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Rift Valley fever in kidney transplant recipient returning from Mali with viral RNA detected in semen up to four months from symptom onset, France, autumn 2015

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A 29-year-old kidney transplant recipient returning from Mali was diagnosed with Rift Valley fever (RVF) in France in autumn 2015. The patient was immunosuppressed due to his renal transplant. IgM and IgG specific to RVF virus (RVFV) were detected in cerebrospinal fluid and blood up to two months after symptom onset, whereas in urine, RVFV genomic RNA was detected by RT-PCR up to three months, and in semen up to four months post symptom onset.

We report a human case of Rift Valley fever (RVF) imported from Mali to France in an immunosuppressed patient after renal transplantation three years earlier, and detail the laboratory findings.

Case report

A 29-year-old kidney transplant recipient originating from Mali and having lived in France for five years, visited friends and relatives in Dionkoulané (Kayes region, Mali) from mid-August 2015 onwards. A month later, he started to have fever, chills and fatigue (day 1) and consulted a general practitioner (GP) in Mali. He was empirically treated with intravenous quinine for three days. His clinical status improved so he was able to return to France eleven days later.

At the end of September 2015 (day 12), he was admitted to Pitié-Salpêtrière University Hospital in Paris for asthenia and hypothermia (35.5 °C). The results of the clinical examination were normal. The liver function tests showed a severe cytolysis (aspartate aminotransferase: 12.44 international units (IU)/L (norm: 20–32), alanine aminotransferase: 1.236 IU/L (norm: 16–35)) with anicteric cholestasis (gamma glutamyl transpeptidase: 652 IU/L (norm: 12–55), alkaline phosphatase: 170 IU/L (norm: 40–120), total bilirubin: 9 IU/L (norm: 2–17)), and without liver failure (prothrombin time: 100%, factor V: 99%). Hepatitis B virus DNA PCR (COBAS AmpliPrep/COBAS TaqMan HBV test v2.0 Roche) was at the positivity threshold, i.e. 20 IU/L. Hepatitis B surface antigen (HBs-AG) was negative, HBs and HBc IgG antibodies (ARCHITECT AgHBs qualitative II, Anti-HBs, Anti-HBc II, Abbott) were previously known positive. PCR detection of other viruses (hepatitis C, D and E, human immunodeficiency virus (HIV), herpes simplex virus) in blood was negative as well as hepatitis A virus IgM, HCV and HIV serologies. Epstein–Barr virus and cytomegalovirus PCR in blood were weakly positive. The patient was treated with entecavir due to suspicion of acute hepatitis B. The treatment was stopped after one week when the diagnosis was ruled out. Meanwhile, liver enzymes had returned to normal limits, and hepatic ultrasound scan did not show gallstones. He was discharged on day 17 with a temperature of 36.8 °C.

On day 44, he was hospitalised again, this time presenting with delirium, fever (38.8 °C), headache, neck and back pain but no other neurological deficit and no visual loss. Lumbar puncture showed a lymphocytic meningitis (Table).

Two further lumbar punctures were carried out on day 48 and day 52. Cerebrospinal fluid (CSF) and serum were sent to the French National Reference Center for Arboviruses in Marseille. A magnetic resonance imaging (MRI) scan of the patient's brain on day 60 showed signs of pachymeningitis. Cryptococcal antigen and JC polyomavirus PCR were negative in blood and CSF. Given that the patient came from a tuberculosis endemic country, tuberculosis was considered and a pulmonary CT-scan and a bronchoscopy were performed for detecting pulmonary localisation. The bronchoscopy showed signs of intra-alveolar haemorrhage but the CT-scan

Table

Results of cerebrospinal fluid, serology and RT-PCR follow-up in a transplant recipient with Rift Valley fever after a travel to Mali, France, January 2016

Day from first physician visit		Day 44	Day 48	Day 52	Day 74	Day 117	Day 145
CSF	Protein(g/L)	1.41	1.02	1.1	ND	0.6	ND
	Cells/mm³	211	71	199	ND	37	ND
	Lymphocytes	60%	97%	99%	ND	ND	ND
	RVF antibodies	ND	ND	IgM pos IgG neg	ND	IgM pos IgG neg	ND
RVF antibodies in blood		IgM and IgG pos	ND	ND	IgM and IgG pos	IgM and IgG pos	IgM and IgG pos
PCR	Semen	ND	ND	ND	Pos	Pos	Neg
	Urine	ND	ND	ND	Pos	Neg	ND

CSF: cerebrospinal fluid; ND: Not done; Neg: negative; Pos: positive; RVF: Rift Valley fever.

was normal. The tuberculin skin test and QuantiFERON test were negative. However, the clinical presentation of lymphocytic meningitis with pachymeningitis in an immunocompromised patient originating from a tuberculosis endemic region, led us to consider tuberculosis meningitis in the absence of an alternative diagnosis. Treatment with anti-tuberculosis drugs was initiated on day 62 but stopped three days later because results for RVF ELISA serology in CSF taken on day 52 were positive for IgM and IgG antibodies.

The in-house qualitative RVFV ELISA is based on the same technic developed by Peyrefitte and al [1]. for dengue virus, including positive and negative serum controls confirmed by serum neutralisation test. RVF antigens are produced from a RVFV strain isolated in 2003 from a patient from Chad. Specificity of the RVFV antibodies detected was confirmed by a 50% neutralisation titre of 1/160 for RVFV (strain Chad 2003) using the virus neutralisation test described by Swanepoel et al. [2].

Semen and urine collected on day 74 were positive (Cycle threshold (Ct) 36.40 and 35.20, respectively) for RVFV, using a RVFV quantitative real-time reverse transcription-PCR assay (Taqman RT-PCR) developed by Bird et al. [3] but faeces and saliva collected on day 82 tested negative. Isolation of the virus was unsuccessful so the RVFV strain could not be sequenced.

Upon establishment of the diagnosis on day 63, we stopped mycophenolate mofetyl and doubled prednisone (from 7.5 mg to 15 mg per day) to facilitate clearance of the virus. The patient improved clinically and headaches, back pain and asthenia disappeared within three weeks. He was discharged without any clinical sequelae in early December, 74 days after he had presented with symptoms to the GP in Mali.

During follow-up, the patient did not report having had any neurological defects or seizures. Neurological and ophthalmologic examination remained within normal limits.

RT-PCR became negative on day 145 in semen i.e. four months after the first physician visit, and in urine on day 117 i.e. three months after the onset of symptoms (Table).

Investigation into possible source of infection

The investigation was limited to an interview of the patient. He arrived in Dionkoulané, Kayes region, 30 km south the Mauritanian border, in Mali, in mid-August 2015. He took care of his father's livestock i.e. sheep and donkeys. No deaths or abortions in these animals were reported. He stated that he may have been in contact with mice living in the area. He did not handle dead animals or abortion products. He consumed raw cow's milk with couscous for a wedding in mid-September, about four days before the onset of the symptoms. No other guests exhibited illness following the wedding.

Background

Rift Valley fever (RVF) is an arthropod-borne zoonotic viral disease affecting mainly domestic and wild ruminants, as well as humans. Outbreaks in humans have been reported in 19 countries across Africa, the Indian Ocean islands, and the Arabian Peninsula [4].

RVF virus is transmitted to humans and animals by (i) direct contact with sick or dead infected animals or their body fluids, (ii) consumption of raw milk or meat from infected animals, and (iii) occasionally by mosquito bites (*Culex, Anopheles, Aedes*) [5]. Livestock i.e. sheep and goats, are highly susceptible to the virus leading to frequent epizootics, causing deaths and abortions and a significant economic impact.

Human infection is typically either subclinical or associated with moderate to severe, non-fatal, febrile illness. Patients can also develop ocular and neurological lesions, hepatitis with hepatic failure or haemorrhagic syndrome associated with infection. Approximately 1 to 2% of RVF infections result in fatal haemorrhagic fever [6].

Discussion and conclusion

This case report highlights that RVF may be acquired in Mali and that the virus was detected in urine and semen after the onset of symptoms for up to three and four months, respectively, in an immunosuppressed patient.

This is to the best of our knowledge the first documented human case of symptomatic RVF acquired in Mali. However, results from a study in 1999 among slaughterhouse personnel working with cattle and sheep in Saudi Arabia during the Hajj, showed a high seroprevalence for workers from Mali [7] and the village where the patient was infected is 30 km south the Mauritanian border, where an outbreak of RVF in sheep and goats was declared in October 2015 [8]. To our knowledge, there was no ongoing outbreak, unusual die-off or abortion reported in livestock in the village during the period of the patient's stay but a silent circulation of the virus cannot be excluded [9]. The patient was frequently bitten by mosquitoes. He also reported raw milk consumption which is considered as a risk factor for human infection [10].

Given the massive affection of the liver, the possibility of acute hepatitis B was ruled out by the low HBV viral load detected by RT-PCR at 20 IU/mL or 1.3 log, corresponding exactly to the threshold of this technique and with no clinical or biological significance, in a patient with a cured hepatitis B. Furthermore, a test for HBV viral load done one month later, was negative.

This case demonstrates the difficulty in diagnosing a little known pathology. The initial clinical presentation was characteristic for acute hepatitis and four weeks later, the patient presented with a lymphocytic meningoencephalitis. The severity of the clinical picture may have been related to the necessary therapeutic immunosuppression in the kidney transplant recipient which might also have slowed down the clearance of the RVFV. We did not precisely identify the mode of infection, however, the time from raw milk consumption to symptom onset was approximately four days. Considering this, and the clinical course with hepatitis symptoms 12 days later, and meningoencephalitis 44 days after having visited the physician in Mali, this is in agreement with the incubation time in the literature [5,6].

We report the first RVF viral RNA detection in urine and in human semen that did not have gross blood contamination. No information about infectivity could be determined as viral isolation failed. The RVFV was previously detected in cattle semen [10] but not in humans. Infectious Ebola virus can be present in semen more than three months after recovery and is transmitted to other persons by sexual route [11]. Similarly, Zika virus has been isolated from the semen of a patient 24 days post onset of symptoms [12]. Ebola virus RNA was detected more than nine months post-exposure [11]. Duration of virus persistence in semen may be linked to the immunosuppressive status of a patient or a viral characteristic as for Ebola and Zika virus. As a precaution, we recommend to abstain or engage only in protected intercourse when RVFV is detected in semen, until complete clearance of the virus.

RVFV genomic RNA was detected in semen four months after the onset of symptoms in an immunocompromised patient. Testing semen could be a new method for diagnosing RVF retrospectively and presence of RVFV genomic RNA raises the possibility that RVF could be transmitted sexually.

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: FH, MM and EC; performed laboratory investigations: MM and IL-G; was actively involved in the reflection on this case: FS; revised the manuscript: FS and VMP; managed the patient and lead the epidemiological investigation: FH, MH and EC.

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Experimental studies of susceptibility of Italian *Aedes albopictus* to Zika virus

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We report a study on vector competence of an Italian population of *Aedes albopictus* for Zika virus (ZIKV). *Ae. albopictus* was susceptible to ZIKV infection (infection rate: 10%), and the virus could disseminate and was secreted in the mosquito's saliva (dissemination rate: 29%; transmission rate: 29%) after an extrinsic incubation period of 11 days. The observed vector competence was lower than that of an *Ae. aegypti* colony tested in parallel.

Zika virus (ZIKV) is an emerging mosquito-borne virus (Flaviviridae family) isolated from different *Aedes* species in the past. In the recent outbreaks that occurred in Latin America, *Aedes aegypti* is believed to be the main vector. The isolation of ZIKV from this mosquito species in Malaysia [1], and early experimental studies [2,3] appear to confirm this hypothesis. Recent vector competence studies have also shown that the American *Ae. albopictus* exhibits similar transmission potential as the American *Ae. aegypti* [4].

Ae. albopictus is widespread in Mediterranean countries, in particular in Italy where it caused an outbreak of Chikungunya virus (CHIKV) (*Togaviridae* family, *Alphavirus* genus) in 2007 [5]. To assess the risk of ZIKV transmission, we evaluated the vector competence of an Italian *Ae. albopictus* population for the virus. Potential vertical (transovarial) transmission of ZIKV was also evaluated.

