Chikungunya virus and West Nile virus infections imported into Belgium, 2007–2012

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SUMMARY

Arboviral infections are emerging among tourists travelling to (sub)tropical regions. This study aims to describe the importation of chikungunya virus (CHIKV) and West Nile virus (WNV) into Belgium over a 6-year period from 2007 to 2012. Clinical samples were obtained from travellers presenting at the outpatient clinic of the Institute of Tropical Medicine (ITM), Antwerp, Belgium or submitted to the Central Laboratory for Clinical Biology of the ITM. Testing was performed by serology and/or by real-time reverse transcriptase–polymerase chain reaction. A total of 1288 returning travellers were investigated for CHIKV infection resulting in 34 confirmed and two probable diagnoses (2·80%). Out of 899 patients, four confirmed and one probable imported WNV infections were diagnosed (0·55%). No locally acquired cases have been registered in Belgium until now and the geographical origin of the imported infections reflects the global locations where the viruses are circulating.

Key words: Arboviruses, epidemiology.

INTRODUCTION

Arboviruses consist of three virus families: Togaviridae, Bunyaviridae and Flaviviridae. Arboviral infections include a broad spectrum of disease, ranging from subclinical infection or a flu-like illness to severe haemorrhagic fever, encephalitis and shock, following a short incubation time of 1–14 days. Prevalence of these viruses is increasing and arboviral infections are emerging among tourists travelling to (sub)tropical regions. In Belgium, dengue virus is by far the most common imported arboviral infection [1]. Recent reports of imported cases of chikungunya virus (CHIKV) [2] and West Nile virus (WNV) [3] indicate the importance of less known arboviruses in travel medicine. This study aims to describe the importation of CHIKV and WNV into Belgium over a 6-year period from 2007 to 2012.

CHIKV, isolated for the first time from humans in Tanzania in 1953 [4], is an *Alphavirus* and member of the Togaviridae family. Chikungunya infection starts with acute-onset of fever and rash and the further course of the disease is characterized by incapacitating joint pain. The proportion of asymptomatic patients is low (10–15%). Three different genetic lineages are prevalent in CHIKV: the West African, the Asian and the East/Central/South African (ECSA) genotype [5]. The latter re-emerged in coastal Kenya in 2004, reached the islands of Comoros and Réunion and

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spread over the Indian Ocean islands in the 2005–2006 CHIKV outbreak [6–9].

WNV, a *Flavivirus* belonging to the Flaviviridae family, had already been isolated in 1937 from the blood of a woman in the West Nile province of Uganda [10]. Human infection by WNV can present as flu-like symptoms and can evolve to neurological disease (e.g. meningo-encephalitis) in less than 1% of cases. As opposed to CHIKV infection, WNV is asymptomatic in the majority (80%) of infected individuals. Its emergence on the North American and European continent in the 21st century contributed to raising the awareness of West Nile infections and it is now recognized as a major cause of public health concern in these regions. In the USA, outbreaks have been reported yearly since its introduction in 1999 in New York City, all caused by lineage 1, with so far three neuroinvasive disease outbreaks in 2002, 2003 and 2012 [11–13]. West Nile fever has affected multiple countries in southeast Europe since the first large outbreak in Romania in 1996 [14]; WNV virus transmission has been reported in Czech Republic (1997), France (2000, 2003, 2004, 2006), Italy (1998, 2008–2012), Hungary (2000–2008, 2010, 2012), Romania (1997–2001, 2008–2012), Spain (2004, 2010), Portugal (2004), and Greece (2010–2012) [15]. Two WNV lineages co-circulate in Europe: initial outbreaks were caused by the epidemic WNV lineage 1, which has a widespread geographical distribution, while WNV lineage 2 was formerly confined to sub-Saharan Africa but has also recently emerged in Europe [16]. Lineage 2 was first reported in Hungary in 2004 with first evidence of human cases in Russia in 2007 and in Italy in 2011 and a first large outbreak in Greece in 2010 [15]. Other WNV lineages are associated with isolated cases of infection [16].

