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Yellow fever in a traveller returning from Suriname to the Netherlands, March 2017

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A Dutch traveller returning from Suriname in early March 2017, presented with fever and severe acute liver injury. Yellow fever was diagnosed by (q)RT-PCR and sequencing. During hospital stay, the patient's condition deteriorated and she developed hepatic encephalopathy requiring transfer to the intensive care. Although yellow fever has not been reported in the last four decades in Suriname, vaccination is recommended by the World Health Organization for visitors to this country.

Yellow fever virus (YFV) is known to be enzootic in South America, causing periodic outbreaks of disease in monkeys and humans in some countries. In Brazil, there has been an outbreak of yellow fever ongoing since December 2016 with 1,500 cases as at 9 March [1,2]. Here we report an imported case of human infection with YFV in a traveller returning from Suriname, on the north-eastern coast of South America, from where the last case of yellow fever was reported 45 years ago.

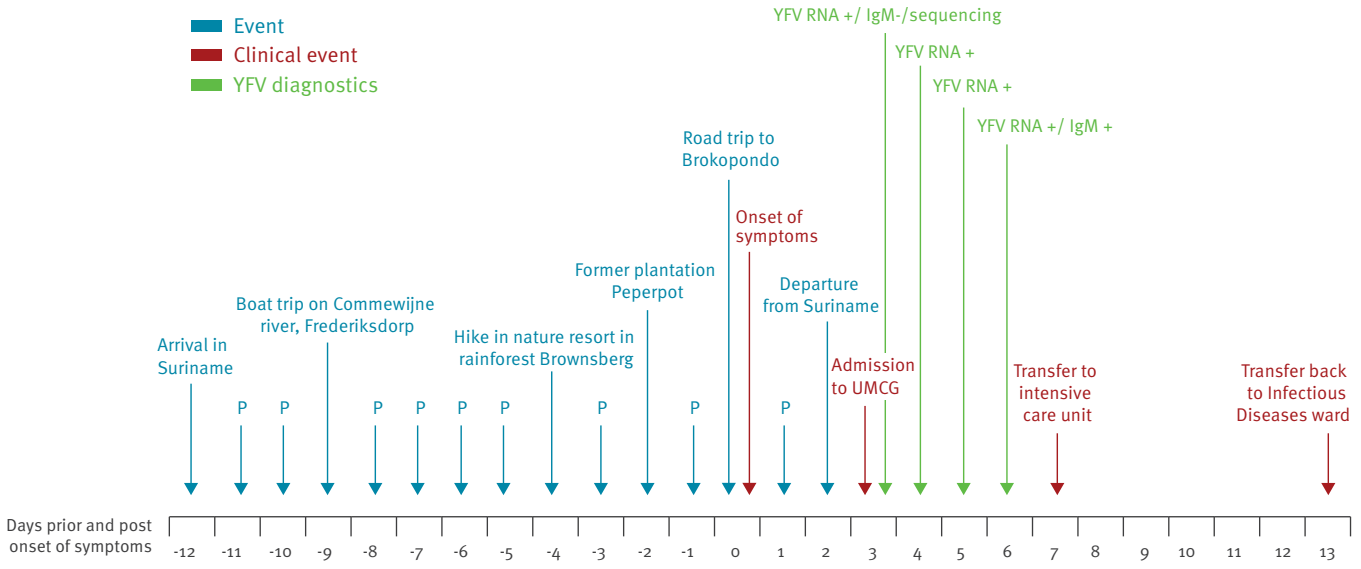
Case description

In March 2017, a Dutch Caucasian female in her late 20s from the Netherlands was referred to the University Medical Center Groningen in the Netherlands because of high fever and signs of severe acute liver injury after returning from a two-week stay in Suriname. She had no co-morbidities apart from obesity (body mass index around 40 kg/m2, norm: 18.5–25 kg/m2). During her visit she stayed in the capital of Suriname, Paramaribo, and she made several daytrips by boat and car, of which two in the tropical rainforest (Figure).

She recalled having been bitten by mosquitoes during her hike at Brownsberg, a nature resort in the rainforest with wildlife. Before her travel, she did not visit a travel clinic and did not receive yellow fever vaccination. On day 12 of her visit in Suriname, she experienced mild muscle pain, headache and nausea and she developed a high-grade fever. She returned to the Netherlands on day 15 and visited the emergency department of a secondary care centre, from where she was referred to our University hospital. At physical examination she was not icteric. Except for a temperature of 39.9 °C, vital parameters were normal. The results of the remaining physical examination were unremarkable. Laboratory testing revealed leukopenia (leukocytes 0.9x109/L, norm: 4.0–10.0x109/L) and massive liver injury (aspartate aminotransferase 5,787 U/L, norm: ‹31 U/L; alanine aminotransferase 4,910 U/L, norm: ‹34 U/L), with mildly elevated bilirubin levels (total bilirubin 20 µmol/L, norm: ‹17 µmol/L). Liver synthesis was impaired as revealed by increased clotting times (activated partial thromboplastin time (APTT): 49s, norm: 23–33s; prothrombin time (PT): 26.6s, norm: 9.0–12.0s) and reduced antithrombin (49%, norm: 80–120%). Fibrinogen was diminished suggestive of diffuse intravascular coagulation. Renal

Figure

Timeline of events and diagnostic results, case of yellow fever in a traveller returning from Suriname to the Netherlands, March 2017