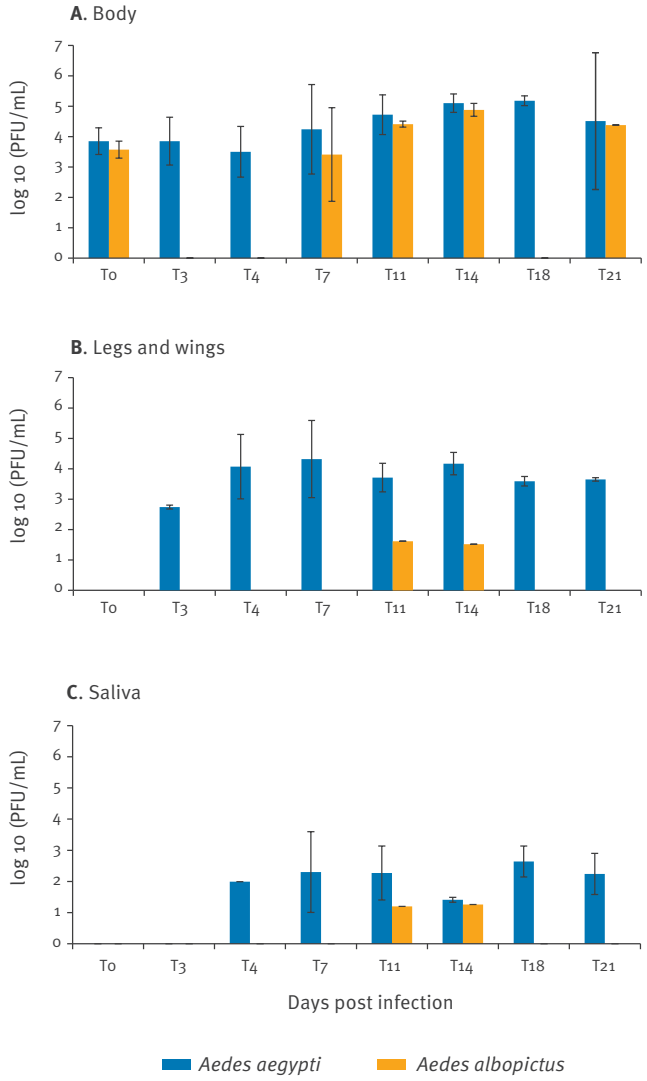
Experimental infection by membrane feeding technique

Oral infection was performed in a BSL-3 laboratory using a ZIKV strain of the Asian genotype (kindly provided by Dr Isabelle Leparc-Goffart of the French National Reference Centre for Arboviruses in Marseille) isolated from a patient returning from French Polynesia in 2013 [6]. Ten-day-old mosquito females from an Italian *Ae. albopictus* population (collected in Scalea town, Calabria region, in the late summer of 2015) and from a long-established colony of *Ae. aegypti* (collected in Reynosa, Mexico, in 1998) were allowed to feed for 1 hour through a membrane feeding apparatus. The virus was diluted in rabbit blood (final virus concentration: 6.46 $\log_{10}$ plaque-forming units (PFU)/mL) and maintained at 37 °C by a warm water circulation system. After the blood meal, fully engorged females were transferred to cages and maintained on a 10% sucrose solution in a climatic chamber (26 ± 1 °C; 70% relative humidity; 14 h:10 h light/dark cycle) for 21 days. Ten mosquitoes from either species were individually processed at 0, 3, 4, 7, 11, 14, 18 and 21 days post infection (dpi). To evaluate viral infection, dissemination and transmission, body (head, thorax and abdomen), legs plus wings, and saliva were analysed, as previously described [7]. ZIKV titre was evaluated by quantitative reverse transcription PCR (qRT-PCR). Specific primers ZIKV 1086 and ZIKV 1162c were used, with 5-FAM as the reporter dye for the probe (ZIKV 1107-FAM) [8]. Crossing point values were compared with a standard curve obtained from 10-fold serial dilutions of virus stock of known concentration [8-10].

Mosquito bodies were analysed in order to evaluate the infection rate (IR), calculated as the number of ZIKV-positive bodies with respect to the total number of fed females. Legs plus wings were tested to assess the dissemination rate (DR), calculated as the number of the specimens with ZIKV-positive legs plus wings among the number of specimens with ZIKV-positive bodies. The saliva of the potentially infected females was processed to assess the transmission rate (TR), defined

Figure 1

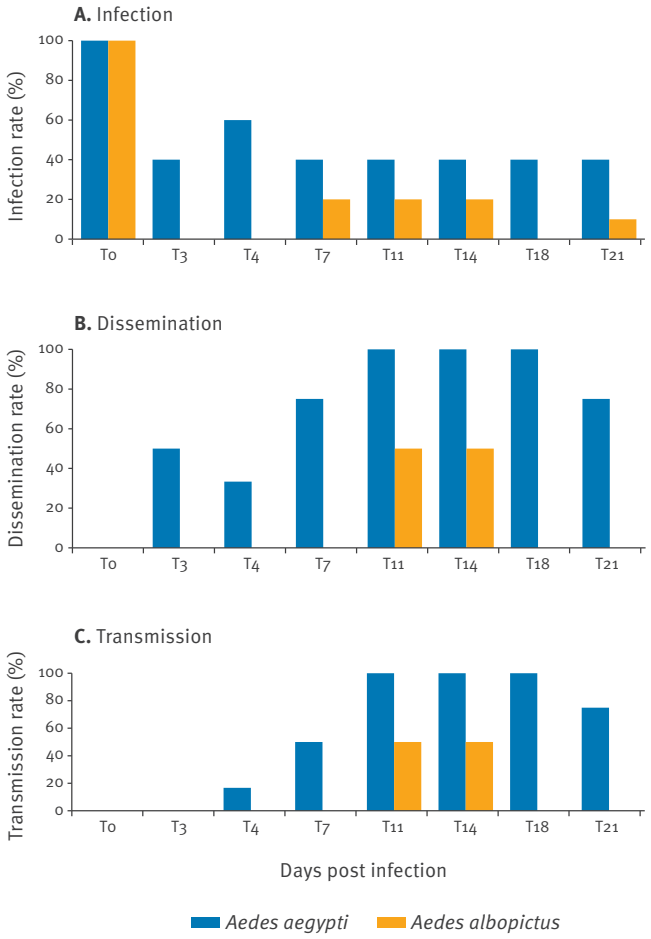
Mean Zika virus titres in different body parts of *Aedes albopictus* and *Ae. aegypti* colonies, at different times post infection, Italy, 2016



PFU: plaque-forming units.

Figure 2

Infection, dissemination and transmission rates in *Aedes albopictus* and *Ae. aegypti* colonies, at different times post infection with Zika virus, Italy, 2016



Infection rate: number of positive bodies/number of tested fed females; dissemination rate: number of positive legs plus wings/number of positive bodies; transmission rate: number of positive saliva/number of positive bodies.

as the number of mosquitoes with ZIKV-positive saliva among the number of specimens with ZIKV-positive bodies [7]. The potential vector competence was expressed as population transmission rate (PTR), calculated as the number of specimens with ZIKV-positive saliva with respect to the total number of fed mosquitoes [9,11].

Vector competence analysis

Mean viral titres and IR, DR, and TR values are shown in Figures 1 and 2.

All of the *Ae. aegypti* and *Ae. albopictus* bodies analysed immediately after the infectious blood meal (day 0) showed positive results, with mean viral titres of 3.85 ± 0.44 $\log_{10}$ PFU/mL and 3.57 ± 0.28 $\log_{10}$ PFU/mL, respectively, confirming the ingestion of infectious viral particles. The viral titres detected in the bodies increased gradually in both mosquito colonies, reaching 5.18 ± 0.16 $\log_{10}$ PFU/mL in *Ae. aegypti* and 4.88 ± 0.21 $\log_{10}$ PFU/mL in *Ae. albopictus* at 18 and 14 dpi (Figure 1A). As expected, differences in IR values between the two species were observed (Figure 2A). In particular, *Ae. albopictus* showed lower IR values than *Ae. aegypti* at all collection times. Whereas an IR of 40% was already detected at 3 dpi for *Ae. aegypti*, infected *Ae. albopictus* specimens were observed starting from 7 dpi, with an IR value of 20%. Cumulative IR values were 43% for *Ae. aegypti* and 10% for *Ae. albopictus* (Table).

Disseminated infection was observed in *Ae. aegypti* starting from 3 dpi, with a mean viral titre of 2.74 ± 0.06 $\log_{10}$ PFU/mL (DR 50%), while in *Ae. albopictus*, the presence of the virus in legs and wings was detected from 11 dpi, with a lower viral titre (1.62 $\log_{10}$ PFU/mL) and an equal value of DR (50%) (Figures 1B and 2B). Starting from 4 dpi, the saliva of *Ae. aegypti* showed ZIKV particles (titre of 1.99 $\log_{10}$ PFU/mL and TR of

Table

Cumulative infection, dissemination, transmission and population transmission rates of *Aedes albopictus* and *Ae. aegypti* experimentally infected with Zika virus, Italy, 2016

	Aedes aegypti	*Aedes albopictus*
Infection rate	43%	10%
Dissemination rate	73%	29%
Transmission rate	60%	29%
Population transmission rate	26%	3%

Infection rate: number of positive bodies/number of tested fed females; dissemination rate: number of positive legs plus wings/number of positive bodies; transmission rate: number of positive saliva/number of positive bodies; population transmission rate: number of positive saliva/number of tested fed females.

17%) and remained positive throughout all collection times. In particular, the viral titres increased reaching the highest levels after 11 dpi (2.64 ± 0.50 $\log_{10}$ PFU/mL). In contrast, virus was detected in the saliva of *Ae. albopictus* at 11 and 14 dpi, with TR values of 50% at both collection points, showing a longer extrinsic incubation period (EIP). Cumulative DR and TR values were 73% and 60% for *Ae. aegypti* and 29% and 29% for *Ae. albopictus*. Finally, PTR values were 26% for *Ae. aegypti* and 3% for *Ae. albopictus* (Table).

After the infectious blood meal, 40 to 50 engorged mosquitoes from each species were kept separate in different cages, under the same laboratory conditions as the ones analysed above, and were allowed to lay eggs (first gonotrophic cycle). Two weeks after the infectious blood meal, a second uninfected blood meal was provided to obtain a second gonotrophic cycle. Pools of 15 to 30 specimens (males and females) from the first and second gonotrophic cycles of both species were processed by qRT-PCR and were negative for ZIKV.

Discussion

Little is known on ZIKV despite its significant epidemic potential [12]. The introduction and dissemination of this previously neglected flavivirus in Latin America, raised concern in temperate climate countries with established *Ae. albopictus* populations [3]. In light of the spread of this mosquito species in Italy, proven vector in the 2007 outbreak of CHIK [5], it is particularly important to evaluate its vector competence for ZIKV and to assess the potential risk transmission in Italy as well as in other Mediterranean countries. Our study shows that the Italian *Ae. albopictus* population is susceptible to ZIKV, allowing viral replication and dissemination also in the salivary glands. The short persistence of the virus in the mosquito's saliva, the PTR value of 3% and the long EIP indicate a low transmission efficiency compared with that of *Ae. aegypti*. In addition, it should be noted that despite the use of a long-established mosquito colony, not representative of a wild population, the vector competence of *Ae. aegypti* for ZIKV was significant in our experiment. A recent modelling study [13] that was based on parameters of susceptibility to infection of the *Ae. albopictus* derived from mosquito populations from the United States and Singapore [4,14], also estimated a low risk of sustained autochthonous transmission of ZIKV in northern Italy. Our results are similar to the above results on American *Ae. albopictus* and substantially confirm the low epidemic potential of ZIKV in Italy. However, the epidemic potential and the capacity to cause long chains of transmission depends on a series of factors such as the abundance of the mosquito population, the density of the human population, feeding host preferences, biting rates and environmental conditions. High mosquito density, day-biting activity, opportunistic feeding behaviour and climatic and environmental adaptability can affect the efficiency of *Ae. albopictus* as a vector, favouring its primary role in epidemics, also in the presence of a limited vector competence [15].

Our results also have important public health implications for preparedness. In fact, the extended EIP, which is consistent with the results of studies using American *Ae. albopictus* mosquitoes [4], would allow the implementation of mosquito control measures that are likely to be more efficient than those implemented in areas infested by the tropical mosquito *Ae. aegypti*. Moreover, our analysis of offspring of both species from the first and second gonotrophic cycle showed no evidence of transovarial transmission of ZIKV; this finding adds knowledge on the bionomics of this vector and may aid the optimisation of vector control management. Finally, ZIKV appears to be less well adapted to the Italian *Ae. albopictus* than the A226V variant of CHIKV (data not shown), which caused more than 250 cases in Italy after a single introduction from Kerala, India [5,16].

In conclusion, this experimentally infected Italian *Ae. albopictus* population appeared to be a competent vector for ZIKV, albeit less efficient than the primary vector *Ae. aegypti*. However, we should not forget the risk posed by CHIKV and dengue virus that remains high in southern European countries, where small outbreaks and clusters of autochthonous cases have been already documented [17,18].

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Conflict of interest

None declared.

Authors' contributions

DLM, SF, TL, BD, RME, SM, VG and FC performed the experiments; DLM, SF, TL, BD, VG and FC analysed the data; DLM, SF, TL, BD, VG, RR, RC, RG and FC wrote the manuscript.

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Research article

Evaluation of the enterovirus laboratory surveillance system in Denmark, 2010 to 2013

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The primary aim of the Danish enterovirus (EV) surveillance system is to document absence of poliovirus infection. The conflict in Syria has left many children unvaccinated and movement from areas with polio cases to Europe calls for increased awareness to detect and respond to virus-transmission in a timely manner. We evaluate the national EV laboratory surveillance, to generate recommendations for system strengthening. The system was analysed for completeness of viral typing analysis and clinical information and timeliness of specimen collection, laboratory results and reporting of clinical information. Of 23,720 specimens screened, 2,202 (9.3%) were EV-positive. Submission of cerebrospinal fluid and faecal specimens from primary diagnostic laboratories was 79.5% complete (845/1,063), and varied by laboratory and patient age. EV genotypes were determined in 68.5% (979/1,430) of laboratory-confirmed cases, clinical information was available for 63.1% (903/1,430). Primary diagnostic results were available after a median of 1.4 days, typing results after 17 days, detailed clinical information after 33 days. The large number of samples typed demonstrated continued monitoring of EV-circulation in Denmark. The system could be strengthened by increasing the collection of supplementary faecal specimens, improving communication with primary diagnostic laboratories, adapting the laboratory typing methodology and collecting clinical information with electronic forms.

