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Laboratory surveillance of arboviral infections in a southern France region colonized by *Aedes albopictus*

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SUMMARY

The establishment of *Aedes albopictus* in southern France, a recognized competent vector for several arboviruses, represents a new threat for the local transmission and spread of what were until recently considered as tropical diseases. A preparedness and response plan, based on vigilance of both clinicians and laboratories, has introduced significant changes in guidelines and behaviour regarding patients' care specifically during the activity period of mosquitoes. In the present study, we report the results of a 1-year activity in arboviral infection diagnosis. A total of 141 patients were included in this retrospective study. The number of suspected imported and autochthonous cases was 69 and 72, respectively. A diagnosis of arboviral infection was confirmed for 15 (21·7%) suspected imported cases, with identification of 13 dengue viruses, one chikungunya virus and one Zika virus. No autochthonous cases were detected. This report illustrates the increase in requests for arboviral infection diagnosis and confirms the challenge with identifying autochthonous arboviral infection cases in many unspecific febrile syndromes.

Key words: Arboviruses, chikungunya virus, dengue virus, surveillance, Zika virus.

INTRODUCTION

Since its first introduction in 2004, the mosquito species *Aedes albopictus* has become well established in southern France with a continuous further spread every year. *Ae. albopictus* is an efficient vector for several arboviruses including dengue virus (DENV), chikungunya virus (CHIKV) and to a lesser extent Zika virus (ZIKV) [1]. DENV and CHIKV with the recent addition of ZIKV might be a threat for mainland France, since all prerequisites for autochthonous transmission are present in southern regions [2]. Indeed, the regular introduction of these viruses through viraemic travellers returning from endemic or epidemic countries to France, where the population is mostly naive for arboviral infection, represents a risk for local transmission in vector-colonized areas during the activity period of mosquitoes (May–November). This situation has previously led to several episodes of autochthonous transmission of both CHIKV and DENV, with up to four small outbreaks in southern France recorded since 2010 [3–6]. In order to prevent transmission and dissemination of these arboviruses in areas colonized by *Ae. albopictus*, the French Institute for Public Health Surveillance (InVS) has implemented a preparedness and contingency plan during the activity period of mosquitoes [7]. This plan relies on both entomological and epidemiological surveillance. All clinically suspected cases must be reported to regional health authorities and notification of confirmed cases are mandatory in

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order to trigger epidemiological investigations and vector-control measures in locations visited by arbovirus-infected patients. The efficiency of this plan involves an increased awareness of physicians about the diagnosis and notification of suspected arboviral fever associated with a close coordination with a network of laboratories in which appropriate diagnostic tools have been implemented. We report here a 1-year experience (2015) in laboratory surveillance of human arbovirus infections at the university hospital of Montpellier, a city located in a southern France area where *Ae. Albopictus* is established.

MATERIAL AND METHODS

Patients and samples

During 2015, all patients who were sampled for a diagnosis of DENV and/or CHIKV infection, with subsequent addition of ZIKV were retrospectively included in the analysis. A suspected case of DENV or CHIKV infection was defined by rapid onset of fever ($\geqslant 38{\cdot}5$ °C) associated with at least one of the following signs: headache, myalgia, arthralgia, retro-orbital pain or cutaneous rash. A suspected case of ZIKV infection was defined by a cutaneous rash with or without fever with at least two of the following signs: conjunctival hyperhaemia, arthralgias or myalgias. Samples collected for viral investigation (serum or plasma) were sent to the laboratory with a notification form completed by the physician that recorded clinical signs, the date of symptom onset and, for travelling patients, the country visited and the return date. This document, edited by the French national surveillance authorities, was used as a patient informed consent for the anonymous research use of both samples and data. The diagnostic strategy followed in the laboratory was based on a consensual algorithm with molecular assays to detect arboviral RNA performed on samples obtained up to 7 days after symptom onset, whereas specific antibody detection was restricted to samples obtained after the fifth day following onset of clinical signs. In the last 2 months of 2015, a urine sample collected up to 10 days after the onset of clinical signs was added for ZIKV RNA detection.