P: Paramaribo; RNA: ribonucleic acid; UMCG: University Medical Center Groningen; YFV: yellow fever virus.

function was normal apart from severe albuminuria (up to 22.6 g/24h, norm: 0g/24h). Malaria, viral hepatitis (A, B, C, E, Epstein Barr virus, cytomegalovirus, herpes simplex virus), dengue, chikungunya and Zika were ruled out (Table). Diagnostic tests to exclude leptospirosis performed on day 6 post onset of symptoms (dps 6) were inconclusive (Table) and a convalescent serum was going to be tested at the time of publication. Because of the combination of fever, leukopenia, thrombocytopenia, liver injury and travel history, yellow fever was included in the differential diagnosis. Real-time reverse transcriptase PCR (qRT-PCR) was positive for YFV in serum taken on dps 3. On dps 7 the patient's condition deteriorated due to hepatic encephalopathy (ammonia 149 µmol/L, norm: 15–45 µmol/L). Cerebral oedema and bleeding was ruled out by computed tomography (CT)-scan. The patient was transferred to the intensive care unit for close observation of vital parameters. Vitamin K was administered. Hepatic encephalopathy was treated with rifaximin and lactulose. Ceftriaxone (2g per day intravenously) was given for 7 days as antibiotic prophylaxis. Consequently, possible leptospirosis was also treated. Her neurological condition stabilised on dps 10 together with the coagulation parameters. On dps 13 the patient was transferred back to the ward.

Virology findings

qRT-PCR and/or pan-flavivirus RT-PCR on blood samples on dps 3 did not detect chikungunya virus (CHIKV), dengue virus (DENV), or Zika virus (ZIKV) (Table) [3,4]. In four consecutive samples of dps 3–6, YFV-RNA was detected (Figure) [4-6], with increasing Ct values (from 23 to 31 from dps 3 to dps 5 [5] and 39 on dps 6 [6]). Sequencing of a 176 bp pan-flavivirus hemi-nested RT-PCR product, targeting part of the NS5 genomic region confirmed YFV infection [4]. The sequence was deposited in the GenBank database under the following accession number: KY774973.

On dps 3, indirect immunofluorescence assays (IFA) was negative for IgM and IgG against YFV (Flavivirus Mosaic, Euroimmun AG, Luebeck, Germany). A convalescent sample of dps 6 was clearly positive for YFV IgM (titre 1:10, Figure), with non-reactive IgG. This anti-YFV IgM response on dps 6 is in line with literature stating that IgM antibodies usually appear during the first week of illness. Neutralising IgG antibodies are likely to appear towards the end of the first week after onset of illness and will be tested for in convalescent serum [7].

Background

YFV is a mosquito-borne virus in the genus Flavivirus, family *Flaviviridae*, related to DENV, ZIKV, tick-borne encephalitis virus and West Nile virus. YFV is maintained in a sylvatic cycle between non-human primates and so-called 'jungle'-mosquitoes (*Hemagogus* and *Sabethes* spp. in South America) [8]. Sporadic infection of humans with sylvatic YFV can occur when unprotected humans are exposed while entering the habitats where the viruses circulate. Subsequent introduction of a viraemic human case to urban areas with high population densities and *Aedes aegypti* mosquitoes can initiate an urban transmission cycle [9]. YFV is endemic in (sub)tropical areas of South America and Africa. The risk for YFV infection in South America is the highest in tropical regions and during the rainy season (January–May) when mosquito population densities peak [10].

Table

Pathogens for which laboratory tests were performed, yellow fever case, the Netherlands, March 2017

Pathogen	Blood (day 3 post onset of symptoms)
Plasmodium spp.	Thick smear negative, antigen test negative
Hepatitis A virus	IgM and IgG negative
Hepatitis B virus	Serological screening negative
Hepatitis C virus	Serological screening negative
Hepatitis E virus	PCR negative
Epstein Barr virus	IgM and IgG negative
Cytomegalovirus	IgM and IgG negative
Herpes simplex virus type 1 and 2	PCR negative
Dengue virus	PCR negative, IgM and IgG negative
Chikungunya virus	PCR negative, IgM and IgG negative
Zika virus	PCR negative, IgM and IgG negative[a]
Leptospira spp.	PCR negative, microscopic agglutination test negative, IgM 1:80[b]

[a] Performed on day 5 post onset of symptoms (dps 5).

[b] ELISA (in-house ELISA Dutch Leptospirosis Reference Center) performed on dps 6 showed IgM 1:80 (cut-off positive IgM ≥1:160). IgM results were negative on dps 3 and dps 7 using Leptocheck-WB (Zephyr Biomedicals, Goa, India).

In 2011, Suriname was identified by the World Health Organization (WHO) as one of 14 South American countries at risk for YFV transmission based on current or historic reports of yellow fever, plus the presence of competent mosquito vectors and animal reservoirs [11].

Since December 2016, an outbreak of sylvatic YFV is ongoing in Brazil; as at 9 March 2017, there were 371 confirmed and 966 suspected human cases, while a total of 968 epizootics in non-human primates have been reported, of which 386 were confirmed [2]. So far, there has been no evidence for a change from sylvatic to an urban transmission cycle [1]. In addition, Bolivia, Colombia and Peru have reported suspected and confirmed yellow fever cases in 2017 [2].