Clinical diagnosis of acute WNV and CHIKV infections may be hampered owing to aspecific symptoms and laboratory diagnosis based on serological tests, nucleic amplification tests and virus isolation is therefore required. WNV viral RNA is often not detectable in serum and CSF as viraemia is very short and the virus may no longer be present at the time of symptom onset. WNV diagnosis generally relies on the detection of specific IgM in serum and particularly in CSF in the case of encephalitis, which appear 3–8 days after symptom onset [17]. CHIKV can be isolated from serum within the first 2–3 days of illness [18–19] and viral RNA is detectable in serum up to the first 12 days of infection [20]. Following the viraemic phase, diagnosis relies on the detection of antibodies, which are most frequently present as soon as 2–7 days after symptom onset for IgM and generally after 5–6 days for IgG [21]. No vaccine, or targeted treatment is available for either arboviruses.

Before 2006, tools to diagnose CHIKV infections were not available at the Institute of Tropical Medicine (ITM) of Antwerp (Belgium). A few samples were sent to the Bernhard-Nocht Institute (BNI) for Tropical Medicine in Hamburg, Germany for serological diagnosis and no laboratory-confirmed infections were detected. As a consequence of the outbreak in the Western Indian Ocean region, 37 imported chikungunya infections were diagnosed in Belgium in 2006, in patients arriving from East Africa (Réunion island, Mauritius, Madagascar or the Seychelles) ($n = 32$), from India ($n = 3$) or unknown destination ($n = 2$). Thirty-four of these have been reported upon before [18]. From 2006 onwards, serological testing for CHIKV has been implemented at the ITM. Diagnosis of WNV infections was based on antibody detection, already available at the ITM for over 20 years, initially performed by an indirect immunofluorescence assay (IFA) and later on by enzyme-linked immunoassay (ELISA). Here we report on CHIKV and WNV infections imported into Belgium over a 6-year period (2007–2012). Results are discussed in comparison to epidemiological data available in the literature.

MATERIAL AND METHODS

Patients and clinical samples

From January 2007 until December 2012 surveillance of WNV and CHIKV infections was performed by examining samples from travellers presenting at the outpatient clinic of the ITM (Antwerp, Belgium) or samples that were submitted by Belgian laboratories to the Central Laboratory of Clinical Biology (CLKB) of ITM for diagnosis of WNV or CHIKV infections. Analyses were performed if requested by the treating physician. If information was available on the country of exposition and the clinical symptoms, the laboratory responsible could propose the performance of other arboviral tests in agreement with the treating physician. If dengue antibody tests were not requested by the treating physician and cross-reaction with dengue was suspected, a comment was added to the result explaining about the cross-reaction within the flavivirus group. Clinical information when available was mostly obtained retrospectively. The day of onset of symptoms was defined as day 0.

Nucleic acid testing

Real-time reverse transcriptase–polymerase chain reaction (RT–PCR) was performed on samples [serum or cerebrospinal fluid (CSF)] from the first 10 days of illness. Total RNA from $140\,\mu l$ of sample was extracted by QIAamp viral RNA mini kit (Qiagen, Germany) and eluted twice with $40\,\mu l$ buffer according to the manufacturer's instructions. One-step RT–PCR reactions were run on a SmartCycler (Cepheid Benelux, Belgium) device in a $25\,\mu l$ reaction volume using $5\,\mu l$ RNA and iScript one-step RT–PCR Master mix (Bio-Rad Laboratories, Belgium). The PCR programme consisted of a RT reaction of 10 min at 50 °C and a denaturation step of 5 min at 95 °C, followed by 50 cycles of 10 s at 95 °C and 30 s at 60 °C. In every run, a non-template control (RNase-free water) served as a negative control ($C_t = 0$) and a species-specific positive control was used to monitor inter-run variability. PCR inhibition was evaluated through an internal RNA control PCR (Diagenode, Belgium). CHIKV PCR targets a specific part of the non-structural protein 1 (NSP-1) gene, according to the protocol of Panning et al. [18]. Two different sets of primers and one probe were used to detect CHIKV of the African prototype (S27, general strain) and the Indian Ocean islands epidemic strain (LR2006_OPY_1), respectively. For WNV detection, a RT–PCR adapted from Linke et al. [22] was used for detection of the capsid gene of WNV, amplifying both WNV lineages (lineages 1 and 2). CHIKV- and WNV-specific PCRs have been available at CLKB since 2007 and 2012, respectively.