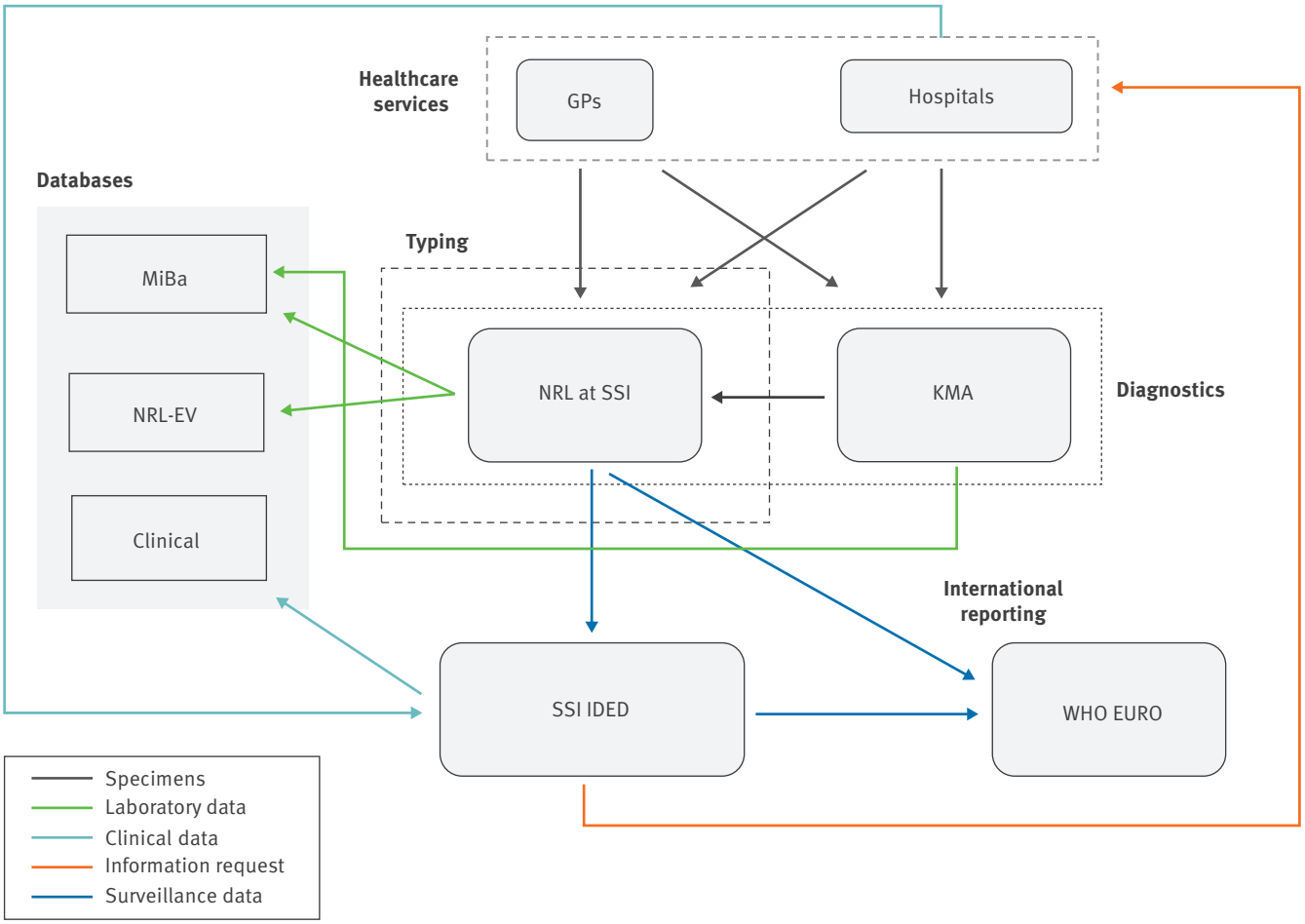
Introduction

Human enteroviruses (EV) is a diverse group of single-stranded RNA-viruses from the *Enterovirus* genus of the Picornaviridae family that includes polioviruses. A number of EVs are among the most common viral infectious agents in humans, with the majority of infections being asymptomatic or mild [1]. However, infection with EV can lead to a wide spectrum of symptoms including; upper respiratory illness, febrile rash, aseptic meningitis, pleurodynia, encephalitis, neonatal sepsis-like disease and acute flaccid paralysis (AFP) which may indicate poliomyelitis [1-3].

The World Health Organisation (WHO) announced the European Region as poliovirus free in 2002 [4]. In 2013 when only three countries had endemic circulation of poliovirus, namely Pakistan, Afghanistan and Nigeria, circulation of wild-type poliovirus was detected in three new regions: Syria, the Horn of Africa, and Israel [5-7]. Due to this recent transmission of poliovirus combined-+ with the armed conflict in Syria and the movement of refugees from this region, there is an increased risk for importation of poliovirus into Europe [7,8]. Considering this risk, there are currently concerns in the European Union (EU) and European Economic Area (EEA) over the quality of EV surveillance, in particular the capacity of countries to detect and respond to poliovirus transmission in a timely manner [9,10]. The European Centre for Disease Prevention and Control (ECDC) therefore advised in a 2014 technical report, entitled 'Detection and control of poliovirus transmission in the European Union and European Economic

Figure 1

Flowchart of the laboratory surveillance system for enterovirus in Denmark, routes of specimen flow, clinical information collection, databases and international reporting.



EV: enterovirus; GPs: general practitioners; IDED: Department of Infectious Disease Epidemiology; KMA: hospital clinical microbiological laboratory; MiBa: the Danish national microbiological database; NRL; Danish National WHO Reference Laboratory for poliovirus; SSI: Statens Serum Institut; WHO EURO: World Health Organization Regional office for Europe

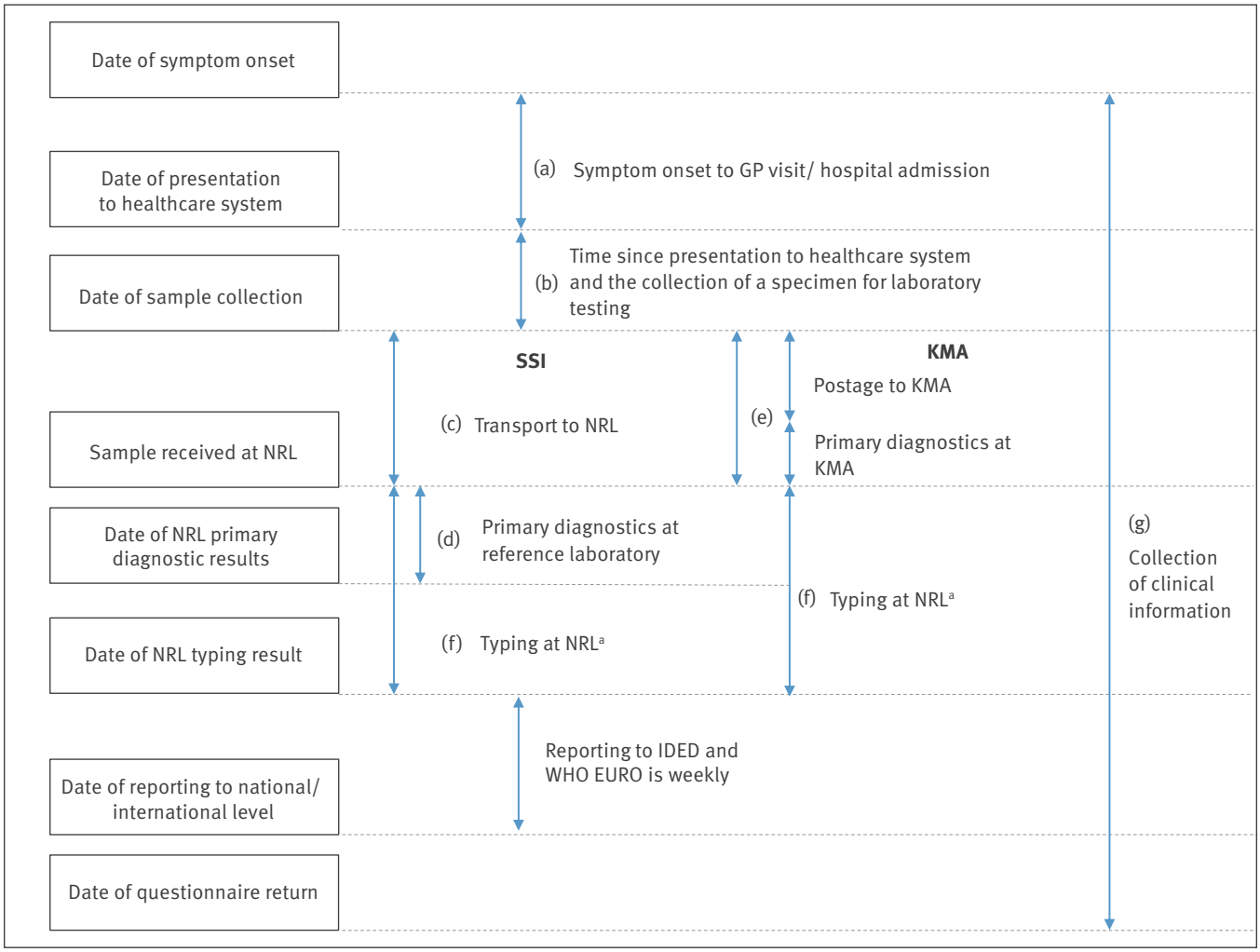
Specimens are sent for primary diagnostics to a KMA or directly to SSI. All data from the KMA and SSI are entered MiBa, all data from SSI is also entered into the NRL-EV database. The NRL reports new EV cases to the IDED. Reporting was monthly up until 2011 and weekly from 2012. The SSI IDED collects detailed clinical information with a letter and standardised questionnaire sent to the patient's hospital or GP. Returned questionnaires are entered into the clinical database by the IDED. Reporting to WHO EURO is carried out on a weekly from the reference laboratory; information is submitted for one sample per patient per day of sample collection. Reporting to WHO-EURO is yearly from the IDED.

Area', EU/EEA countries should assess the quality of their poliovirus surveillance and determine whether it needs to be strengthened [9]. A surveillance system allowing for rapid detection and a short time between specimen collection and outbreak response, would permit reducing transmission faster, while a slower response would conversely be associated with more widespread-transmission and therefore a greater cost and effort of containment [11]. This project aimed to address these points with regards to aspects of poliovirus surveillance currently operating in Denmark; the laboratory EV surveillance system and collection of detailed EV clinical information.

Poliomyelitis is a mandatorily notifiable disease in Denmark and a laboratory surveillance system based at the Danish National WHO Reference Laboratory for poliovirus (NRL) at Statens Serum Institut (SSI), in collaboration with the Danish clinical microbiology laboratories (Klinisk Mikrobiologisk Afdeling (KMA)), ensures year-round surveillance of all cases of EV-positive aseptic meningitis. The laboratory surveillance system is case-based and covers the entire population of Denmark; including all general practitioners (GPs), all hospitals and all KMAs. The system was initially set up as a component of the national poliomyelitis eradication plan, with the main objective of documenting the absence of poliovirus transmission in Denmark. This surveillance system collects data on EV to the genotype

Figure 2

Time intervals in the reporting of laboratory confirmed enterovirus cases in Denmark



EV: enterovirus; GP: general practitioner; IDED: Department of Infectious Disease Epidemiology; KMA: hospital clinical microbiological laboratory; NRL: Danish National WHO Reference Laboratory for poliovirus; SSI: Statens Serum Institut; WHO EURO: WHO Regional office for Europe.

[a]Could be calculated for 2013 only, relevant dates were not available before 2013.

The timeliness of interval (a) and (b) were calculated from the clinical database. For specimens where primary diagnostic was carried out at SSI, the intervals (c) and (d) were calculated from the NRL-EV database. For specimens where primary diagnostic was carried out at a KMA (e) was calculated from the NRL-EV database. For all samples subject to typing analysis the time interval (f) was calculated from the BioNumerics database and files containing sequencing run history, and was the time interval between arrival at the reference laboratory and the date when the first sequence result was obtained. The final time interval, (g) symptom onset to reporting of clinical symptoms to the department of epidemiology was calculated from the clinical database

level, as well as patient demographic information and clinical details; including symptoms, presentation with AFP and date of symptom onset. With this information the system also serves the objective to monitor national trends in circulating EV.

The surveillance system consists only of EV surveillance, is run on a voluntary basis and involves components that are passive, and there are consequently concerns with regards to underreporting. It has never been evaluated, and the completeness and timeliness are unknown.

The overall aims of the study were to describe the EV surveillance system and to determine whether it meets its surveillance objectives. In order to achieve this, we assessed the surveillance system and its characteristics, described the data sources, data providers, flow of diagnostic specimens, and mapped the routes of reporting to the national level and to WHO Regional office for Europe (WHO EURO). Hereafter, we evaluated the EV surveillance system for the attributes completeness and timeliness and made recommendations for improvements to the current system with regards to

firstly, documenting the absence of poliovirus transmission and secondly monitoring national trends in EV circulation.