A subclinical infection with YFV is believed to occur in most infected people. In symptomatic cases, symptoms of general malaise occur after an incubation period of 3–6 days (range 2–9 days), followed by remission of the disease in the majority of patients. However, 15-25% of symptomatic persons develop a complicated course of illness, in which symptoms recur after 24–48 hours, with a reported mortality of 20-60% [7,12]. This phase is characterised by fever, abdominal symptoms, severe hepatic dysfunction and jaundice, multi-organ failure and haemorrhagic diathesis. As no specific antiviral treatment is currently available, treatment consists of supportive care [7,12].

Discussion

Although Suriname is considered to be endemic for YFV, no human cases have been officially reported since 1971 [13]. With a population of ca 570,000 people, Suriname has a YFV vaccination coverage of 80–85% in infants [14]. Although WHO recommends vaccination for travellers to countries with risk of YFV transmission like Suriname, sporadic cases of imported yellow fever in returning travellers have been reported for example in Europe, the United States and Asia [15-17], with three reported cases related to the ongoing YFV outbreaks in South America in European travellers since 2016 [18,19]. The establishment of ongoing YFV circulation in Suriname extends the current YFV activity in South America to five countries [2]. However, despite the presence of competent *Ae. albopictus* mosquitoes in France [20] and *Ae. aegypti* in Madeira, the risk for YFV transmission in Europe is currently considered to be very low due to the lack of vector activity [18]. An effective, safe live-attenuated YFV vaccine is available for people aged ≥ 9 months and offers lifelong immunity [7]. Vaccination is advised by the WHO for all travellers to Suriname, for the coastal area as well as the inlands [21]. With regard to yellow fever, pre-travel health advice should take into account destination, duration of travel, season and the likelihood of exposure to mosquitoes (in rural areas, forests versus urban areas), and potential contraindications for vaccination with a live-attenuated vaccine.

The multi-country YFV activity might reflect current, wide-spread ecological conditions that favour elevated YFV transmissibility among wildlife and spill-over to humans. Thorough sequence analysis of currently circulating strains in Brazil, Bolivia, Colombia, Peru and Suriname should provide insight whether the human cases in these countries are epidemiologically linked or represent multiple, independent spill-over events without extensive ongoing community transmission. Because of its potential public health impact, our case of yellow fever was notified to the WHO and the European Union Early Warning and Response System on 9 March 2017, according to the international health regulations [22].

Conclusion

Clinicians in non-endemic countries should be aware of yellow fever in travellers presenting with fever, jaundice and/or haemorrhage returning from South America including Suriname. This case report illustrates the importance of maintaining awareness of the need for YFV vaccination, even for countries with risk of YFV transmission that have not reported cases for decades.

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Conflict of interest

None declared.

Authors' contributions

MWB: treating physician of case, wrote initial manuscript. MK: consultant-virologist of case, co-wrote manuscript. APvdB: consultant-hepatologist of case. CHGvK: consultant-virologist of case, serology, data interpretation. MPGK: reference diagnostics, expertise. CvLB: consultant-virologist of case. BOV: treating physician of case on Intensive Care Unit. SDP: molecular diagnostics, data interpretation. WLMR: coordination WHO notification. JSC: confirmatory diagnostics. SGSV: consultant-infectious diseases Suriname. TSvdW: treating physician, expertise. CBEMR: reference diagnostics and expertise, sequencing, data interpretation, co-wrote manuscript. WFWB: consultant-infectious diseases of case, co-wrote manuscript. All authors reviewed, provided comments and approved the final manuscript.

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Increasing evidence of tick-borne encephalitis (TBE) virus transmission, the Netherlands, June 2016

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We present a case of endemic tick-borne encephalitis (TBE) occurring in June 2016 in the eastern part of the Netherlands in an area where a strain of TBE virus, genetically different from the common TBE virus strains in Europe, was reported in ticks in 2016. With the start of the tick season in spring, this second autochthonous Dutch TBE case should remind physicians to consider the possibility of endemic TBE in patients with respective symptoms.

We report the second autochthonous human case of tick-borne encephalitis (TBE) ever diagnosed in the Netherlands in an area where a strain of TBE virus, genetically different from the common TBE virus strains in Europe, was identified in ticks in 2016.

Case description

On 14 July 2016 (day 1), a 44 year-old male patient was admitted to the hospital with tinnitus, malaise, vomiting, muscle aches, and headache, that had started on 8 July. Shortly before symptom onset, starting on 25 June, he had also experienced a short episode of malaise and fever, from which he recovered spontaneously. A week before the first symptoms, he had been bitten by a tick, without having developed erythema migrans. The patient lives in the Sallandse heuvelrug area in the eastern part of the Netherlands, where tick-borne encephalitis virus (TBEV) had been detected in ticks in 2016 [1]. He maintains a garden in which he frequently encountered ticks. He also reported frequent outdoor activities such as running and walking in the area where he lived. Six weeks before the first symptoms, he had travelled to Bad Salzungen in Thuringia, Germany, for 2 days, but did not reside in a wooden area, and was not bitten by ticks. He was vaccinated against yellow fever in 1996 but not vaccinated against TBEV. The patient did not report any consumption of unpasteurized milk products in the month before illness onset.