Serological testing

From 2006 to February 2012 sera for CHIKV were tested by an IFA for IgG produced at and provided by the BNI. Since March 2012, two commercial IFAs for IgM and IgG (Euroimmun, Germany) have been used. Samples were screened at a titre of 1:16 for IgG and 1:10 for both IgM and IgG for the BNI and Euroimmun tests, respectively. Samples positive for IgG, were diluted further by twofold dilutions. All IgG-positive sera tested before March 2012 were analysed retrospectively with the IFA to detect IgM antibodies. Serological testing for WNV IgM and IgG was performed by ELISA (WNV IgM Capture DxSelect™ and IgG DxSelect™; Focus Diagnostics, USA).

To exclude cross-reactions with other flavivirus infections, data were compared to other serology results when available. Detection of dengue IgM antibodies was performed by Dengue Virus IgM Capture DxSelect™ (Focus Diagnostics, USA). Dengue IgG antibodies were detected by the Dengue IgG capture ELISA test (PanBio Diagnostics, Australia) until June 2009 and by the Dengue Virus IgG Dx Select™ (Focus Diagnostics) from June 2009 onwards. Antibodies for yellow fever were tested by a commercial IFA (Euroimmun, Germany) for both IgM and IgG.

For confirmation of WNV infection, samples were sent to the Robert Koch Institute (RKI) (Berlin, Germany) for testing for the presence of neutralizing antibodies by the sero-microneutralization test (SMNT) [23] and in some cases for IgG avidity testing as a marker to differentiate recent from past infections. A low avidity index indicates acute infection (<20 days), intermediate index indicates recent infection (30–60 days), and a high avidity index indicates a past infection (>30 days) [24].

Case definitions

European Centre for Disease Control (ECDC) criteria were used to define possible, probable or confirmed cases [25, 26]. Laboratory results were interpreted according to flavivirus vaccination status when possible and cross-reactions of WNV with other (flavi)viruses were taken into consideration.

Data were analysed anonymously. ITM's policy is that unused samples of patients presenting at the ITM polyclinic can be used for research unless the patients explicitly state otherwise. The Institutional Review Board of ITM approved this institutional presumed consent policy provided that patients' identities are not disclosed to third parties.

Statistics

Mann–Whitney exact test for comparison of age distributions was performed using SPSS v. 17.0 (SPSS Statistics, IBM Corporation, USA).

RESULTS

Chikungunya imported infections

In the 6 years following the Indian Ocean epidemic (2007–2012), 1407 serum samples from 1288 returning travellers (male:female ratio 1:0·98) were investigated resulting in a total of 34 confirmed and two probable diagnoses (2·80%) of CHIKV infection
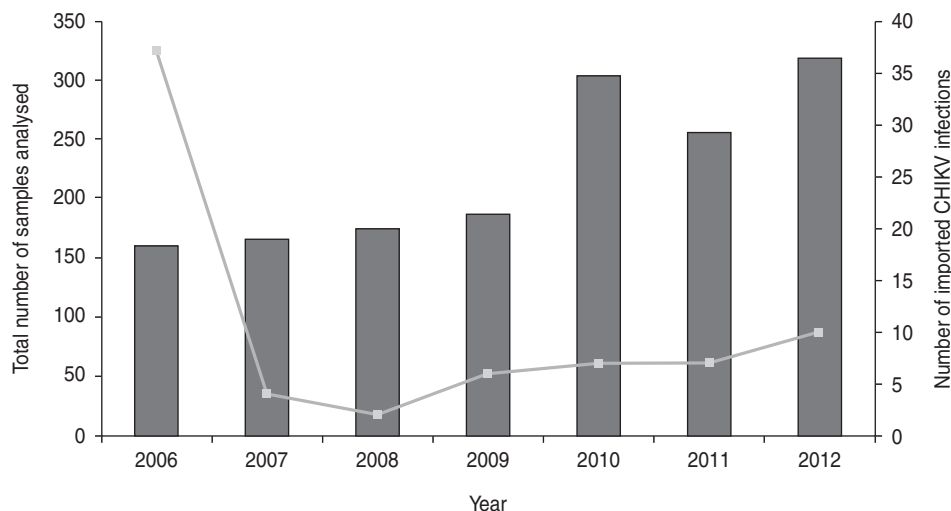
Fig. 1. Overview of total number of chikungunya virus (CHIKV) serology analyses (■) and confirmed and probable diagnoses (──■──).