Methods

Routes of reporting

The Danish EV surveillance system is run collaboratively by the NRL at the Department of Microbiological Diagnostics and Virology (MDV), SSI and the Department of Infectious Disease Epidemiology (IDED), at SSI. Although the system is voluntary it is highly recommended that EV-positive faecal or cerebrospinal fluid (CSF) specimens from patients with aseptic meningitis are sent to SSI. Between July 2014 and January 2015, interviews were conducted with staff of the NRL, IDED and the WHO EURO group lead for the Regional Laboratory Networks. Questions were asked in regards to the laboratory practices; the diagnostic and genotyping workflow and the surveillance system infrastructure; data sources, analysis and outputs. Finally, questions were asked about the system routes of reporting.

Laboratory identification and typing of enteroviruses

Interviews were additionally conducted in November 2014 with section chiefs or laboratory personnel responsible for EV testing, at the primary diagnostic level, at the KMAs. All 11 of the national KMAs were invited to take part in the study, either by telephone or by a paper-based questionnaire. Seven of the eleven KMAs took part in the interviews.

Four KMAs did not respond to the invitation for a telephone interview and did not return the paper questionnaire. Three of these KMAs: Mid-Vest, Esbjerg and Vejle did not test for EV during the study period. Only one of the non-respondent KMAs, Odense, did test for EV during the study period.

Questions were asked to ascertain whether the laboratories carried out EV testing and if so by what techniques. Additionally, KMAs were asked what criteria they followed for selecting specimens for forwarding to SSI.

Evaluation the surveillance system

The CDC 'Updated guidelines for evaluating a public health surveillance system' were used as a framework for the evaluation [12]. Completeness and timeliness were chosen as surveillance performance indicators for the analysis.

Data sources

Several central data sources were accessed for this evaluation and are outlined in Figure 1. Briefly, specimens are sent for primary diagnostics to a KMA or directly to SSI. Not all KMAs have the capacity to test for EV therefore specimens for EV testing may be forwarded directly to SSI or to another KMA. If a specimen is found EV-positive at a KMA it can be sent to the NRL for viral typing. Laboratory data for all EV primary diagnostic testing are contained in the Danish national microbiological database (MiBa). An extract was obtained from MiBa for all EV-positive serological, culture and RNA-based diagnostic tests and their results from 2010 to 2013. Details relating to laboratory testing carried out by the NRL are stored in the SSI laboratory information and management system (LIMS). All entries coded as EV culture, diagnostic PCR, and typing PCR were extracted from LIMS for the period of 01 January 2010 to 31 December 2013. This dataset is called the NRL-EV database herein.

Information on sequencing results for all EV-positive specimens typed at SSI for the corresponding study period was obtained in from an extract of the NRL sequencing results database, which is maintained with BioNumerics software and herein called the NRL-sequencing database.

The NRL reports new EV cases to the IDED, who in turn collects clinical information with a letter and standardised paper-based questionnaire sent to the patient's hospital or GP, and collects details on patient's symptoms in particular neurological symptoms including acute flaccid paralysis. The letter also reminds clinicians to send faecal samples from the patient to the NRL for characterisation.

The collected clinical data are stored in a database internally at the IDED. All entries in this clinical information database corresponding to the years 2010 to 2013 were extracted.

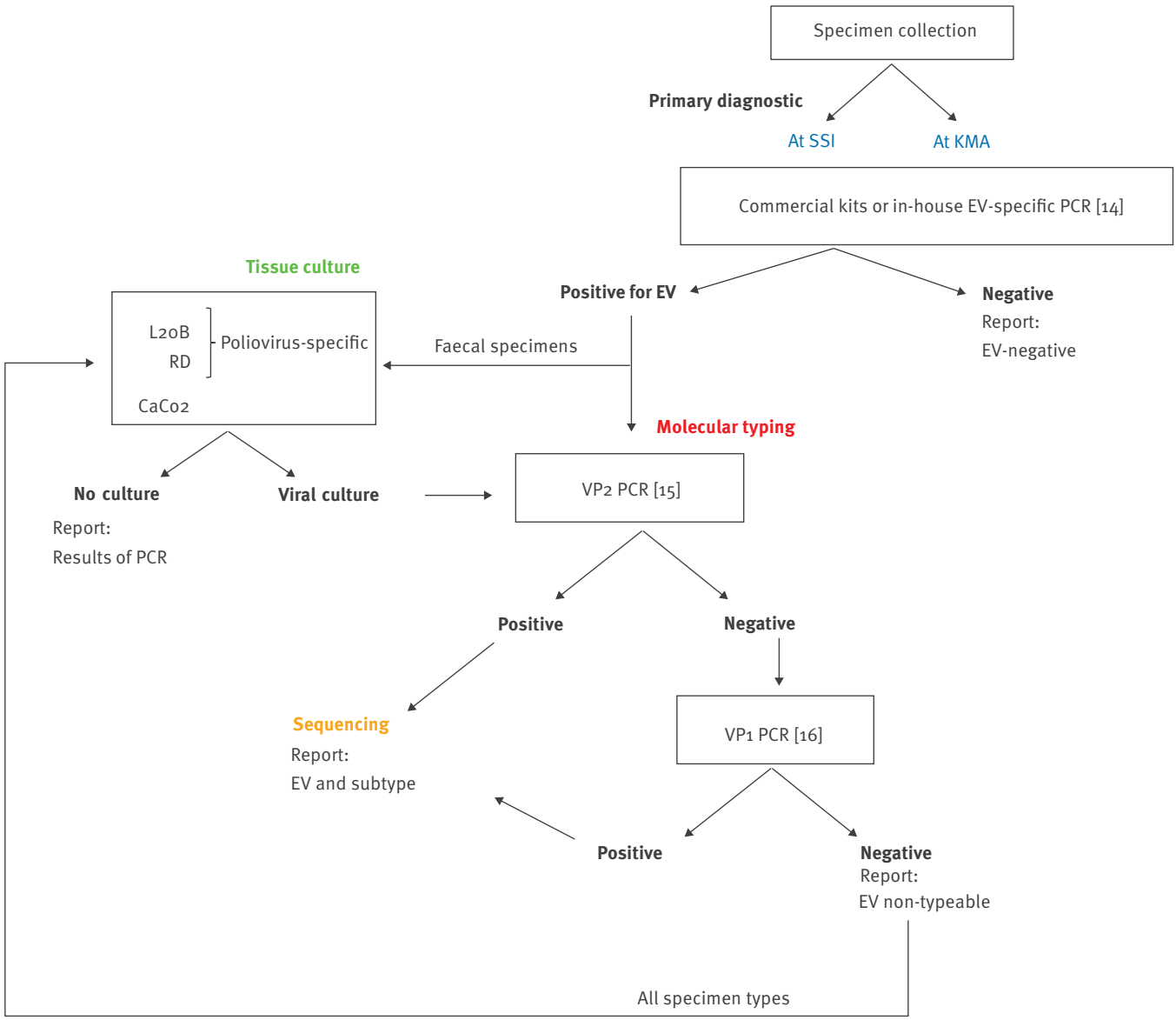
Data linkage between the various databases was possible and efficient due to the use of the Danish civil registry number (CPR numbers) as a unique identifier. This number is used in all four databases.

Completeness of the surveillance system

Completeness was assessed for the first component of the laboratory-based surveillance system; the forwarding of EV-positive specimens from primary diagnostic facilities to the NRL. All specimens that tested EV-positive on primary diagnosis, present in the MiBa database, were overlapped with the NRL-EV dataset, containing those specimens forwarded to SSI. If multiple specimens were taken from the same patient only one overlapping specimen was needed for all specimens associated with the patient to be considered overlapping. Samples were considered overlapping if there was no more than a 14 day difference between the sampling dates indicated in the two databases. If more than one specimen was collected from the same patient on the same day, only one was counted in the analysis. The proportion of specimens positive in MiBa present in the NRL-EV dataset was calculated as the performance indicator. This calculation was performed for CSF and faecal specimen types only, and repeated for all specimen types that were submitted

Figure 3

Flowchart of the laboratory diagnostic workflow, for the surveillance of enterovirus in Denmark



CSF: cerebrospinal fluid; EV: enterovirus; KMA: hospital clinical microbiological laboratory; SSI: Statens Serum Institut

KMAs differ in their methods for primary diagnostics. At SSI primary diagnostics is carried out with a multiplex real-time PCR. A reverse-transcriptase PCR targeting the VP2 capsid protein is the primary EV typing assay. If VP2 typing fails, a reverse-transcriptase semi-nested PCR targeting the VP1 capsid protein is used. PCR amplicons are sequenced. All faecal specimens, as well as CSF and other specimens where PCR has failed are subject to viral cultivation assays. Specimens are cultured in three cell lines, two poliovirus specific cell lines; L20 and RD and one cell line for the non-specific cultivation of EV, CaCO2. Positive viral cultures are then subject to the EV typing workflow. If a typing result was obtained from either VP2 or VP1 the results are reported as EV and the genotype is given. If no product is obtained from either assay the isolate is reported as EV non-typeable. If poliovirus is detected from the cultivation process, further characterisation based on virus neutralisation as well as RT-PCR will be applied to determine the poliovirus type as well as to discriminate between wild-type or vaccine-derived poliovirus, respectively.

for EV surveillance; faecal samples, CFS, blood, serum, plasma, biopsy, swabs, bronchoalveolar lavage, expectorate, naso-pharyngeal secretions, pulmonary secretions, pericardial fluid, pus, saliva and urine. Results were stratified by primary diagnostic laboratory, patient age and analysed for statistical differences using a Chi-squared test. The completeness over the test period was analysed for trends using Poisson regression. Binomial proportion 95% confidence intervals (CIs) were calculated.

Secondly, the completeness of the typing analysis carried out at the NRL was determined. A dataset extracted from the NRL-EV database corresponding to all samples that were EV-positive was overlapped to the NRL-sequencing database, containing typing results. The

Table 1

Completeness of EV-positive CSF and faecal specimen forwarding from primary diagnostic laboratories to the National Reference Laboratory; by year, patient age and primary diagnostic laboratory, Denmark, 2010–2013 (n=2,202)

	Positive specimens[a]	Sent to reference laboratory	Not sent to reference laboratory[b]	Percentage Completeness (%)	95% Confidence Intervals
All sample types					
Total number of specimens	2,202	1,712	490	77.7	75.9-79.4
CSF and faecal specimens					
Total number of specimens	1,063	845	218	79.5	76.9-81.8
KMA of primary diagnostic[c]					
Aalborg	22	10	12	45.5	26.9-65.3
Herlev/ Hillerød/ Hvidovre	103	45	58	43.7	34.5-53.3
Odense	216	203	13	94.0	89.9-96.4
Region Sjælland	15	11	4	73.3	48.1-89.1
Rigshospitalet	70	39	31	55.7	44.1-66.7
SSI[d]	377	377	0	100	98.9-100
Aarhus	249	151	98	60.6[e]	54.5-66.5
Sønderborg	11	9	2	81.8	52.3-94.8
Patient age[c]					
Less than one year	293	258	35	88.1	83.8-91.2
Aged 1 year or older	770	587	183	76.2	73.1-79.1
Year					
2010	227	185	42	81.5	75.9-86.0
2011	314	267	47	85.1	80.6-88.6
2012	305	244	61	80.0	75.1-84.1
2013	217	149	68	68.7	62.2-74.4

KMA: hospital clinical microbiological laboratory; SSI: Statens Serum Institut.

[a]The calculation counts one specimen per patient per 14 days (specimens taken within a 14-day period are considered to be relating to the same episode of illness).

[b]This value reflects different practices in the KMAs, including differences in the selection criteria for sending specimens where low volume remains following the KMA's primary diagnostic workflow.

[c]Indicates groups where completeness values were statistically different between strata

[d]The SSI primary diagnostic laboratory and the NRL are located in the same building

[e]All specimens are checked, and only those with sufficient material are sent to the NRL.

performance indicator was the number of EV-positive specimens subject to typing analysis over the total number of EV-positives. The completeness of the genotype result was also determined; and the performance indicator was the total number of specimens where a subtype could be determined over the total number of EV-positive specimens.

Thirdly, completeness of the clinical data (e.g. symptoms of CNS affection, paralysis etc) for EV-positive cases was determined by comparing the number of patients with clinical information available to the number of patients with no clinical information available. An extract was made of the NRL-EV dataset containing all EV-positive cases. This was overlapped with the clinical information database. The performance indicator was the number of EV-positive cases where detailed clinical information was collected over the total number of EV-positive cases. The results were stratified for patient age and analysed for statistical differences using a Chi-squared test. The completeness by year was analysed for a trend with Poisson regression. Binomial proportion 95% CIs were calculated.

Timeliness of the surveillance system

The time intervals between six steps in the surveillance system were evaluated using various time variables from the four databases described above. The six steps (a) to (g) are outlined in Figure 2. Intervals for steps (a) to (e) and (g) were calculated for the years 2010 to 2013, intervals for step (f) could be calculated for 2013 only, dates were unavailable for the previous years. The time intervals were calculated in days. For each time interval the median, 25% and 75% quartiles and the interquartile range (IQR) were calculated using STATA V12.1 software. The timeliness calculations were stratified for patient age, geographical location, hospital of patient origin, laboratory of primary diagnostic and laboratory result. Strata were compared for differences with a one-way analysis of variance. Trends in the timeliness values over the test period were analysed with linear regression.