Physical examination revealed no abnormalities. Laboratory tests showed signs of inflammation: C-reactive protein (CRP): 45 mg/L (norm: <10 mg/L); leukocytes: 10.4 x 109/L (norm: 4–10 mmol/L). A slightly lower haemoglobin (Hb) concentration (7.8 mmol/L; norm: 8.5–11.0 mmol/L) possibly indicative of an infection ongoing for some time. The result of the thorax X-ray examination was normal.

The dehydrated patient was hospitalised with differential diagnosis being gastroenteritis, borreliosis, or a bacterial infection. Ceftriaxone therapy intravenously (2 g per day) was initiated. On day 2, his condition did not improve, and an overt mental slowness became evident as well as a tremor in both hands, with concurrent loss of strength.

Lumbar puncture was performed and cerebrospinal fluid (CSF) analysis revealed leukocytosis (99 x 106/L (norm: <5 x 106/L); 85% mononuclear cells; 15% polynuclear cells (norm: <6%); glucose and protein concentrations of 3.3 mmol/L (norm: 2.2–3.9 mmol/L) and 1,101 mg/L (norm: 250-800 mg/L) respectively. The working diagnosis now was a meningoencephalitis, possibly caused by a virus, or neuroborreliosis. Acyclovir, intravenously 3 mg/kg, 3 times per day, was added to the treatment regimen.

ELISA tests on both CSF and serum did not elicit Borrelia-specific antibodies. Nucleic acid detection of various potential pathogens in the CSF turned out negative (Table 1). Due to a report by the Dutch National Institute for Public Health and the Environment (RIVM), on 30 June 2016, of the first time detection of TBEV in ticks in the Sallandse heuvelrug region [1], serum was

Table 1

Nucleic acid detection of pathogens in cerebrospinal fluid, tick-borne encephalitis (TBE) case, the Netherlands, June 2016

Pathogen	PCR result
Borrellia burgdorferi	Negative
Mycoplasma pneumoniae	Negative
Haemophilus influenzae	Negative
Neisseria meningitidis	Negative
Streptococcus agalactiae	Negative
Streptococcus pneumoniae	Negative
Herpes simplex virus	Negative
Varicella zoster virus	Negative
Adenovirus	Negative
Enterovirus	Negative
Human parechovirus	Negative

investigated for TBEV-specific antibodies. Pending the results, doxycycline, 100 mg twice per day, was added to the regimen, because rickettsiosis could not be excluded at the time. A brain magnetic resonance imaging (MRI) was performed, showing an enlargement and staining of the dura mater, which may suggest viral meningitis.

On day 7, the results of the TBEV serology confirmed the following: anti-TBEV IgM and IgG were detected in serum with the ELISA method, suggesting a recent TBEV infection (Table 2). Also, the CSF turned out positive for anti-TBEV IgG and borderline positive for anti-TBEV IgM. The antibiotics and acyclovir were stopped. By day 9 the patient gradually improved. However, the tinnitus persisted.

The ELISA results were confirmed with a virus neutralisation assay, by the Department of Virology of the Medical University Vienna, Austria. A second serum sample taken on 8 August showed an increase in virus neutralising antibody titres (from 1:10 to 1:480), which is proof of an acute infection.

Background

TBE is a zoonotic infectious disease, caused by TBEV. Natural hosts are small rodents while larger mammals like deer and humans may become accidentally infected. Infection of humans mainly occurs by transmission of the virus through the bite of an infected tick (*Ixodes* species). The chance of transmission depends on the activity of ticks, and increases during spring. Alternatively, infection may occur by ingestion of milk and milk products from viraemic ruminants [2]. TBE caused by the European subtype TBEV (TBEV-Eu) characteristically has a biphasic course, starting with influenza-like symptoms such as fever, malaise, headache and myalgia (average 2–7 days). After a successive symptom-free interval (average 2–10 days), approximately one third of the cases develop neurological symptoms, varying from mild meningitis to severe encephalitis, with or without myelitis and paralysis of extremities and breathing muscles [3,4].

In Europe, areas of risk for TBE are predominantly situated in central and eastern Europe, and the Baltic and northern countries [5]. In the Netherlands, TBE was only considered as an imported disease until the first case of autochthonous TBE was reported, occurring in June 2016, shortly before our case [6]. In the first case, the virus was recovered and showed strong homology with the common TBEV-Eu strains that cause disease in Europe. The infection likely occurred in a forested area between Driebergen en Maarn, in the province of Utrecht. There was no link to the Sallandse heuvelrug region, in the province of Overijssel, which was at that time the only region in the Netherlands known to harbour TBEV infected ticks. There are currently no proven infections described with the TBE-Eu strain of the Sallandse heuvelrug, which is genetically distinct from the common TBE-Eu strains (data not shown and [6]).

Discussion

We present a confirmed case of TBE, according to the European Union case definition (symptoms of meningoencephalitis combined with a rise in TBEV-specific antibody titres) [7]. The typical biphasic clinical course in our case fits the diagnosis TBE. Vomiting, a prominent symptom in our case, is usually not described in association with TBE, nor is tinnitus [3,4].

The microbiological diagnosis of TBE is based on serological tests. Because of the strong likelihood of cross reactivity with antibodies against TBEV-related viruses, other flaviviruses, as for instance yellow fever virus, positive ELISA results need to be confirmed with a virus neutralisation assay [8]. In this way, confirmed ELISA results combined with a rise in TBEV-specific antibody titres, make a different explanation for the positive test results, as for instance a past yellow fever vaccination, unlikely.