(Fig. 1). Three of the confirmed cases were diagnosed by a combination of RT–PCR and serology and 31 solely by serology. Of the latter 31, two patients demonstrated a seroconversion of IgG antibodies and 29 showed a combined IgM and IgG response in a single serum sample. Retrospective detection of IgM could not be performed in two cases with a single high IgG titre (1:1024) that met the epidemiological and clinical criteria for classification of a probable CHIKV infection.

Median IgG titre at diagnosis was 1:512 (range 1:16 to 1:10 240). For 20 patients exact dates from the onset of symptoms and sampling were available. Median time between onset of symptoms and sampling was 41 days (range 2–175 days). Twenty-five (69%) of the patients were female (male:female ratio 1:2·3). Median age of the male and female patients was 45 and 38 years, respectively (range 6–75 years), with the highest numbers noted in the 20–60 years age groups (Fig. 2). The yearly geographical distribution of all 36 cases is illustrated in Figure 3. Overall, the Democratic Republic of Congo (DRC) accounted for the majority of travel destinations of patients with a CHIKV infection ($n = 8$), followed by India ($n = 7$) and Thailand ($n = 5$). Infections were reported in patients coming from Sri Lanka and Indonesia from 2007, Thailand and Myanmar from 2009, the DRC and Gabon from 2010 and Congo-Brazzaville from 2011. Information on symptoms at presentation was available for 35 patients: fever at onset was reported by 32 (94·1%), arthralgia by 28 (80·0%), myalgia by 10 (28·6%) and rash by 17 (50·0%). A statistical difference ($P = 0·013$) in age distribution

between patients presenting with ($n = 28$) and without ($n = 7$) arthralgia was noted with median ages of 45 years (range 13–75 years) and 24 years (range 6–53 years), respectively. Of those aged >30 years, 88·9% (24/27) reported suffering from arthralgia, whereas in patients aged <30 years only 44·4% (4/9) complained of joint pains. No statistical differences depending on age were registered for the other symptoms.

West Nile imported infections

In the period 2007–2012, 983 samples from 899 patients were tested for WNV (male:female ratio 1.03:1).

Two patients were confirmed by RT–PCR, of which one was diagnosed with WNV encephalitis by PCR (lineage 2) and serology in 2012 [3] and the second by PCR on CSF at RKI (Berlin, Germany) (Antoine-Moussiaux, unpublished data).

Serology revealed both WNV IgM and IgG antibodies in 17 patients which were considered as cross-reactive with dengue antibodies in 14 of them. Four samples from the remaining three patients were sent to the RKI, for WNV SMNT followed by avidity testing. Infection in two patients (three samples) was confirmed by the presence of WNV neutralizing antibodies. Avidity was high for one patient, indicating an infection older than 30 days and the other yielded an equivocal result, probably demonstrating an infection between 30 and 60 days old.