Table 2

Completeness of patient clinical information for enterovirus-positive specimens, by year and age for all sample types, Denmark, 2010–2013 (n=1,430)

	Total no. positive specimens	Total no. clinical information collected	Total no. clinical information not collected	Percentage completeness (%)	95% Confidence Intervals
All cases	1,430	933	497	65.1	62.7-67.7
Year					
2010	318	270	48	84.9	80.6-88.4
2011	285	92	193	32.3	27.1-37.9
2012	452	263	189	58.2	53.6-62.7
2013	375	308	67	82.1	77.9-85.7
Patient age[a]					
Less than one year of age	948	605	343	63.8	60.7-66.8
Aged 1 year or older	482	328	154	68.0	63.8-72.1

[a]Indicates groups where completeness values were statistically different between strata

Surveillance standards

Surveillance standards were based on those for AFP along with the objectives of the EV surveillance system [13,14]. The following surveillance standards were considered acceptable for the system:

≥80% of positive CSF or faecal specimens, determined on primary diagnostics should be submitted to the NRL for viral typing;

- ≥80% of positive CSF or faecal specimens, should arrive at the NRL within 7 days of specimen collection;
- the final laboratory typing result should be available for≥80% of specimens within 28 days of sample arrival;
- clinical information should be collected for≥80% of cases with a positive specimen sample.

Results

Surveillance system structure and laboratory workflow

From the interviews the structure of the system, including specimen flow, data flow, databases and routes of reporting was elucidated (Figure 1).

At the six KMAs that were interviewed, the EV primary diagnostics differed in terms of assays used and range of sample material they had capacity to test. One of the interviewed KMAs did not test for EV, four of the KMAs used a commercial, automated real-time multiplex reverse transcription-PCR for the diagnosis of EV from CSF, but did not test other sample materials. One KMA used an in-house PCR for all sample materials. In all cases KMAs sent positive CSF samples to SSI and supplementary faecal samples from the positive patients should also be sent to SSI. Interviews elucidated different practices locally at the KMAs with regards to criteria for sending low-volume CSF specimens to the NRL. Some KMAs sent all specimens including low volume whereas others did not. These differences impacted on the completeness values.

Specimens from all regions of Denmark were sent to SSI for primary diagnostics. Primary diagnosis at SSI was carried out with a one-step multiplex real-time PCR as described by Nielsen et al. in 2013 [15].

VP2 and VP1 regions of EV-positive specimens were PCR amplified, as described by Nasri et al. and Nix et al., respectively [16,17]. Amplicons from these PCRs were sequenced and genotypes obtained by sequence comparisons within the NRL-sequencing database, and the NCBI, using the BLAST software. Genotyping was carried out at the NRL only. The laboratory workflow for EV typing at SSI is outlined in Figure 3.

Surveillance data

During the study period a total of 23,720 samples were tested for EV in Denmark; 9.3% (2,202/23,720) were positive. Of these samples 16,538 (931 positive samples) were tested at KMAs while 7,182 (1,271 positive samples) were submitted directly for testing at SSI.

A total of 10,945 CSF specimens were tested, 7.7% (844/10,945) were EV-positive; of 2,211 tested faecal specimens, 9.9% (219/2,211) were EV-positive; 17.6% (149/844) positive CSF specimens had a faecal submitted for analysis also. The remaining 10,564 samples, comprised blood, serum, plasma, biopsies, swabs, pulmonary secretions, pericardial fluid, pus,

saliva and urine and unknown sample types and 10.4% (1,106/10,564) were EV-positive.

During VP2 and VP1 amplification and subsequent typing and alignment, no poliovirus (wild-type or vaccine-derived) was detected.

Completeness

Submission of EV-positive specimens to the National Reference Laboratory

The completeness of submission of EV-positive specimens to NRL for further characterisation was 77.7% for all specimen types (1,712/ 2,202 positive specimens) (Table 1). For CSF or faecal specimens, completeness of 79.5% was obtained (834/1,063 positive specimens) (Table 1).

There was no increasing or decreasing trend in the numbers of CSF or faecal specimens forwarded to the NRL over the four years). However, the completeness varied by year and was above the adopted surveillance standard cut-off of ≥ 80% in three of the years and was below the cut-off in 2013 only (Table 1). The level of completeness also varied according to the location of primary diagnostics; the KMA of sample origin, (p-value < 0.001), and was above the surveillance standard of ≥ 80% for three KMAs (Table 1). Some KMAs check the samples for sufficient material before sending them to the NRL and withhold samples that would not be processed further by the NRL. Thus, the number of EV-positive specimens evaluated by the KMAs is higher than those actually sent to the NRL. The completeness of this step for all samples, including those with too little sample material remaining, was significantly higher, and above the surveillance standard threshold, for patients aged one year or younger compared with those older than one year, (p-value < 0.001) (Table 1).

EV genotyping carried out at the National Reference Laboratory

There were 1,430 EV-positive specimens in the NRL-EV database; 863 following primary diagnostic at SSI and 567 from KMAs. Of these, 1,344 were genotyped, 86 specimens were not genotyped because they lacked sufficient sample material. All samples where sufficient material remained following primary diagnosis were subject to genotyping. The completeness of this component of the surveillance system was therefore 94.0% (1,344/1,430). Of these, no amplicon could be obtained with the genotyping PCR for a total of 365 specimens; they were therefore reported as non-typeable EV. The completeness of a final genotyping result was therefore 68.5% (979/1,430).

Detailed clinical information for entereovirus-positive cases

Completeness of detailed clinical information for EV-positive cases over the four year period was determined as 65.2% (Table 2). Although there was no trend in the completeness detected over the four year period, the completeness of this step varied throughout the years; two years 2010 and 2013 were above the surveillance standard while the values for 2011 and 2012 were well below the standard (Table 2). The collection of detailed clinical information was not different for cases over and under one year of age.

Timeliness

The median timeliness of the six steps in the surveillance system is summarised in Table 3. The median for the first interval symptom onset and presentation to the health system was significantly shortened for cases under 1 year of age; with a median of 1 day (IQR 0 to 2 days), compared with cases aged one year or over, with a median of 2 (IQR 1 to 4 days, p-value 0.024. The timeliness of presentation to the health system to the collection of a specimen for laboratory testing did not vary by patient age, or between hospitals. The interval for the time taken for transport of a specimen from the healthcare facility to SSI varied by geographical location, (p-value < 0.001), and was shorter for samples from facilities within the Capital region, where the NRL is located, median of 1.02 days (IQR 0.8 to 2.9) and was longer for samples from regions further from the NRL laboratory, with the longest from North-Jutland, median of 1.93 days (IQR 1.10–3.10). However, overall 98.1% (7,809/7,691) of the specimens arrived at the NRL laboratory within 7 days of sample collection. The interval for the time for specimens to be transferred to a KMA, for primary diagnostics to be carried out and for positive samples to then be forwarded to the NRL also met the surveillance standards, with 80.6% (1,094/1,357) of submitted specimens arriving at the NRL within 7 days of sample collection. For these samples the KMA of origin and relevant dates was known for 644 specimens, and the interval differed significantly between the various KMAs, p-value < 0.001, the shortest interval recorded, had a median of 1 (IQR 0.80 to 3) and the longest had a median of 31.5 days (IQR 0.91–92.64). Five of the KMAs met the surveillance standard, however two did not, with 62.6% (47/75) and 71.4% (5/7) of specimens arriving at the NRL within 7 days. The timeliness of step (f) sample arrival to results of viral genotyping could be calculated for 2013, results for 82.5% (193/234) of specimens were available within 28 days.

The final time interval was described before symptom onset and reporting of clinical symptoms to IDED. There was no difference between patients aged over and under one. There was a decreasing trend detected in the value of the interval overtime (p-value < 0.001). The year with the longest interval for the collection of clinical data was 2011, with a median of 100 days (IQR 60-183), decreasing to a median of 19 (IQR 5-42) in 2013.

Discussion

This study aimed to review the Danish EV surveillance system and determine whether the system fulfils its objective to document the absence of poliovirus

Table 3

Value of examined time intervals in days, enterovirus laboratory surveillance system, Denmark, 2010–2013

Interval description	N	Median days (IQR)
From symptom onset and presentation to the health system (a)	497	1 (0–3)
From presentation to the health system to the collection of a specimen (b)	475	1 (0–2)
From specimen taking to arrival at the reference laboratory for primary diagnostic (c)	7,961	1.4 (0.9–2.5)
From sample arrival to results of the primary EV diagnostic laboratory test (d)	7,961	1.3 (1–3)
From specimen taking to arrival at the reference laboratory for typing analysis (e)	1,378	3 (1–5)
From sample arrival to results of viral typing[a](f)	1,378	17 (11–26)
From symptom onset and reporting of clinical symptoms to the department of epidemiology (g)	770	33 (17–63)

IQR, 25–75% interquartile range

a-g: Time intervals as indicated in figure 2.

[a]Data for 2013 only.

transmission in Denmark and monitor genotypes of EV in circulation.

From 2010 to 2013, the EV laboratory surveillance system performed detailed surveillance of EV in circulation in Denmark; over 20,000 specimens were tested with the primary aim of documenting the absence of poliovirus transmission. No wild-type poliovirus was detected during this period. The laboratory system has the ability to detect the occurrence of a poliovirus infection; ensured by poliovirus proficiency panels from WHO correctly serotyped through this system within a 14-day time period, following WHO guidelines [18]. The surveillance system additionally gathers the necessary information to facilitate an investigation if a case of poliomyelitis occurs. As a secondary objective this surveillance system also monitors the circulating EV genotypes in Denmark and allows to study long-term patterns and epidemiological characteristics of EV transmission and it facilitates the detection of outbreaks through analysing data for individual genotypes. In addition, the data collected by this system enables the identification of molecular targets for the development of more specific diagnostic assays should they be required, for examples in the case of an outbreak as demonstrated by recent investigation on EV outbreaks in Denmark [3,19,20].

The second part of this study aimed to evaluate the EV surveillance system from 2010 to 2013. Firstly, the completeness was determined as a means to whether the data collected was meaningful and included the entire population that should be covered by the surveillance system [9,10]. Secondly, the timeliness was calculated to detect time-delays in the system, and the identification of factors associated with this delay [21].

The initial step of the EV surveillance system, the forwarding of EV-positive specimens for further viral characterisation from KMAs to the NRL, is a passive step in the surveillance system and therefore a major concern with regards to the data completeness of the system [22]. This evaluation documented that the forwarding of EV-positive CSF and faecal specimens to the NRL for viral typing was overall just short of the surveillance standard specified for the completeness of this step. However, some KMAs participate in the sorting of samples and only send samples with sufficient material for further processing at the NRL. The timeliness of this step was however, despite difference between various regions of Denmark, within the surveillance standards for all regions. When the completeness and timeliness values were stratified a number of factors associated with underreporting were identified. The completeness value varied between the KMAs meeting the surveillance standard in some of the laboratories and not in others. Similarly, there were differences in the timeliness values between the various KMAs, ranging from the shortest median of 0.8 days to the longest of 31.5 days. Differences in surveillance performance on a sub-national level, have been reported on previously in AFP surveillance in the United States (US). In the US, regional reporting was also variable and in some instances did not fulfil the surveillance standards, even though the national system on a whole did so [23]. The difference in the distribution of samples from the various KMAs indicated that the surveillance of EV may not be representative of EV circulation in the entire Denmark. The quality of the data obtained in this initial step of the surveillance will determine the representativeness at all subsequent stages [22]. Therefore, improved forwarding of samples and supplementary faecal sampling from patients with too little residual material in the primary sample would allow for a more representative and timely system.

Furthermore, the completeness was significantly higher for patients one year or younger as compared with those older than one year of age, meeting the surveillance standard for those one year or younger only. Often, EV infections in younger patients tend to be associated with a more severe illness and a specific diagnosis considered more urgent; specimens from younger patients tend to be submitted for laboratory testing more frequently [24,25]. This age-based surveillance bias was similarly reported in the US [24].

These results indicate that in order to obtain a more representative overview of EV circulation an increased awareness in the importance of forwarding specimens from older patients is needed by clinicians and primary diagnostic laboratories.

The completeness of the second step of the surveillance system was adequate. All specimens where sufficient quantity remained after primary diagnosis, above 90%, were subject to genotyping. However, since the completeness of the first step of the surveillance system was 79.5%, the percentage of the total number of EV-positive specimens subject to viral typing analysis was closer to 75% and would increase if more specimens were forwarded to the NRL.

The timeliness met the surveillance standard with a median time interval between specimen arrival and a typing result of between two and three weeks. The overall aim of the viral typing is not for the real-time detection of outbreaks but rather to document the absence of poliovirus transmission along with the detection of general trends in the circulation of EV. The system has met its surveillance objective for EV and poliomyelitis, and enabled the detection of recent outbreaks of EV 71 and D-68 [3,16]. However, our results highlighted a high proportion of › 25% of non-typeable EV among EV positive samples sent to the NRL. The proportion of non-typeable isolates is thought to vary by sample type and typing may be more successful for faecal samples. A somewhat high proportion of non-typeable EV specimens reported in studies that include many sample types is not unusual and has been reported previously [26,27]. This study therefore demonstrated that virus genotyping procedures, need to be improved to increase the number of EV where an amplicon can be obtained to increase the typing result-completeness rate.

