1349–1352
10.1111/j.1469-0691.2011.03544.x

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Dengue virus (DENV) is an arthropod-borne RNA virus (*Flaviviridae* family) and is transmitted by *Aedes* mosquitoes [1]. There are four DENV serotypes, and infection results in dengue fever or dengue haemorrhagic fever (DHF) [2]. According to the WHO, the annual incidence of dengue fever/DHF has soared recently [3,4].

In Europe, DENV is not endemic, but DENV-competent vectors exist [5]. Although this competence is lower for *Aedes albopictus* than for *Aedes aegypti* [6], new autochthonous cycles of infection can be established by infected travellers returning from endemic areas to regions with high *A. albopictus* density [7].

Similarly, Chikungunya virus (CHIKV) was introduced to northern Italy in 2007; *A. albopictus* was the vector [8], and a mutation in the CHIKV E1 envelope protein increased CHIKV infectivity in *A. albopictus* [9], favouring its survival in mosquitoes. Thus, vector-borne diseases, typical of tropical environments, can be introduced and establish themselves in Europe in appropriate climates [10].

An active regional surveillance programme, comprising clinicians, general practitioners, and virologists, was established in 2007 in Emilia-Romagna, north-eastern Italy, to ensure early diagnosis of DENV infections in individuals returning from high-risk areas. Travellers to endemic countries with symptoms compatible with DENV or CHIKV infection were diagnosed by the Regional Reference Centre for Microbiological Emergencies, Clinical Microbiology Unit, St Orsola-Malpighi University Hospital, Bologna. Suspected cases were based on clinical and epidemiological criteria according to the Regional Plan for Control of CHIKV and DENV infection (http://www.saluter.it/; accessed 21 January 2011).

From 1 January to 31 December 2010, DENV infection was examined in 83 patients. Laboratory confirmation of DENV infection entails molecular and serological testing, based on clinical history [11,12]. If symptom onset is ≤9 days, nucleic acid identification, antigen detection or viral isolation is used to diagnose the infection; over >9 days, serology is preferred (Fig. 1).

DENV non-structural protein (NS1) was measured by enzyme immunoassay (Platelia Dengue NS1 AG Kit; BioRad, Segrate, Italy). Multiplex real-time RT-PCR was performed for DENV RNA [13]. Cell culture is the most common method for DENV isolation, for which we used Vero cells [4]. DENV-specific IgG and IgM were detected by immunofluorescent assay (Euroimmun, Lübeck, Germany). All results, excluding viral culture, were generated within 24 h of sample arrival to isolate positive patients and implement environmental vector control.

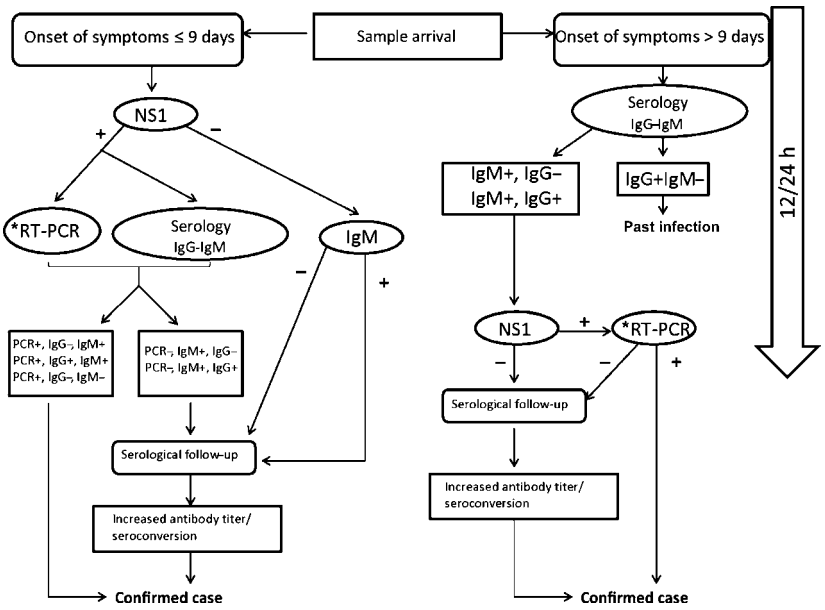

FIG. 1. Workflow of the diagnosis of dengue virus infection in febrile travellers returning from endemic areas. *Viral isolation was performed when RT-PCR kits were unavailable. NS1, non-structural protein.

Five (6.0%) of 83 samples were positive for DENV-specific IgG, and were considered to represent past flavivirus infection/vaccination; one (1.2%) was positive for DENV-specific IgG and IgM, and was considered to represent a recent DENV infection; and 60 (72.3%) did not harbour specific antibodies or viral components. Seventeen patients (20.5%) were in the acute phase of infection; 11 samples tested positive for NS1 and DENV RNA, and two cases were RNA-negative and NS1-positive. Only RT-PCR or antigen detection was performed in four cases, owing to a temporary shortage of kits—one was tested only by PCR and three were analysed for NS1; two of the three cases also harboured DENV-specific IgG or IgM, and virus was isolated in the remaining case. Ten of 17 patients (58.8%) with viral protein or RNA in the blood had specific IgG and IgM; seven (41.2%) tested negative for DENV antibodies.