In 13 patients serology solely revealed IgM antibodies which were considered cross-reactions with dengue antibodies in nine and false-positive reactions
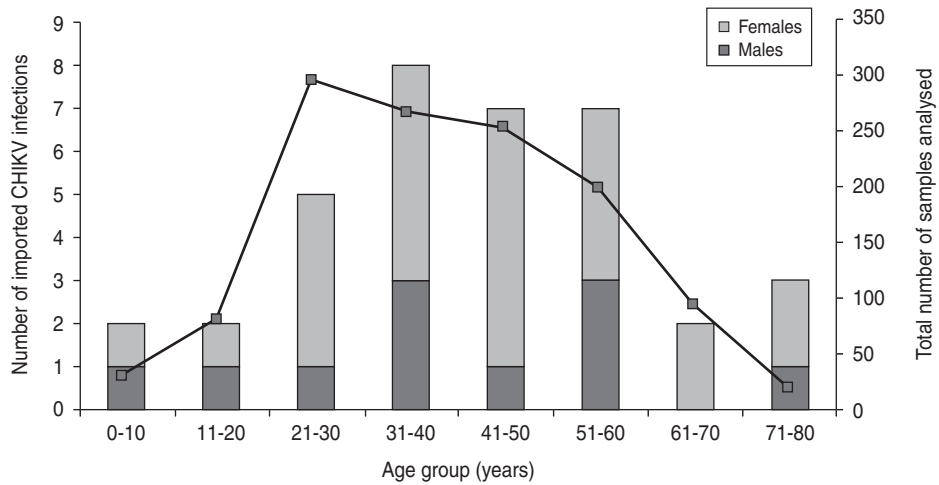
Fig. 2. Age and sex distribution of imported chikungunya virus (CHIKV) infections in Belgium. The curve indicates the total number of samples analysed by age group.
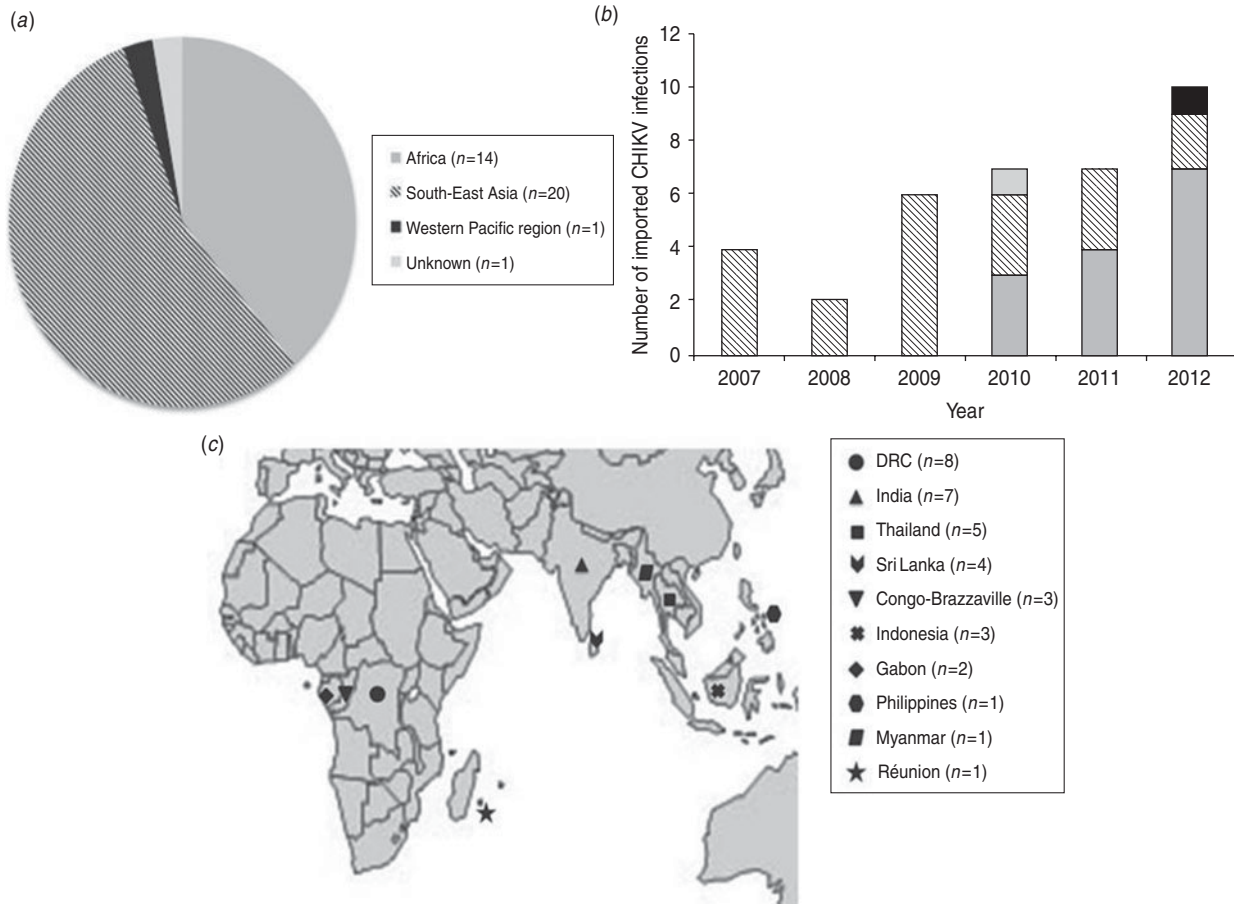