Viral laboratory investigations

RNA was extracted from 200 μl samples (plasma, serum, urine) with the MagNA pure Compact apparatus running the MagNA pure compact nucleic acid isolation kit 1 (Roche Diagnostics, France). DENV and CHIKV RNA detection were performed on a LC480 apparatus (Roche Diagnostics) using the RealStar Dengue kit 2.0 and Chikungunya kit 2.0 assays (Altona Diagnostics, France), respectively. ZIKV RNA was detected using an in-house real-time RT–PCR based on primers and probes previously described [8].

The DENV serotype was subsequently determined in DENV-positive samples through sequence analysis of a NS5 PCR fragment as described by Lanciotti *et al.* [9]. Briefly, The NS5 PCR product of the F gene was purified with the QIAquick PCR purification kit (Qiagen, France) and sequenced on an ABI 310 sequencer with a fluorescent dye terminator kit (Applied Biosystems, France). Sequencing primers were the PCR primers. A phylogeny analysis was submitted to the French phylogeny website (http://www.phylogeny.fr) using the 'one click version' for analysis. The nucleotide sequences were aligned using MUSCLE software, cured with Gblocks and subsequent phylogeny performed with PhyML software. One hundred bootstrap datasets with random sequence addition were computed to generate consensus trees through TreeDyn.

DENV- and CHIKV-specific antibodies

Serological investigations were performed using the PanBio Dengue duo cassette for DENV IgG and IgM (Standard Diagnostics, Korea) and a commercial indirect immunofluorescent assay (EuroImmnun, Germany) for CHIKV-specific IgM and IgG. ZIKV-specific antibodies were assayed by the National Reference Center for Arboviruses (Marseilles, France), with in- house IgM antibody-capture ELISA (MAC-ELISA) and IgG indirect ELISA assays.

RESULTS

Population studied

Samples were obtained from 141 patients (121 adults, 20 children) with a suspected arboviral infection. Most patients were seen soon after the acute phase with a median delay of 7 days (interquartile range 4–16·5 days) between the onset of clinical signs and sampling. Sixty-nine (49%) patients were returning from DENV-, CHIKV- or ZIKV-endemic or epidemic areas (Table 1) and constituted the group of suspected

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Table 1. *Origins and types of imported arboviruses*

		Caribbean and South America	Pacific Islands	Africa and Middle East	Indian Ocean	Asia
No. of cases	69	25	5	15	9	15
No. of confirmed arbovirus cases	15	6	3	0	2	4
Virus detected		DENV3, DENV2, ZIKV	DENV1, CHIKV		DENV1, DENV4	DENV3, DENV4