Whenever possible, a second serum sample, obtained during the convalescent phase, was analysed, because increased DENV antibody titres or seroconversion help to confirm positive cases (Fig. 1). Follow-up samples were available for nine of 17 DENV-positive subjects; seroconversion or increased antibody titre was detected in all nine cases. Thus, all 17 were confirmed cases of DENV infection, because they tested positive by RT-PCR, viral isolation, or the presence of specific DENV antibodies with seroconversion or increased antibody titre in the follow-up sample [4].

Fever, rash, headache, asthaenia, arthralgia, retro-orbital pain and myalgia were the chief symptoms in acute DENV-infected subjects; three subjects exhibited bleeding with suspected DHF. The mean age of viraemic patients was 38.1 years (range 15–63 years); 12 were male (70.6%). Because DENV and CHIKV can cause fever and exanthem, both diagnoses are frequently considered in febrile patients returning from high-risk areas. Of 23 samples that were positive for DENV components or specific antibodies, only DENV identification was requested in 15 cases, whereas DENV and CHIKV were tested in eight cases; one of these eight subjects tested positive for CHIKV and DENV antibodies, and was deemed to have had previous exposure to CHIKV and DENV.

All subjects in the acute phase of DENV infection were residents of the Emilia-Romagna region and had recently visited a DENV-endemic area: the Caribbean (n = 1), India (n = 3), Indonesia (n = 3), Brazil (n = 2), Thailand (n = 4), Venezuela (n = 3), and Nicaragua-Honduras (n = 1). Twelve of 17 viraemic patients tested positive for DENV serotype 1 (6/17, 35.3%) or serotype 3 (6/17, 35.3%); typing was not possible for the five remaining NS1-positive cases in the blood, because PCR was not performed or gave negative results.

TABLE 1. Imported cases of dengue virus (DENV) infection diagnosed by the Regional Reference Centre for Microbiological Emergencies in 2008, 2009, and 2010

Years	Total no. of tested samples	No. of positive cases	
		Subjects in acute phase of DENV infection	DENV serology-positive
2008	46	3	4
2009	26	6	2
2010	83	17	6

The frequency of DENV infection (20.5%) was high in febrile patients returning from endemic areas to Emilia-Romagna in 2010, demonstrating the efficacy of our regional surveillance system for DENV. The number of imported DENV cases has rapidly increased in Emilia-Romagna (Table 1), which is alarming, considering that the *A. albopictus* density is high in much of the region in summer [14] and that local DENV transmission has been reported in a French region that has a comparable environment to that of Emilia-Romagna [15].

Because the *Aedes* mosquito remains uncontrolled in endemic areas, and because no vaccine exists [1], DENV infections will continue to escalate, leading to more infected travellers returning from high-risk areas; this underscores the need to rapidly diagnose and identify such cases, and prevent local outbreaks in non-endemic areas that are infested with *A. albopictus* or other competent vectors.

Transparency Declaration

This study was supported by Fondi Lab P3 from Regione Emilia-Romagna. The authors declare no conflict of interest.

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Update on emergence of HIV-1 resistance to antiretroviral drug classes in an Italian national database: 2007–2009

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Abstract

We analysed trends of human immunodeficiency virus type 1 (HIV-1) drug resistance during 2007–2009 in the Italian national HIV drug resistance database 'ARCA'. Prevalence of resistance in each year was examined on the basis of the presence of major International AIDS Society-2009 mutations. Predictors of resistance were analysed by multivariable logistic regression. Nine hundred and sixty-six patients were selected. Resistance to nucleoside reverse transcriptase inhibitors and protease inhibitors showed a significant decline with respect to previous surveys. Resistance to any class of drug and three drug classes remained stable. Independent predictors of three-class resistance were the number of treatment regimens experienced, prior suboptimal nucleoside reverse transcriptase inhibitor therapy and the current use of ritonavir-boosted protease inhibitors.

Keywords: Antiretroviral therapy, drug resistance mutations, human immunodeficiency virus-1

Original Submission: 30 October 2010; **Revised Submission:** 9 April 2011; **Accepted:** 12 April 2011
Editor: G. Antonelli
Article published online: 25 April 2011

Clin Microbiol Infect 2011; **17:** 1352–1355
10.1111/j.1469-0691.2011.03563.x

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Background

The introduction of combination antiretroviral therapy (cART) has dramatically improved the natural history and prognosis of human immunodeficiency virus type 1 (HIV-1) infection and AIDS [1]. However, the high propensity of HIV-1 to develop drug resistance often leads to treatment failure and progressive exhaustion of treatment options. As a consequence, continuous surveillance of antiretroviral drug resistance in large cohorts is warranted [2–5]. In an analysis of patients failing cART in Italy between 1999 and 2006, we previously found that the prevalence of any drug resistance