Fig. 3. (*a*) Overall and (*b*) yearly geographical distribution of imported chikungunya virus (CHIKV) infections in Belgium. (*c*) An overview of the origin of imported cases.

on the basis of additional examination for other viruses and/or absence of seroconversion of IgG antibodies in three patients. In one patient a high ratio for WNV IgM antibodies was present, but no IgG antibodies were detected. Dengue serology was not performed and the sample was no longer available for

Table 1. *Laboratory results of confirmed imported WNV infections at diagnosis*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Country	Florida, USA	Senegal/Guinea	DRC	Greece	Sudan
Year (month)	2007 (Sept.)	2008 (Aug.)	2012 (Mar.)	2012 (Aug.)	2012 (Dec.)
Sample type	Serum	Serum	CSF	Serum	Serum
Time after onset	5 days	?	?	1 week	3 weeks
Neuroinvasive case	Yes	No	Yes	Yes	No
ELISA IgM (ratio)	Positive (10·3)	Positive (6·00)	n.t.	Positive (25)	Positive (2·61)
ELISA IgG (ratio)	Negative	Positive (3·31)	n.t.	Negative	Positive (4·75)
SMNT (titre)	n.t.	Positive (1:640)	n.t.	n.t.	Positive (1:40)
IgG avidity index	n.t.	High	n.t.	n.t.	Intermediate
Polymerase chain reaction	n.t.	Negative	Positive	Positive	n.t.
ECDC case definition	Probable	Confirmed	Confirmed	Confirmed	Confirmed
Reference	Present study	Present study	Antoine-Moussiaux (unpublished data)	[3]	Present study

DRC, Democratic Republic of Congo; SMNT, Sero-microneutralization test; ECDC, European Centre for Disease Control; n.t., not tested.

SMNT. However, the patient presented with fever (39·5 °C) and meningitis, rendering it as a probable case of WNV infection.

In conclusion, in 4/899 patients (0·44%) tested, the diagnosis of WNV infection was confirmed and one patient (0·11%) was diagnosed with a probable infection. Laboratory results are summarized in Table 1. The patient with the probable infection was residing in Florida, USA and presented with symptoms in 2007. One patient with a confirmed infection lived in Guinea Republic and was diagnosed after a visit to Senegal in 2008. Three other patients travelled to or resided in Greece [3], the DRC (Antoine-Moussiaux, unpublished data) or South Sudan and were diagnosed in 2012.

DISCUSSION

After the emergence of CHIKV infections in travel medicine in 2005–2006, infections are continuously registered in Belgium at a low level as illustrated in this study (2–10 infections yearly). A trend towards more diagnoses is seen from 2010 onwards. This might at least partially be explained by the growing awareness of CHIKV infections as demonstrated by the increasing numbers of analyses performed. From 2007 to mid-2010 all chikungunya imported infections in Belgium were of South-East Asian origin, mainly from India, Sri Lanka and Thailand. The Indian outbreak from 2005 to 2006 occurred shortly after the epidemic on the Indian Ocean islands with more than 1 400 000 cases reported in India since 2006 [9]. Subsequently CHIKV spread over South-East Asia