The final aspect of the surveillance system to be evaluated was the collection of clinical information in relation to neurological symptoms from EV-positive cases, as an indicator of potential poliomyelitis. Our results show that the collection of this information was below the target ≥ 80% over the four-year period. However, the completeness varied by year and was lowest during 2011. An explanation for the low value that year was that there was a shortage of staff at SSI in 2011. This resulted in a backlog in laboratory testing and a delayed reporting to IDED. A decision was taken not to collect detailed clinical information from cases confirmed as EV-positive, where specimen collection had taken place more than 6 months previously, therefore negatively impacting the completeness of data obtained. The system was revised hereafter, and reporting from the NRL to IDED was changed from monthly to weekly in 2012. The completeness subsequently increased and it was above the surveillance standard for 2013. There was however, a long time interval associated with the collection of clinical information (median 2–3 weeks). The current system is paper-based, and requires clinicians to fill in a standardised form and return it by post to SSI. This time interval could be shortened if it would be replaced by an electronic system, as has been demonstrated for numerous other systems [28-30].

This study demonstrated that there were a number of factors that could be addressed to improve the current laboratory EV surveillance system with regards to its primary objective to document the absence of poliovirus transmission. A detailed report on results of this study will be directly communicated to all KMAs, to ensure that all laboratories are aware of the national recommendation to forward positive CSF and faecal samples to the NRL. This report will highlight the fact that specimens should be sent as soon as possible after the primary diagnostic result, and that specimens from patients of all ages should be forwarded for viral characterisation to rule out infections with poliovirus. This fact will also be communicated to clinicians to ensure the importance of collecting faecal samples from EV-positive patients is understood. In addition, a dialogue between the NRL and the KMAs is required to ensure the NRL is fully aware of the selection criteria for sending specimens at all the KMA. A modification of the surveillance system to collect clinical information on EV-positive cases, from a paper-based to an electronic system is additionally suggested as a means to improve the timeliness of the system.

To improve the representativeness of the EV surveillance in Denmark with regards the secondary surveillance objective to track circulating EV genotypes, a decrease is needed in the proportion of isolates where a non-typeable result is reported. A revision of the laboratory work flow is currently ongoing to address this issue and is focussed on redesigning the assay to first differentiate between species A-D and secondly to develop sequencing primers specific for each species.

Conclusion

The current laboratory surveillance system for EV in Denmark is an important tool to document the absence of poliovirus transmission in the country as well as informing public health officials about nationwide alterations in EV trends. The system is currently operating within all but one of the surveillance objectives adopted for this study. Due to the increased risk of an imported poliomyelitis case in Europe, it is recommended to improve certain steps in the EV surveillance system with regards to timeliness and representativeness. Specifically, an increased awareness might result in increased focus and participation of some KMAs, a revision of the EV typing laboratory methodology to decrease the number of non-typeable EV. Finally, the collection of stool and detailed clinical information in case of any EV-positive CSF should be organised in a manner to make it easy for the clinician and the patient to collect and send the material. For example, the use of an immediate electronic prompting system would address these issues and optimise the surveillance system with regards to its objectives.

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This study was approved by the Danish Data Protection Authority as part of a general permission for performing surveillance studies (registration number 2008–54–0472) Permission was obtained from the MiBa Board for the use of national microbiology data on EV diagnostic outcomes for this evaluation.

Conflict of interest

Not relevant

Authors' contributions

Orla Condell: Responsible for the data analysis, drafting of the manuscript and figures and revision of the manuscript.

Sofie Midgley: Advised and contributed to the study design, data analysis plan and results, reviewed drafts of the manuscript critically and contributed to the figures.

Claus Bohn Christiansen, Ming Chen, Xiaohui Chen Nielsen, Svend Ellermann-Eriksen, Mette Mølvadgaard, Kristian Schønning, Silje Vermedal Hoegh: Each is a representative of a clinical microbiological laboratory and part of the collaborative efforts for EV surveillance in Denmark, each revised the manuscript and approved the final version of the paper.

Peter Henrik Andersen: Responsible for the operation of the clinical data component of the surveillance system, revised the manuscript and approved the final version.

Marianne Voldstedlund: Responsible for the operation of the MiBa database, provided advice and guidance on the use of MiBa data, revised the manuscript and approved the final version.

Thea Kølsen Fischer: Head of the Danish National WHO reference laboratory for Poliovirus laboratory at the Department of Microbiological Diagnostics and Virology, engaged in the project design, reviewed the data analysis and drafts of the manuscript critically.

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Research article

Imported chikungunya cases in an area newly colonised by *Aedes albopictus*: mathematical assessment of the best public health strategy

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We aimed to identify the optimal strategy that should be used by public health authorities against transmission of chikungunya virus in mainland France. The theoretical model we developed, which mimics the current surveillance system, predicted that without vector control (VC), the probability of local transmission after introduction of viraemic patients was around 2%, and the number of autochthonous cases between five and 15 persons per hectare, depending on the number of imported cases. Compared with this baseline, we considered different strategies (VC after clinical suspicion of a case or after laboratory confirmation, for imported or autochthonous cases): Awaiting laboratory confirmation for suspected imported cases to implement VC had no significant impact on the epidemiological outcomes analysed, mainly because of the delay before entering into the surveillance system. However, waiting for laboratory confirmation of autochthonous cases before implementing VC resulted in more frequent outbreaks. After analysing the economic cost of such strategies, our study suggested implementing VC immediately after the notification of a suspected autochthonous case as the most efficient strategy in settings where local transmission has been proven. Nevertheless, we identified that decreasing reporting time for imported cases should remain a priority.

Introduction

Environmental changes are a significant cause for concern for public health authorities [1,2]. Global warming [3], decline in biodiversity [4], urbanisation [5] or globalisation [6] can facilitate the spread of vectors and increase pathogen transmission. While some of these changes occur over the course of several years or decades and could therefore represent a problem in the long term, invasive vector species that can transmit pathogens such as dengue or chikungunya viruses expose new human populations to new pathogens already today [7-10].

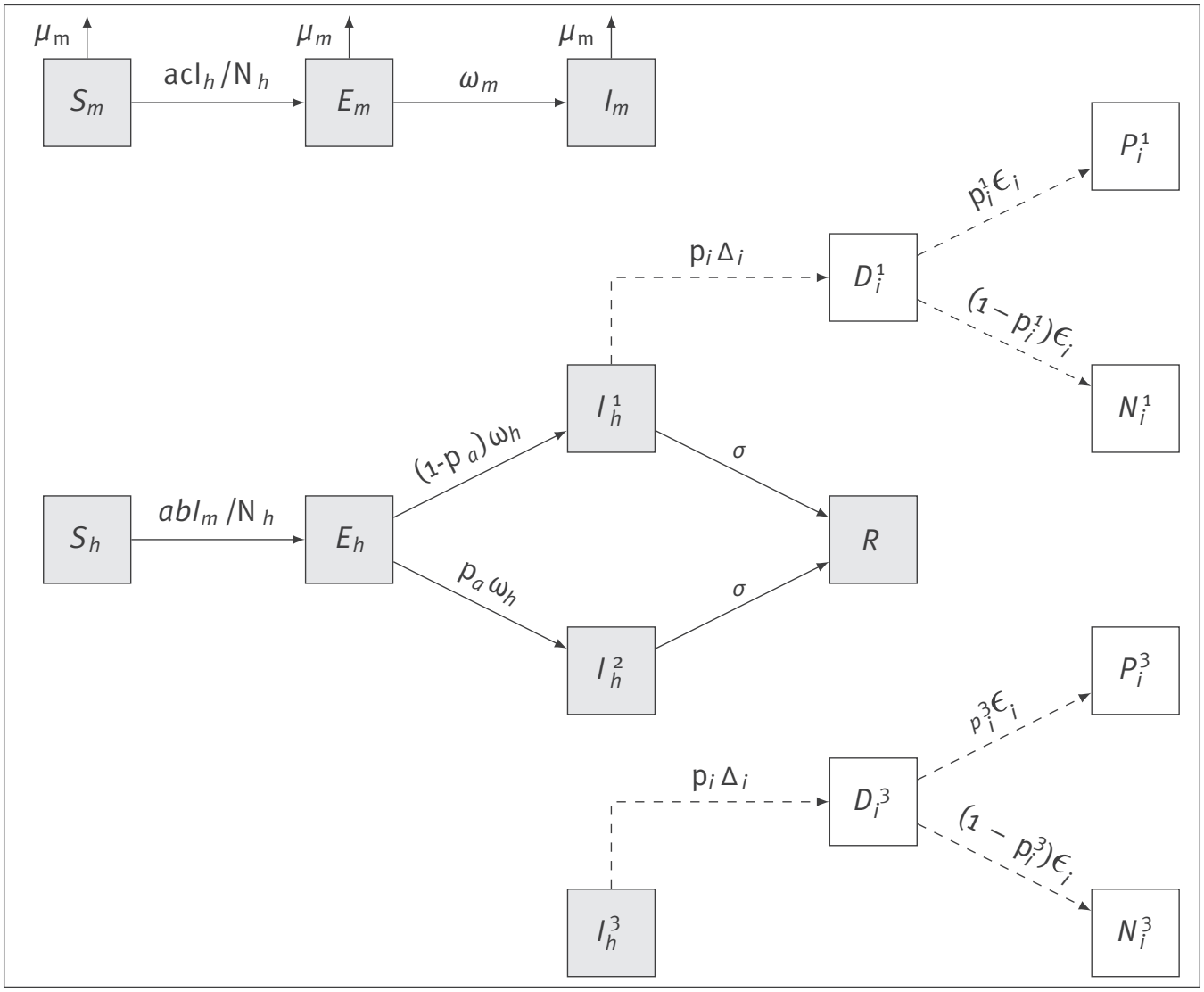
This risk of disease transmission by invasive vectors was particularly significant in 2014. Indeed, the occurrence of a large chikungunya outbreak on several Caribbean islands [11-13] during the season of *Aedes albopictus* vector activity in southern France [14] became problematic for public health risk management because of the high number of imported cases recorded [15]. While virus transmission remained under control in 2014, with moderate local transmission of dengue and chikungunya viruses [16], this situation can be expected to repeat in the future and requires an efficient public health strategy rooted in quantitative risk assessment under the current and alternative strategies.

For chikungunya virus, the current strategy in vector-infested areas and during the season of vector activity comprises the following components [15]: Suspected cases (defined by the presence of acute fever and joint pains not explained by another medical condition) are immediately reported to public health authorities. Such cases are then confirmed by serology (IgM-positive or a fourfold increase in IgG titre) or detection of viral nucleic acids in plasma by real-time reverse-transcription PCR (RT-PCR).

If the suspected case is imported from a chikungunya-endemic or -epidemic area, vector control (VC) measures, such as spraying adulticides to temporarily reduce the density of adult mosquitoes and removing stagnant water to decrease the number of breeding sites, are implemented within 200 m of the places in mainland

Figure 1

Structure of the mathematical model assessing public health strategies again chikungunya virus transmission, mainland France



France visited by the patient during the likely viraemic period (from the day before until seven days after the onset of symptoms), without waiting for laboratory confirmation. In the absence of proof of local transmission (i.e. without any previous biological confirmation of an autochthonous case), VC measures around suspected autochthonous cases are delayed until these cases are laboratory-confirmed.