Chances are high that the TBEV infection was acquired in the Sallandse heuvelrug region. The patient was bitten by a tick in that region during the incubation phase, which ranges from 4 to 28 days (average 7–10 days) [4], and he had neither been in the Utrechtse heuvelrug region, nor in a TBEV-endemic area abroad. His visit to Bad Salzungen was two weeks before the maximum incubation time of 28 days, it lasted only shortly, and was without at-risk activities. Moreover, Bad Salzungen is not regarded one of the risk areas in Germany [9].

For a definite proof that the infection was caused by the TBEV-Eu strain from the Sallandse heuvelrug, nucleic acid sequence analysis of the virus, recovered from the patient or tick, would be required. This is of clinical importance, because the TBEV-Eu strain from the Sallandse heuvelrug genetically diverges from the commonly found TBE-Eu strains, and it is not known whether this strain infects people and

Table 2

Tick-borne encephalitis (TBE) virus diagnostic test results, TBE case, the Netherlands, June 2016

	Material	CSF	Serum	Serum	Urine
	Date (2016)	15 July	15 July	8 August	8 August
RIVM	Serology (ELISA)	IgM borderline positive OD_{450} 0.421	IgM positive 355 VIEU/mL	IgM positive 341 VIEU/mL	NA
		IgG positive OD_{450} 3.064	IgG positive >650 VIEU/mL	IgG positive >650 VIEU/mL	
	PCR	Negative	ND	ND	Negative
Medical University, Vienna	Serology (ELISA)	ND	IgM positive	IgM positive	NA
			IgG positive >1,000 VIEU/mL	IgG positive 50,600 VIEU/mL	
	Virus neutralisation assay	ND	Positive titre: 1:10	Positive titre: 1:480	NA

CSF: cerebrospinal fluid; NA: not applicable; ND: not done; OD^{450} Optical density at 450 nm (IgM norm: <0.360 (negative); IgG norm: <0.270 (negative); RIVM: Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment); VIEU: Vienna Units.

causes symptoms. There is 93% homology between the Sallandse heuvelrug TBEV-Eu strain and the prototype TBEV-Eu Neudörfl strain (data not shown), which is found in large parts of Europe. For comparison, the TBEV-Eu strain from the first Dutch TBE case, reported in August 2016, showed 99% homology with this common TBEV-Eu strain [6].

Recovering of nucleic acid sequences was, however, not possible. The aforementioned tick had not been saved, and the chance of detecting the virus in patient materials at the time of neurological symptoms is extremely small [8] (also Table 2). Based on the place of residence and travel history of the patient, it seems however likely that the acquired infection was caused by the Sallandse heuvelrug TBEV-Eu strain because this strain represents the single strain that has been found in this region so far (data not shown).

Although a large national survey is lacking, based on the current knowledge, the percentage of TBEV infected ticks seems to be low (<0.1% in the Sallandse heuvelrug region). In comparison, the average percentage of ticks in the Netherlands infected with *Borrelia burgdorferi* was estimated between 15 and 20% [2]. Besides *Borrelia burgdorferi* and TBEV, ticks in the Netherlands have been shown to harbour other potential human pathogens, like *Anaplasma*, *Rickettsia*, *Candidatus Neoehrlichia*, *Babesia* spp., and *Borrelia miyamotoi*, but their clinical relevance is still unclear [10].

Worldwide, TBEV infections have been on the rise in recent years, and endemic areas have been expanding [11]. When and from where TBEV was introduced into the Sallandse heuvelrug area is unclear. Deer from this region were shown to carry TBEV-specific antibodies, in a survey dating from 2010 (data not shown). It is therefore possible that TBEV has been present in the Netherlands for already some years, and TBEV infections may have been overlooked. The majority of TBEV infections do not lead to TBE symptoms. This suggests that for the two identified autochthonous TBE cases in the Netherlands, a larger number of (undiagnosed) TBEV infections might be expected.

Currently, no specific therapy that targets TBEV exists. Effective vaccines against TBE are available, and are on the market since the 1980s. No advice on TBE vaccination exists for the Netherlands [2]. The risk of TBEV infection in the Netherlands is currently being assessed by the RIVM.

Conclusion

This is the first described case of TBE associated with the Sallandse heuvelrug region, where TBEV is endemic in ticks. Yet, definite proof that the infection was caused by the specific TBEV-Eu strain from that region is lacking. Being the second autochthonous TBE case in the Netherlands, however, it provides more evidence for endemic TBEV transmission in this country. With the new tick season approaching, clinicians in the Netherlands should consider TBE in the differential diagnosis of patients with meningoencephalitis symptoms especially after a tick bite, even if there has been no recent travel to a known TBE-endemic country.

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Conflict of interest

None declared.

Authors' contributions

ACG Weststrate and F Geeraedts wrote the initial drafts of this article. All authors were involved in correcting the paper on critical points concerning their specific field of expertise and involvement in this case. D. Knapen, GD Laverman, B

Schot, JJW Prick were responsible for the clinical management, SA Spit for providing detailed socio-geographical information; B Rockx, J Reimerink and F Geeraedts were responsible for microbiological laboratory testing.