with different outbreaks reported [27]. Late 2006 a significant outbreak in Sri Lanka occurred, which is reflected by the cases found in our study in the first months of 2007. Four out of six imported CHIKV cases in 2009 originated in Thailand. Between June 2010 and June 2011, three patients were infected in Central Africa, of which two came from Gabon, where an outbreak was registered in 2010 [28], and one from the DRC where no infections were reported. The latest outbreak in the DRC before 2010 dated from 1999/2000 [29]. In June 2011 an outbreak affected 8000 people in Congo-Brazzaville [30] and three family members were diagnosed after their return to Belgium. Remarkably, the DRC accounted for the majority (6/10) of travel destinations of patients with a CHIKV infection in Belgium in 2012. Moreover, in France two imported cases from the DRC were reported in July 2012 [31]. The fact that a substantial part (±8%) of the patients consulting at ITM are travellers and/or residents from this former Belgian colony, might explain why more imported cases originating from the DRC are seen in Belgium compared to other countries. Overall, women were more often affected than men, which is in contrast to the equal attack rates reported by the ECDC. The reason for this remains unclear. The majority of cases were registered in patients aged between 20 and 60 years and no specific age category stood out, which is comparable to age distributions reported by the ECDC, Germany, Italy and The Netherlands [32–35] and probably the result of a combination of travel preferences in this age group, and a more severe disease with prolonged recovery in patients aged >30 years [36, 37]. Based on data from

the Réunion outbreak, severe initial joint pain, age ⩾45 years and underlying osteoarthritis were defined as predictors of slow recovery [38]. Although the persistence of arthralgia was not registered in this study, there was a significant difference in median age for patients presenting with and without arthralgia. Data should be interpreted carefully as clinical information was obtained retrospectively and underestimation of cases with arthralgia is possible.

CHIKV is transmitted by mosquitoes belonging to the genus *Aedes*. One decade ago, before the resurgence of CHIKV, *Aedes aegypti* was the primary transmission vector in Asia where CHIKV infections were maintained by a mosquito–human–mosquito cycle, whereas in Africa a sylvatic cycle between non-human primates and forest *Aedes* spp. (*A. furcifer*, *A. tylori*, *A. luteocephalus*, *A. africanus*, *A. neoafricanus*) is believed to exist [39]. *A. albopictus*, another transmission vector, has a wide geographical distribution, is present in both rural and urban regions and has superseded *A. aegypti* in many places [40]. The escalation of CHIKV infections since the Indian Ocean islands outbreak and convergent emergence in different regions is probably explained by the envelope glycoprotein E1-A226V adaptive mutation that increases transmissibility by *A. albopictus* [41]. *A. albopictus* mosquitoes are also present in Southern Europe. They were introduced in Italy in 1990 as a result of the global trade in car tyres and ornamental plants and they were first detected in France in 1999 and spread from 2004 onwards [42]. *A. albopictus* was introduced in Belgium in 2004 [43] and recently re-introduced in July 2013 [44], but no established population has been recorded, mainly because the Belgian winter climate does not allow egg survival. It is estimated that monthly average temperatures of 10 °C and 20 °C are required for development of adult *A. albopictus* populations and CHIKV transmission, respectively [45]. Predictive models on future climate changes suggest that northern Europe is at risk of becoming an appropriate habitat for invasive mosquitoes by 2030 [46].

From 2007 up to 2012, four confirmed and one probable imported WNV infections were diagnosed, of which three were neuroinvasive. Two patients were confirmed by PCR upon presentation, while for two others WNV infection could only be presumed based on positive ELISA results and a definite diagnosis was subsequently made by SMNT. The latter patient resided in South Sudan, and a Guinean patient had a recent travel history to Senegal. Data on circulation of WNV in these regions are scarce. Confirmed cases have been described in Guinea in 2006 [47], Senegal in 2001 [48] and a southern province of Sudan in 2002 [49]. A probable infection was imported from Florida in 2007 where only three cases were reported by the Centre for Disease Control (CDC) in the same year [50]. Since the emergence of WNV in 1999, no imported case from the USA had been registered in Belgium before. This is probably explained by the limited proportion of patients consulting the ITM after visiting the USA (0·1%).