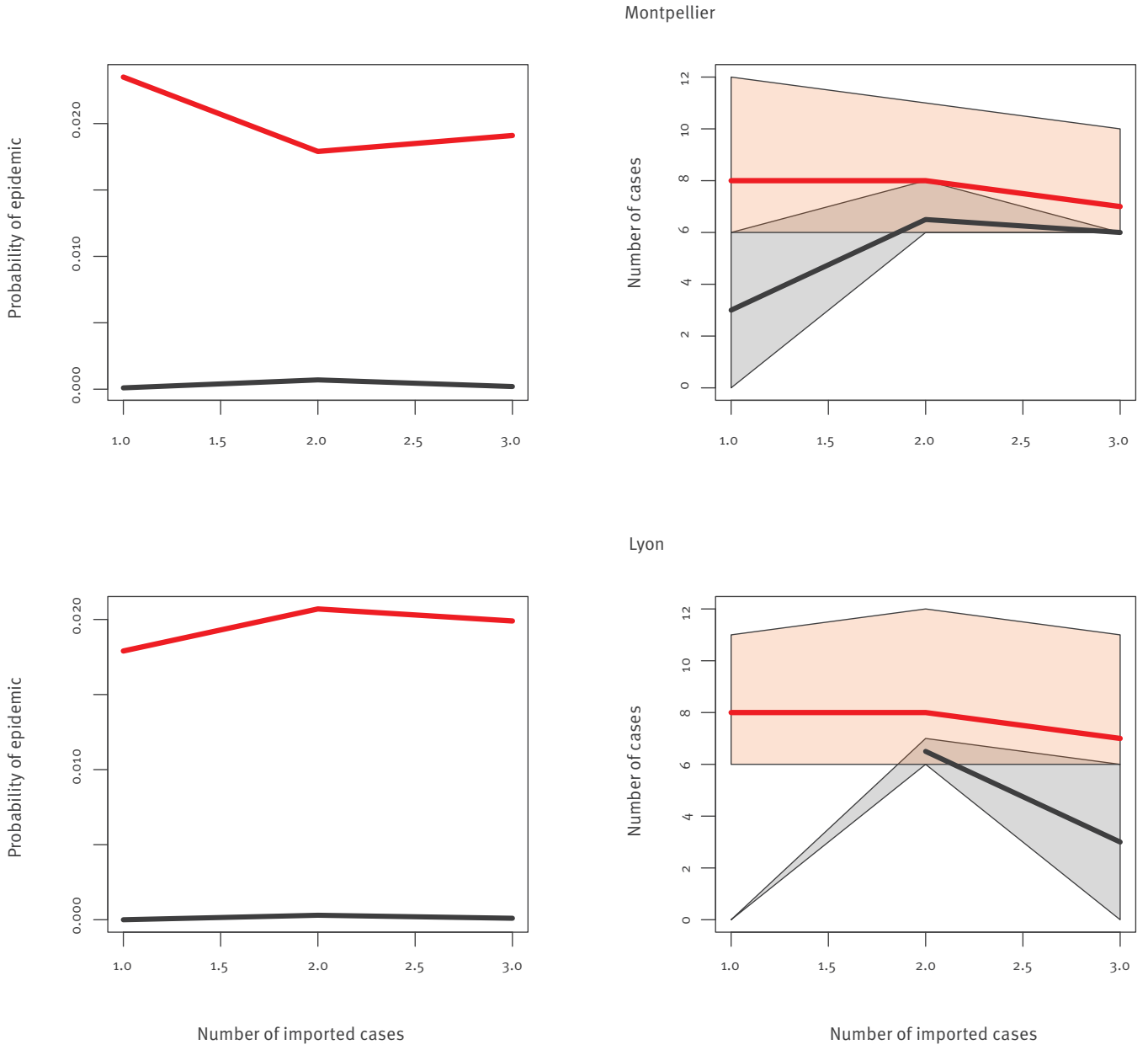
The notification of a laboratory-confirmed autochthonous case triggers immediate epidemiological and entomological investigations and control measures, including active case finding in the neighbourhood of the case's residence and in other areas visited by the case. VC is then implemented around the confirmed cases and around any further suspected autochthonous cases.

One of the main questions is whether or not waiting for laboratory confirmation is relevant before implementing VC locally. Intuitively, the most efficient strategy in epidemiological terms would be to implement VC as soon as possible, i.e. just after the notification of a clinically suspected case, whether the case is imported or locally acquired (autochthonous). However, the number of false notifications (clinical suspicion of chikungunya cases where chikungunya virus is not involved) is substantial because of the low specificity of the symptoms: in 2015, only six cases were confirmed among 532 notified cases (personal communication: Alexandra Septfons, INVS, April 2015). Therefore, the efficiency of such strategies has to be assessed. In order to design the most efficient public health strategy, it is important to quantify how many secondary cases occur because VC is delayed until laboratory confirmation for the suspected imported and autochthonous cases.

We designed a mathematical model for chikungunya control, which could be easily extended to other

Figure 2

Modelled probability of epidemics (defined as simulation with at least five local cases) and number of autochthonous cases (in simulations identified as epidemic) in simulations without vector control



Red lines: maximum mosquito density (800 females/ha); black lines: minimum density (20 females/ha). The confidence intervals are the 95% interval over the 10,000 simulations defined by the 2.5th and 97.5th percentiles.

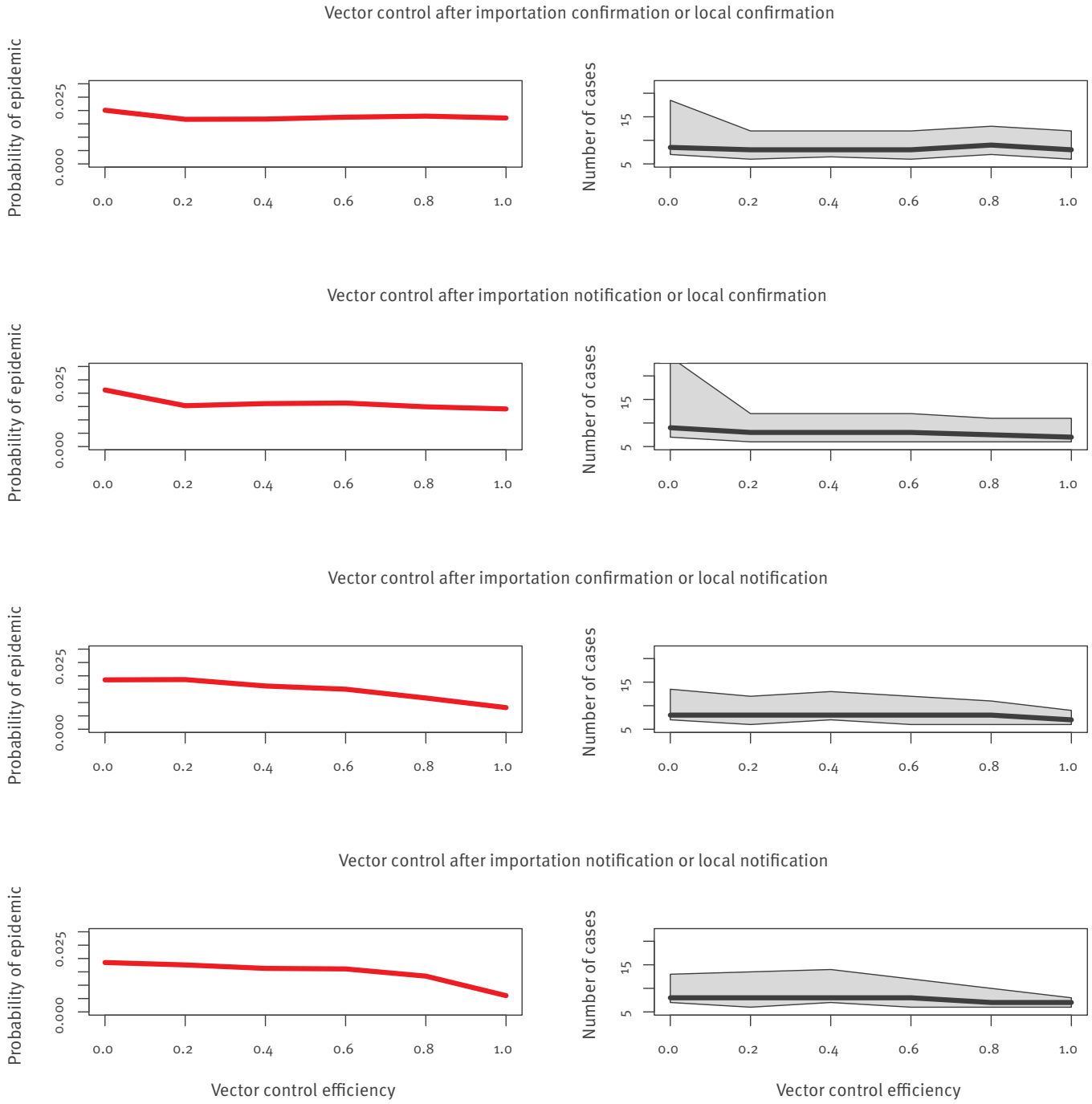
vector-borne diseases (e.g. dengue or Zika virus infections) and/or surveillance systems, in order to analyse theoretically four possible public health strategies (VC after clinical suspicion or after laboratory confirmation, for imported or autochthonous cases). We include in this model the surveillance data obtained during the 2014 season, suggest what could be the optimal strategy for the following years and make recommendations how to improve the surveillance system.

Methods

We developed a stochastic epidemiological model [17] rooted within the SIR (susceptible-infectious-recovered) framework where populations of humans and vectors are compartmentalised according to their infection status (Figure 1). Human individuals start simulation in a susceptible state (S_h) and then can be infected through bites of infectious vectors (I_m) at rate a and with the probability b. When infected (E_h), these individuals become, at a rate ω_h, infectious and symptomatic (I_h^1) with the probability $(1 - p_a)$, or asymptomatic (I_h^2) with the probability p_a. Finally, infectious individuals

Figure 3

Impact of vector control strategies on probability of epidemics (defined as more than three locally acquired cases) and number of locally acquired cases after importation of one case

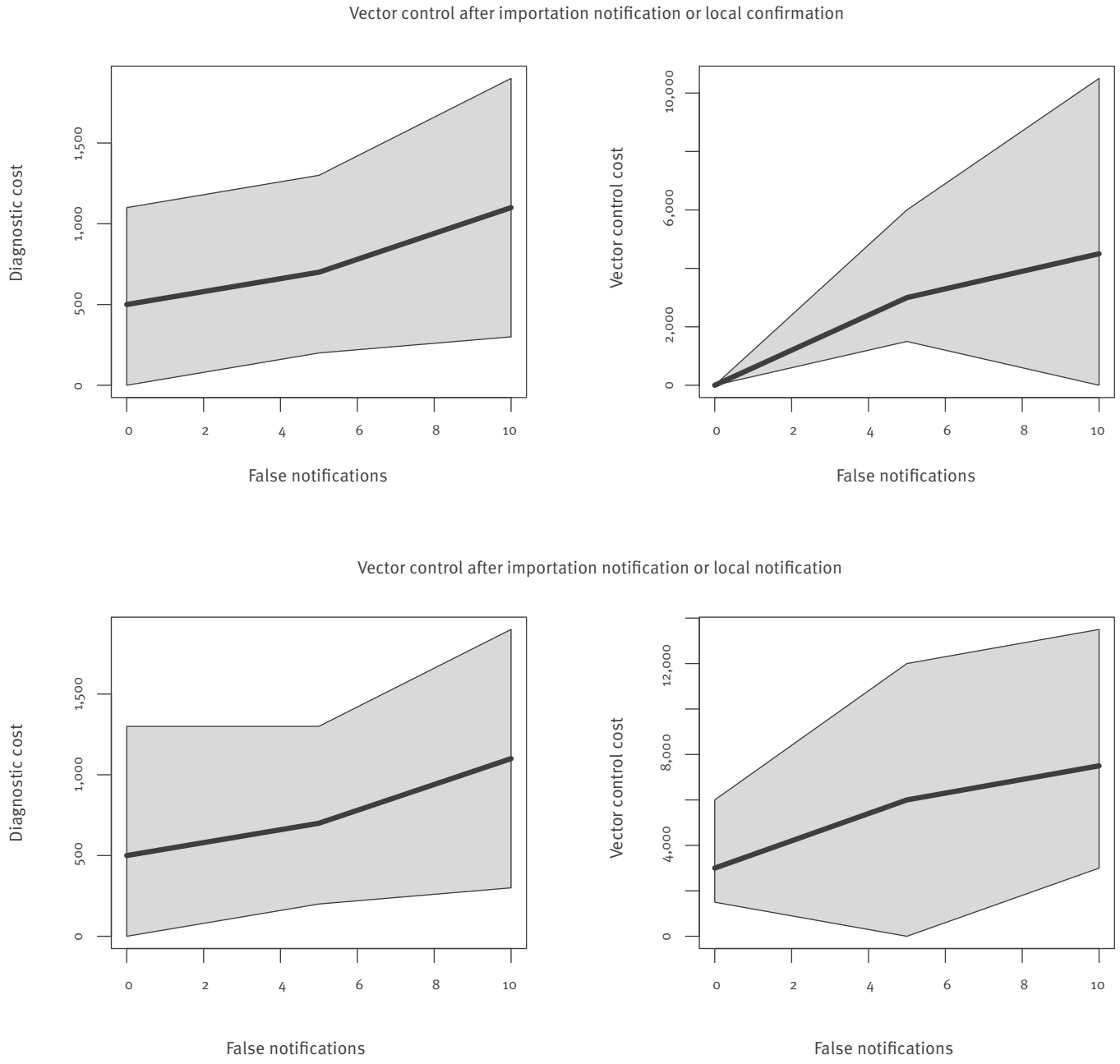


Solid lines represents the median of the simulations.

While starting vector control (VC) after suspicion or confirmation of an imported case did not significantly change the epidemiological outcomes, implementing VC immediately after the notification of a suspected locally acquired case rather than awaiting laboratory confirmation allowed the greatest reduction in the probability of an epidemic. The efficiency of VC represents the percentage of the vector population that was removed by VC operations.

Figure 4

Costs of diagnostics (left panels) and vector control (right panels) by number of false notifications



Top panels: vector control (VC) triggered after biological confirmation; bottom panels: VC triggered only after suspicion. Costs are in EUR/ha.

become recovered (R) at rate σ and cannot be infected again. Similarly, if a susceptible mosquito vector (S_m) bites an infectious human (I_h^1, I_h^2) at rate a, the mosquito can become infected (E_m) with the probability c and then infectious (I_m) at the rate ω_m, characterised by the inverse of the extrinsic incubation period.

In order to quantify the direct economic costs of false notifications of autochthonous cases to the health system, a third class of individuals (I_h^3) was considered which represented non-infected individuals who appear symptomatic. These erroneous suspicions may trigger VC if laboratory diagnostic is not required a priori. Furthermore, laboratory tests may return false-positive results, resulting in erroneous confirmation and autochthonous case notification, triggering VC implementation.