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Research article

Use of WHONET-SaTScan system for simulated real-time detection of antimicrobial resistance clusters in a hospital in Italy, 2012 to 2014

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Resistant pathogens infections cause in healthcare settings, higher patient mortality, longer hospitalisation times and higher costs for treatments. Strengthening and coordinating local, national and international surveillance systems is the cornerstone for the control of antimicrobial resistance (AMR). In this study, the WHONET-SaTScan software was applied in a hospital in Italy to identify potential outbreaks of AMR. Data from San Filippo Neri Hospital in Rome between 2012 and 2014 were extracted from the national surveillance system for antimicrobial resistance (AR-ISS) and analysed using the simulated prospective analysis for real-time cluster detection included in the WHONET-SaTScan software. Results were compared with the hospital infection prevention and control system. The WHONET-SaTScan identified 71 statistically significant clusters, some involving pathogens carrying multiple resistance phenotypes. Of these 71, three were also detected by the hospital system, while a further 15, detected by WHONET-SaTScan only, were considered of relevant importance and worth further investigation by the hospital infection control team. In this study, the WHONET-SaTScan system was applied for the first time to the surveillance of AMR in Italy as a tool to strengthen this surveillance to allow more timely intervention strategies both at local and national level, using data regularly collected by the Italian national surveillance system.

Introduction

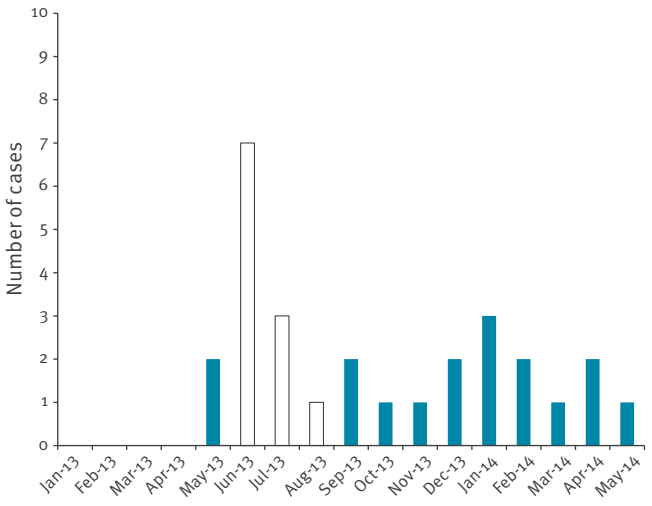
Antimicrobial resistance (AMR) is considered a public health threat as it is increasingly hampering effective treatment of bacterial and fungal diseases worldwide [1,2]. According to the Global Report on Surveillance of Antimicrobial Resistance, rates of resistance are increasing in all World Health Organization (WHO) Regions in pathogens causing infections in both healthcare and community settings [3]. A better strategy to enhance surveillance and strengthen collaborations at a global level is needed in order to coordinate efficient control strategies and to complete the current gaps in surveillance caused by lack of standard methodologies for data collection and failure of data sharing at local, national and international levels [4].

Despite multiple efforts for harmonisation and centralisation of clinical data, lack of data standardisation and poor data accessibility still constitute a worldwide problem. There is also a current need for a standardised interpretation of microbiology data as exemplified by the recent breakpoint harmonisation process promoted by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5]. Clinical microbiology reports represent an important resource for the detection of ongoing dissemination of resistant (and even susceptible) pathogens. In spite of this, they are often underutilised not only at local hospital level, but also in national surveillance systems or across countries [6].

With the aim to centralise and coordinate European surveillance of AMR, the European Centre for Disease Prevention and Control (ECDC) coordinates the European Antimicrobial Resistance Surveillance Network (EARS-Net), a network of national surveillance systems [7]. This network collects routine clinical antimicrobial susceptibility data from 28 European Union (EU) and two European Economic Area (EEA)

Figure

Distribution of *Acinetobacter baumannii* XDR cases and cluster alerts, San Filippo Neri Hospital, Italy, January 2013–May 2014



XDR: extensive drug-resistant.

The figure shows number of cases per month and the empty bars represent the signals that generated the alert in 2013 by WHONET-SaTScan; the sharp increase in number of cases in June 2013 compared with the previous months is statistically significant (see Table 4). The cases in July and August are part of the same cluster, as detected by the SaTScan temporal window and possibly part of the same epidemiological cluster.

countries (Norway and Iceland) concerning invasive isolates (blood and cerebrospinal fluid, CSF) of eight organisms considered of public health concern [8]. This network has promoted the regular collection of clinical data in the participating countries and further highlighted the need of a standardised data format. To address such a need, and to facilitate data sharing, the WHO Collaborating Centre for Surveillance of Antimicrobial Resistance based at the Brigham and Women's Hospital and Harvard Medical School, Boston (United States) developed a software to manage microbiology test results, the WHONET software [9], free to download, (www.whonet.org/software.html) that allows data entry into a standard format, or via BacLink utility software, a conversion tool [10]. Thanks to the software's automated data entry and its ability to handle large datasets as well as to rapidly generate trends and patterns, WHONET has become the official component of many national surveillance programmes and is now used as a support tool in up to 120 WHO member states [11].