Culex spp. mosquitoes are the main vector for WNV transmission, which is influenced by the geographical distribution of both vectors and reservoir birds [51]. *Culex* spp. are mainly ornitophilic mosquitoes but some species take a larger fraction of their bloodmeals on mammals. Some *Culex* spp. can act as a bridge vector and transmit WNV from birds to mammals, resulting in incidental mammalian (mainly horses) and human WNV infections [52]. *Culex* is widespread across the world and it is the predominant mosquito in Belgium [53]. Seasonal migration of birds coming from an endemic WNV zone to a more temperate region or trade of birds could allow the spread of WNV. Currently, WNV has not been found in birds in Belgium and no reservoir for WNV is known to be present. In 2010 and 2011, 48 serum samples from 1125 horses were confirmed as containing antibodies, but vaccinated horses could not be discriminated from infected horses [54]. Viraemia in mammals and humans is too low for transmission by mosquitoes and they are considered as 'dead-end' hosts, although immunocompromised patients may be possible sources of transmission [55]. Moreover, *Aedes* spp. have the ability to transmit WNV, but are not considered a primary vector in nature [56]. As opposed to *Culex* spp., *Aedes* mosquitoes feed primarily on mammals, holding a greater risk for expansion among humans. In Belgium, *A. albopictus* was found to be present in 2004 and two other exotic mosquito populations, *A. japonicus japonicus* and *A. koreicus*, have been able to maintain themselves in the Belgian climate [57, 58], most likely, after introduction through trade with second-hand tyre companies, as described for *A. japonicus* found in a tyre depot in the province of Namen in 2012 [59]. Both species are competent vectors for WNV and *A. japonicus* in particular, has a high vector potential and is continuously spreading in central Europe [42, 60].

Autochthonous CHIKV and WNV infections have not been registered in Belgium until now, but have

become reality for other European countries. In 2007 a CHIKV outbreak with over 200 cases occurred in Italy in the province of Ravenna as a consequence of simultaneous presence of the E1-A226 V CHIKV strain imported by a patient returning from India and high levels of *A. albopictus* [61]. Three years later (2010) in southern France two indigenous cases of CHIKV infection were described, transmitted from an index case who had travelled to India [62]. Since 2007, the numbers of reported WNV infections in Europe have been increasing with several outbreaks on the European continent: in Italy, Hungary and Romania since 2008 and in Greece since 2010 [35]. Although southern European countries are popular travel destinations for Belgian tourists, none of the imported CHIKV infections in Belgium and only one WNV infection (Greece) occurred on the European continent between 2007 and 2012. This is probably explained by the lower incidence in Europe compared to other regions, but it is not excluded that physicians may not consider CHIKV and WNV infections in patients travelling to temperate regions.

Importation of CHIKV and WNV into Belgium may pose a risk of autochthonous infections, provided that the vector would become established in the case of CHIKV. Preliminary calculations, based on flight and passenger numbers returning from Chikungunya hotspots like India and the DRC, estimate an annual 1777 potentially viraemic cases passing through Belgium [45]. Hypothetically, an immunologically naive population, such as in Belgium, is at risk for rapid spread following CHIKV/WNV introduction when ideal conditions for transmission are present. Both human and entomological, and also for WNV, bird surveillance warrants prompt control measures to be taken when necessary.

Due to the retrospective nature of this work, some drawbacks are associated with its methodology. The use of WNV ELISA without additional testing did not allow confirmation of infection with certainty in one sample from 2007, which therefore remained as a probable case. Although CHIKV IgG antibodies were shown to appear only 1 day later than IgM antibodies [18], the number of CHIKV infections between January 2007 and March 2012, might have been underdiagnosed by the detection of IgG antibodies alone at the time of diagnosis and performing the detection of IgM antibodies only retrospectively. Clinical information was obtained retrospectively. The broad clinical spectrum probably contributes to underdiagnosis as physicians do not always consider arboviral infections in the differential diagnosis.

In conclusion, cases imported into Belgium correlate well with the timeline of outbreaks described in the literature. Both CHIKV and WNV infections are emerging across the world and we consider it our task as a reference laboratory to inform and report on surveillance data in Belgium. Raising awareness among physicians on the theoretical (provided vector establishment) and real possibility of autochthonous CHIKV and WNV infections in Belgium, respectively, would enable timely diagnosis and subsequent control measures to be taken to prevent an outbreak.

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DECLARATION OF INTEREST

None.

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