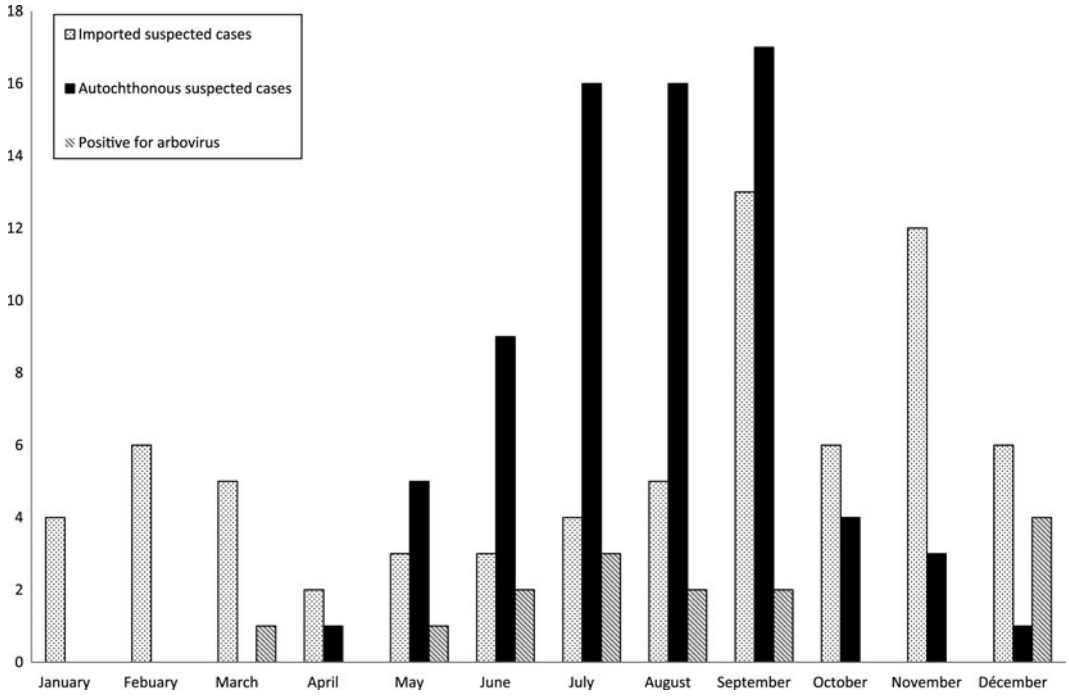

Fig. 1. Number of requests for arbovirus diagnosis during 2015.

imported cases (group I), whereas 72 (51%) patients who did not report any recent travel in areas at risk for arboviral infections constituted the group of suspected autochthonous cases (group II). The distribution of cases throughout the year is presented in Figure 1. The retrospective analysis of clinical data revealed that 49 (35%) patients did not strictly fit the suspected case definition; however, there was no significant differences regarding imported *vs.* autochthonous cases.

Arboviral infections

A diagnosis of acute arboviral infection was confirmed for 15 (10·6%) patients. All these cases belonged to group I comprising 69 (21·7%) patients with suspected imported cases. The viral aetiology was DENV for 13 cases plus one case for each CHIKV and ZIKV. For these cases, viral RNA was detected for 10 (76·9%) DENV-infected patients as well as in the case of CHIKV infection. The two remaining DENV infections and the ZIKV infection were diagnosed based on detection and kinetics of specific IgM and IgG antibodies. Among these confirmed cases, nine were detected during the May–November surveillance period, and were from patients infected with DENV.

For the DENV-infected samples, viral sequences were obtained for nine strains and the phylogenic analysis showed that the four serotypes of viruses were

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Table 2. *Comparison of diagnosis recorded between suspected imported and suspected autochthonous cases*

	Imported suspected cases ($n = 69$)	Autochthonous suspected cases ($n = 72$)	
Fever of unknown origin	33	40	$P = 0{\cdot}66$
Confirmed arboviral infection	15	0	
Other infectious diagnosis	15	16	$P = 0{\cdot}97$
Virus			
	Measles (2) Cytomegalovirus (2) Viral gastroenteritis (2) Viral meningoencephalitis (2)	Parvovirus B19 (4) Cytomegalovirus (1)	
Bacteria			
	Pneumonia (*Streptococcus pneumoniae*) (1)	Mediterranean spotted fever (*Rickettsia conorii*) (5)	
	Sore throat (*Steptococcus pyogenes*) (1)	Q fever (*Coxiella burnetti*) (1)	
	Sepsis (including 1 *Rickettsia typhi*) (2)	Sinusitis (2)	
	Leptospirosis (1)	Salmonellosis (1)	
Parasites, fungi			
	Malaria (*Plasmodium falciparum, Plasmodium ovale*) (2)	Folliculitis (*Trichophyton mentagrophytes*) (1) Toxoplasmosis (1)	
Overall infectious explanation	30	16	$P = 0{\cdot}01$
Non Infectious diagnosis			
	Inflammatory diseases (4) Paraneoplastic syndrome (2)	Inflammatory diseases (12) Paraneoplastic syndrome (4)	
			$P = 0{\cdot}06$

detected (data not shown). As shown in Table 1, DENV strains were mainly detected in imported cases from South America, the Caribbean, Pacific Islands and Asia whereas CHIKV and ZIKV were identified in imported cases from Tahiti and from Martinique island respectively.