As a further application, WHONET has embedded the free SaTScan software (www.satscan.org) developed by Martin Kulldorff together with Information Management Services, Inc. and supported by various United States' National Institutes of Health for the detection of spatial and temporal data clustering, using spatial, temporal or space-time scan statistics [12]. This algorithm is designed to evaluate random distribution or spatial and temporal clustering of diseases and to test their statistical significance, applied to surveillance of diseases and their geographic/spatial determinants or prospectively to timely detect outbreaks [13,14]. In combination, the WHONET-SaTScan system allows for timely detection of clusters of AMR pathogens in space and time facilitating outbreak investigations locally in a single hospital [15], in the community [16], or at national scale for real-time surveillance purposes [17]. The system also enables to study transmission of resistance between wards [18].

In this study, the WHONET-SaTScan software was applied for the first time within the Italian surveillance system. Since 2001, Italy has in place a national antibiotic resistance surveillance project coordinated by the Istituto Superiore di Sanità (AR-ISS), based on sentinel microbiological laboratories, integrating more than 50 hospitals throughout the country. Approximately 20 laboratories have been part of a sub-network called MICRONET and until the end of 2014, automatically submitted clinical data every night to a central server [19]. MICRONET included clinical data for all bacterial pathogens and all kind of samples. Furthermore, in Italy, the WHONET-BacLink software was already used at national level to aggregate and analyse data collected from all the laboratories belonging to the AR-ISS, making the Italian system ideal for the application of the WHONET-SaTScan system. In this work, data collected retrospectively between 2012 and 2014 from one hospital in Rome were analysed using a simulated prospective method to detect statistically significant clusters of pathogens of public health importance. The alerts generated by this method were then compared with the ones generated by the detection method currently in place in the hospital to assess the validity of the WHONET-SaTScan for a possible future implementation within the surveillance of AMR in a real-time and predictive manner.

Methods

Setting of the study

San Filippo Neri Hospital (SFNH) is a public hospital, with predominant surgical activity, located in the northern urban area of Rome with a capacity of 457 beds. Control and response to infections are responsibility of a hospital infections control team (Commissione Prevenzione e Controllo delle Infezioni Ospedaliere, CPCIO), composed of clinicians, microbiologists and virologists, infection preventionists, pharmacists and nurses. The CPCIO is coordinated by the hospital's health manager, and collects microbiology data, detects epidemiological alerts and implements standardised control measures within the hospital. A procedure called 'EpiD' is activated when the definition of an outbreak is met ('three or more samples of the same organism, isolated from three different patients within 5 days in the same operating unit'; '3 by 5' rule) and containment measures are then set in place.

Table 1

Characteristics of isolates from San Filippo Neri Hospital extracted from MICRONET, Italy, January 2012–May 2014 (n = 7,994 isolates)

Isolates characteristics	Number of isolates	Percentage
Year		
2012	3,419	42.7
2013	3,327	41.7
2014[a]	1,248	15.6
Sex		
Female	4,340	54.3
Male	3,616	45.2
Missing information	38	0.5
Specimen type		
Urine	2,972	37.2
Pus	1,598	20.0
Blood	893	11.2
Tracheal aspirate	578	7.2
Vaginal swab	367	4.6
Cervical test	254	3.2
Sputum	238	3.0
Aspirate[b]	203	2.5
Nasal swab	168	2.1
Throat swab	161	2.0
Others	562	7.0
Organism group		
Gram-negative	4,483	56.0
Gram-positive	2,984	37.3
Mycoplasma	272	3.4
Anaerobe	183	2.3
Fungi	57	0.7
Mycobacterium (non tuberculosis)	15	0.2
Department of origin		
Outpatient	2,720	34.0
Medicine	1,970	24.6
Surgery	1,767	22.1
Intensive/intermediate care unit	1,108	13.8
Obstetric/gynaecology	160	2.0
Neonatology	121	1.5
Haematology/oncology	85	1.1
Emergency	49	0.6
Psychiatry	14	0.2

[a] Data are from the first 5 months of 2014 only.

[b] Aspirates other than tracheal aspirates.

Extraction of microbiology data and susceptibility test results

Microbiology data were extracted from the MICRONET database, using date of test request as main parameter and setting restrictions to location (SFNH) and time (between January 2011 and the most recent data available at the time of the study, i.e. 30 May 2014). Data fields extracted included laboratory identity (ID), patient ID, sex, date of birth, age, pathogen type, ward, institution code, department, ward type, specimen number, specimen date, specimen type, specimen code, isolate number, admission date and susceptibility test results which were further described qualitatively as resistant (R), intermediate (I) and susceptible (S) based on minimum inhibitory concentration (MIC) test results and assigned as per EUCAST breakpoints [20]. Data were then converted to WHONET compatible format using the BacLink software. During the conversion the dataset was restricted to the first isolate per patient – including outpatients and inpatients admitted to the hospital any time before specimen collection (i.e. with no distinction between hospital-acquired or community infections) – over a 365 days period and all R and I results were combined as 'non susceptible' (NS) for purposes of resistance phenotype analysis.

Resistance profiles were adapted to this setting by choosing a panel of antibiotics for the main groups of pathogens, according to SFNH's frequency of performed/reported antimicrobial tests per each group. The number of tests was obtained by performing a per cent resistant-intermediate-susceptible (%RIS) analysis on a sample of data from January to June 2013, assuming consistency of testing protocols across years. A 75% frequency was chosen as cut-off value.