A sub-model considering specifically the case detection and diagnosis process was added on top of this epidemiological framework. When a new individual is added (through importation or local contamination) to

Table

Parameters used in the model assessing public health strategies again chikungunya virus transmission, mainland France

Parameters	Description	Values	Source
Diagnostics and vector control			
p_i	Proportion of each diagnostic method	0.29; 0.29; 0.42	INVS[a]
p_i^3	Proportion of false positives for each method	0.027; 0.05; 0.05	INVS[a]
$1 - p_i^1$	Proportion of false negatives for each method	0.03; 0.03; 0.03	INVS[a]
ε_i	Confirmation rate for each method	6; 7; 14 days per individual	INVS[a]
Cost for each diagnostic		EUR 200; 100; 100	Arbitrary values[b]
Cost of Vector control		EUR 1,500	Arbitrary value[b]
Pathogen			
σ	Recovery rate	4 days per individual	[20]
a	Biting rate of vector species	0.25 days per individual	Arbitrary value (no study exists today on *Aedes albopictus* in southern France)
μ_m	Vector mortality rate	19 days per individual	[27]
b	Infection probability of a susceptible human exposed to the pathogen	0.3	Arbitrary value[b]
c	Infection probability of a susceptible mosquito exposed to the pathogen	0.3	Arbitrary value based on a virus poorly adapted to the local mosquito ([22] suggests 0.6 in a well-adapted environment)
Θ	Efficiency of vector control	Variable	Variable
ω_h	Incubation rate in the human host	3 days per individual	[22]
Ω_m	Incubation rate in the mosquito	2days perindividual	[22]
Δ	Physician consultation rate when infectious	6days per individual	INVS[a]
p_A	Proportion of locally acquired cases not detected (including asymptomatic)	0	Arbitrary value[b]

INVS: Institut de Veille Sanitaire (French Institute for Health Surveillance).

[a] Values from INVS cover the whole 2014 season in France and have been averaged over this season.

[b] All arbitrary values have been decided in consultation with the Centre National d'Expertise des Vecteurs.

the number of individuals in class I_h^1 (truly infected) or in class I_h^3 (wrong suspicion; individual not infected with chikungunya virus), the number of individuals ready for laboratory tests (class D_i^1 or D_i^3) will increase at a rate Δ_i, where Δ_i represents the notification period of the diagnostic method *i*. The diagnostic methods considered here are PCR confirmation and serology. Each diagnosis is executed at rate ε_i (representing diagnostic time) and the suspected cases can become confirmed (P_i^j) or reversed (N_i^j) with the probability p_i^j or $(1 - p_i^j)$ respectively. For the diagnostic method *i*, $(1 - p_i^j)$ represents the proportion of false negatives when $j=1$ (real infectious individuals), while p_i^j represents the proportion of false positives when $j=3$ (false notification). Finally, each diagnostic method has an associated cost in order to estimate the total direct cost for biological confirmation.

Finally, VC can be implemented when D_i^j increases by 1 (i.e. when a new infected individual is detected) if the strategy is to implement VC after suspicion ($j=1$ represents real infection while $j=3$ represents erroneous notification). If the strategy is to await laboratory confirmation for both locally acquired and imported cases, VC will be implemented when P_i^j increases. Tracking the changes allows us to address the four possible strategies, namely implementing VC after clinical suspicion and/or after laboratory confirmation for autochthonous and/or imported cases. VC is based on an integrated approach (source reduction, social mobilisation for destruction of breeding sites such as stagnant water, as well as ultra-low volume (ULV) and thermal fogging for control of adult mosquitoes). Based on experience with mosquito control operators and the biology of *Ae. albopictus* in temperate areas, we considered that the mosquito population decreases by θ%, representing the efficacy of VC, during 10 days. After this period, the mosquito population recovers its previous level. Finally, we assumed that a VC operation has an average cost and that the total VC cost is the sum of all VC operations implemented multiplied with this average cost. Details on all transition events can be obtained from the corresponding author and are also available at https://sites.google.com/site/rocheben/sochakiEtAlAppendix_V3.docx*.

For each studied scenario, we replicated 10,000 simulations through the Gillespie's direct method [18]. In order to have a mosquito population that could be fully reached by VC, we considered a very fine spatial scale

of one hectare. Similarly, we simulated one single season (six months) because we were focussing here on the short-term impact of the VC strategies. Over this single season, the number of imported cases, which could be of different infectious status, were assumed to arrive uniformly in the population (e.g. one imported case at the beginning, one imported case in the middle and one at the end of the season, if we consider three imported cases). We considered two specific situations for the vector density (ranging from 20 to 800 females per hectare [19]), and two human densities corresponding to the cities of Montpellier and Lyon (46 and 102 inhabitants per hectare, respectively) which are both exposed to increasing vector abundance. All parameters are detailed in the Table and are estimated at the spatial scale of one hectare. Finally, throughout the manuscript, we focus on the probability of the establishment of a local transmission chain (defined as the occurrence of more than five autochthonous cases), called the probability of epidemic, and on the number of autochthonous cases during an outbreak.

Results

Based on this model, we could derive the basic reproductive number (R_0) that quantifies the number of secondary infections that arise when a single infective host is introduced in a fully susceptible host population. Based on previous results from a chikungunya outbreak in Italy [20], we characterised the R_0 as:

Formula 1

$$R_0 = \frac{N_V}{N_h}\,\frac{bc}{\sigma\mu_m}\,\frac{\omega_m}{\omega_m+\mu_m}$$

Based on the parameters detailed in the Table, we consider in this study R_0 values ranging from 0.16 to 6.72 in Montpellier and from 0.07 to 2.99 in Lyon (according to vector and human densities), which is consistent with current estimations [20].

In order to quantify the net epidemiological impact of imported infectious individuals, we first considered a simple case without VC but with perfect tracking of infectious individuals (no asymptomatic cases nor false notification). As could be expected, we found that an increasing vector density significantly increased the probability of an epidemic (Figure 2), from 0% to 2%, and therefore the size of the epidemic (number of locally acquired cases). However, the number of imported cases had a very weak impact on the probability of an epidemic, from 0% to 0.1% for low vector density, yielding a large confidence interval for the size of the ensuing epidemic because the smaller number of simulations on which size of the epidemic could be calculated.

Based on the high vector density in Lyon (with a R_0 of 2.99), we then focused on the four possible strategies that could be implemented, namely implementing VC (i) after notification of clinical suspicion of imported case(s) and laboratory confirmation of autochthonous transmission, (ii) after laboratory confirmation for either imported or autochthonous cases, (iii) after laboratory confirmation of an imported case or after clinical suspicion of an autochthonous case without laboratory confirmation or (iv) after a clinically suspected case, either imported or autochthonous. VC efficacy had a considerable influence on epidemiological outcomes, although the impact was stronger on the probability than on the size of epidemics (Figure 3).

Our model shows that not all strategies are equivalent. While the size of the epidemic remained roughly similar across the strategies, the probability of an epidemic was reduced when VC was started as soon as autochthonous transmission was suspected, without awaiting laboratory confirmation of cases. Starting VC upon suspicion or after confirmation of imported cases did not alter the epidemiological outcome.

Assuming a VC efficacy of 80% and one imported case within the area, we focused on direct economic costs of the two strategies for autochthonous cases. For this, we introduced non-infectious individuals (I_h^3) who nonetheless were entered in the surveillance system through erroneous suspicion and tested for laboratory confirmation (with the possibility of false positives). Figure 4 shows that the cost of VC increased significantly when VC was implemented after suspicion of cases rather than after their confirmation. However, the impact on diagnostic cost was weak.

Discussion

This first theoretical exploration chiefly aimed to highlight the various factors influencing the probability of a chikungunya epidemic and its spread in southern France and to show the possible consequences of different public health strategies. In this work, we considered four strategies: (i) VC after suspicion of an imported or autochthonous case, (ii) VC after suspicion of an imported case or after confirmation of a locally acquired case, (iii) VC after confirmation of an imported case or suspicion of autochthonous transmission or (iv) VC after confirmation of an imported or locally acquired case. We found that implementing VC immediately after notification of a suspected case did not significantly change the size of the epidemic, compared with delaying implementation of VC until laboratory confirmation. In other words, awaiting confirmation before implementing VC is not expected to result in a larger epidemic (with the current notification delay). However, our results suggest that awaiting laboratory confirmation before implementing VC around autochthonous cases may result in higher probability of an epidemic. However, the optimal strategy is also subject to economically constraints. In our model, implementing VC readily after suspicion was more expensive than after laboratory confirmation because of erroneous suspicions. Moreover, repeated VC operations are generally not feasible in the field for logistical

reasons and concerns of the population about repeated insecticide spraying.

The fact that the different strategies for imported cases did not significantly change the probability of an epidemic or its size, was mainly due to the delay between the infectious individuals entering the surveillance system and the notification of these imported cases. In 2014, this delay was six days on average, i.e. when the infectious individual has almost finished their infectious period and had potentially transmitted the virus to local *Aedes* mosquitoes. In addition, we observed that this delay had to be decreased significantly if we aimed to decrease significantly probability of epidemic following the introduction of imported cases (data not shown; details can be obtained from the corresponding author and are also available at https://sites.google.com/site/rocheben/sochakiEtAlAppendix_V3.docx*).

Some assumptions made by this model deserve to be discussed. Firstly, some epidemiological parameters were unknown and were fixed arbitrarily. This is especially relevant for the probability that a susceptible vector gets infected after biting an infectious human (parameter *c*). Indeed, the vector competence of *Ae. albopictus* populations in Metropolitan France will depend on the chikungunya strain infecting the human population and therefore on the geographic origin of the imported case. Nevertheless, we have conducted a sensitivity analysis (details can be obtained from the corresponding author and are also available at https://sites.google.com/site/rocheben/sochakiEtAlAppendix_V3.docx*), in which our main conclusions remained valid for a large range of parameters. Secondly, our estimated costs, for both diagnostics and VC, allowed to compare between the different scenarios but they underestimated the real costs since only direct costs were included. Finally, we considered here a very fine spatial scale (one hectare). As a consequence, we neglected the important component of human movements. The situation described here corresponds to outbreaks very localised in space, for which this scale is relevant. Nevertheless, in the case of a more diffuse outbreak, this model would need to be extended to a broader scale.

An important result arising from our paper is the influence of vector density on both probability and size of epidemics. While this result could have been expected based on the existing literature [17,21], it is nevertheless worth pointing out that such a relationship is strongly dependent on how the transmission force between vector and hosts is modelled. Here, we used the most popular way of vector-borne disease modelling (asymmetric frequency-dependent transmission). However, we could also show that another way of modelling this transmission force, through a symmetric frequency-dependent process, can result in the absence of a relationship between vector density and size of the epidemic (data not shown; details can be obtained from the corresponding author and are also available at https://sites.google.com/site/rocheben/sochakiEtAlAppendix_V3.docx*), as empirical data suggest for dengue [22]. Nevertheless, the only qualitative change arising with this other kind of transmission force is the influence of vector density. Therefore, although we cannot draw a definite conclusion regarding the influence of vector density, the main conclusion (implementing VC after suspicion for autochthonous cases is the best way to reduce the consequences of an epidemic) remains valid.

Other modelling studies have focused on chikungunya virus transmission in other epidemiological settings [20,23,24], some with a focus on VC [25]. Some studies have highlighted that certain areas are more suitable than others for chikungunya epidemics [26]. Nevertheless, this study is as far as we know, the first to focus on such concerns (large number of imported cases combined with local presence of vector) in Europe. While specifically designed the model to mimic the French surveillance system, we believe that it can easily be adapted to other surveillance systems.

It is worth pointing out that the outcomes of our model matched the size of the epidemic observed in Montpellier in 2014 [15] where 11 autochthonous cases were recorded after the introduction of a single imported case. While such agreement cannot validate the whole framework on its own, it nevertheless underlines that the approach is worth exploring and may provide useful insights for public health strategy.

The current strategy in France foresees the implementation of VC after suspicion of imported cases or after confirmation of autochthonous cases. Nevertheless, when local transmission is proven, current strategy assumes that VC is applied around every suspected autochthonous case without awaiting laboratory confirmation. This strategy is used because imported cases are relatively easy to discern (travel in an endemic area) and the positive predictive value of the clinical case definition is higher in this high-prevalence population group. Conversely, the low predictive power of the clinical case definitions in the resident human population that has not travelled to endemic areas leads to reporting of many patients not infected by chikungunya virus, creating high costs for unnecessary interventions. Overall, our theoretical framework suggests that such a strategy, in the current conditions (and especially with the current delay in case reporting), could be the most efficient one, both in economic and epidemiological terms, if proof of local transmission can be established rapidly enough. The model developed here aimed to be as generic as possible, so that it can be used as a general framework to test other scenarios or to analyse VC strategies for other diseases.

*Note

Supplementary information made available by the authors on an independent website is not edited by Eurosurveillance,

and Eurosurveillance is not responsible for the content. The material can be accessed at: https://sites.google.com/site/rocheben/sochakiEtAlAppendix_V3.docx.

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Conflict of interest

None declared.

Authors' contributions

BR, DF, FS and MCP have designed the study. TS and BR did the modelling analysis. FJ and YP have improved the entomological component of the model. HN, MCP AS improved the surveillance component of the model and analysed the surveillance data during the 2014 season. All authors have significantly contributed the writing of the manuscript.

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