Differential diagnosis

An alternative infectious aetiology could be included for 15 patients in the imported cases group and 16 patients in the autochthonous cases group ($P = 0{\cdot}97$). A non-infectious likely origin of clinical signs (inflammatory, neoplasm) was recorded for respectively 6 and 16 patients from groups I and II, respectively ($P = 0{\cdot}06$). The main final diagnosis was fever of unknown origins with no significant difference between the two groups (Table 2).

DISCUSSION

In 2015, the epidemiology of arboviruses was dominated by the extensive spread of ZIKV in South America and the Caribbean [10]. In our series, more than one third of the suspected imported cases were patients returning from these areas; however, all but one of the confirmed cases, including those from Brazil, were infected with DENV. One ZIKV infection was detected in late 2015, in a patient returning from Martinique concomitantly with the first description of autochthonous cases in French West Indies [11]. This suggests that despite the epidemic spread of ZIKV, DENV remains extensively circulating. This is further illustrated by the panel of various DENV serotypes detected throughout the year from most tropical parts of the world. Imported cases reflected the worldwide arbovirus epidemiology and confirm the need for an efficient surveillance in area where the conditions of local transmission are met [2]. The laboratory part of that surveillance includes the investigation for arboviral aetiologies in travellers returning from epidemic or endemic regions. In febrile travellers returning from risk area, the diagnosis of arboviral infections as well as malaria falls within normal guidelines with additional relevance during the

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May–November period of activity of the locally established vector *Ae. albopictus* in southern France. This surveillance and report of imported cases allows rapid vector-control measures in the environment of viraemic patients in order to limit the spread of the disease. Another part of the plan is to increase awareness of clinicians regarding the detection of what could be autochthonous cases. In 2015, the previous experience of a 12 autochthonous CHIKV case outbreak in Montpellier in 2014 [11] had probably reinforced clinicians' vigilance. As illustrated by our data, this clearly adds more activity for laboratory involvement in arboviruses diagnosis, since during the surveillance period the number of requests for arbovirus assays more than doubled (Fig 1).

The lack of specificity for clinical signs associated with arboviral infections adds further complications to the identification of locally acquired cases. Most of the suspected cases were not subsequently associated with any specific diagnosis, and more than half of the cases were labelled as fever of unknown origin as observed in previous studies [12]. Our observations also confirm that many infectious aetiological agents share the same clinical features with DENV, CHIKV or ZIKV. These agents include common community-acquired viral diseases such as parvovirus B19 or cytomegalovirus infections, various infectious syndrome (sepsis, meningitis, gastroenteritis) or, as expected, other local vector-borne diseases like Mediterranean Spotted fever (Table 2). This illustrates that many differential diagnoses may interfere with that of arbovirus infections and that the overestimation of suspected autochthonous cases would be very difficult to reduce without impairing the chances of recovering confirmed autochthonous cases. However, a strict respect of the suspected case definition could improve the pertinence of diagnosis since in our studies some criteria were lacking for up to 35 % of the tested patients.

The increasing frequency of requests for arbovirus diagnosis in patients not returning from epidemic/endemic areas results from the presence of a competent vector in our region. Although no autochthonous cases have been detected during 2015 in patients seen at our hospital, the same preparedness plan has allowed the rapid identification and management of a small seven-case DENV outbreak in the neighbouring town of Nîmes [13]. Furthermore, regarding the threat of the recent increasing spread of ZIKV, the same preparedness plan should be reinforced and improved each year.

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DECLARATION OF INTEREST

None.

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