Statistical analysis

The SaTScan cluster detection tool integrated into the WHONET software was used to retrospectively identify clusters of antimicrobial resistant pathogens in SFNH. SaTScan can identify clusters of cases in terms of spatial only, temporal only, or combined spatial and temporal distributions. In this work, we used the SaTScan space-time permutation scan statistics for the evaluation of the statistical significance of identified clusters [14]. In this analysis, the temporal parameter was the 'specimen date' while the spatial parameters included a specific location within the hospital, such as the actual 'ward' or a group of wards with communal care characteristic defined as 'service'. Non-spatial variables were the 'pathogen type' or 'resistance profile' based on antibiotic susceptibility test results. Clusters were identified using the categorical variables 'pathogen type', 'resistance profile', 'ward' and 'service' plus a combination of such variables. The statistical significance of clusters was evaluated by a Monte-Carlo maximum likelihood test using SatScan's space-time permutation model. The parameters chosen for this analysis had been already assessed in previous studies [15,17]. A maximum cluster length of 60 days cut-off was chosen, corresponding to the maximum temporal scanning window size for signal generation. The statistical likelihood of signals is determined by the recurrence interval, which corresponds to the inverse of the p-value, expressed in days, signifying the time during which a similar signal would occur by random variation only. In this study, only clusters with a recurrence interval of›365 days were included in the analysis. The baseline parameter (i.e. the temporal baseline

Table 2

Summary characteristics of clusters generated by WHONET-SatScan in San Filippo Neri Hospital, Italy, 2012–2014 (n = 71 clusters)

Cluster characteristics	Number	Percentage[a]
Total number	71	100
Average number of clusters per month	4.5	NA
Year		
2012	17	24.0
2013	42	59.1
2014	12	16.9
Pathogen type		
Escherichia coli	18	25.4
Enterococcus faecalis	13	18.3
Klebsiella pneumoniae	7	9.9
Pseudomonas aeruginosa	5	7.0
Staphylococcus aureus	4	5.6
Acinetobacter baumannii	2	2.8
Other	22	31.0
Type of alerts		
Ward and resistance profile	24	33.8
Resistance profile	21	29.5
Service[b] and resistance profile	16	22.5
Service[b]	4	5.7
Pathogen type	4	5.7
Ward	2	2.8
Mean number of signals per cluster (95% CI)	1.73	(1.53–1.93)
Number of cases		
Total	700	100
Median per cluster (range)	4	(2–143)
Cluster length in days		
1	10	14.1
2–5	17	24.0
6–10	10	14.1
11–50	21	29.5
>50	13	18.3

CI: confidence interval; NA: not applicable.

[a] Unless otherwise specified in the row heading.

[b] A group of wards with communal care is defined as 'service'.

preceding the maximum temporal window against which is compared) was set to 365 days. Thus data from 2011 were considered exclusively as baseline data (as they contributed to the first 365 days of the baseline) for the subsequent 2012 time period, and any clusters detected in 2011 were not included in the analysis.

Dataset generation and comparison of WHONET-SaTScan results with the SFNH infection prevention and control system

Overlapping signals generated by the WHONET-SaTScan analysis were combined into a single 'signal cluster'. In particular, clusters including more information (more types of signal at the same time), more epidemiologically relevant (in terms of duration, number of cases etc.) and with higher recurrence interval, were chosen as representative clusters provided by the system. Cluster summary and cluster detail tables were generated and line listings of all the isolates involved in the alerts were also produced. The summary table of the alerts compiled by WHONET-SaTScan was compared with the CPCIO official list of microbiology alerts from 2012 to 2013 and an extract of the semester report of 2014. Because the CPCIO's analysis of the alert reports from previous years revealed that more than 75% of all episodes within the hospital were caused by three pathogens: *Clostridium difficile*, multidrug resistant (MDR) *Acinetobacter baumannii* and carbapenem-resistant *Klebsiella pneumoniae*, the latest hospital reports, including the ones covered in our study, were restricted to such pathogens. Moreover, as *C. difficile* was not included in the SaTScan-WHONET list of organisms at the time of this study, our comparison could only be based on *A. baumannii* and *K. pneumoniae*.

A questionnaire, adapted from a Brigham and Women's Hospital's, was used to assess whether there were any clusters detected by the WHONET-SaTScan of epidemiological or clinical importance. These alerts were further classified according to the level of concern caused (1 – no concern, disregard; 2 – low concern, await more cases; 3 – moderate concern, action; 4 – high concern, action) and for moderate and high concern, on the type of action (1 – notify other members of the CPCIO to increase awareness; 2 – assess background frequency of organism; 3 – start investigating by assessing medical records to find a common source; 4– activate containment measures). The questionnaire was completed by the head of the microbiology and virology laboratory who was a member of the CPCIO at the time of this study.

Results

Dataset

The microbiology dataset from SFNH collected from the beginning of 2011 to the end of May 2014 included a total of 11,777 samples, of which 7,994 from 2012 and 2014 were included in the final analysis, while 3,783 from 2011 were used as baseline data only. Specimen types were mainly urine (37.2%), pus (20.0%), and blood (11.2%). Table 1 depicts a summary of isolates' characteristics between 2012 and 2014. Overall, isolates included 139 species, the most common being *Escherichia coli* (n = 2,092, 26.2%), *Staphylococcus aureus* (n = 742, 9.3%), *Enterococcus faecalis* (n = 656, 8.2%), *K. pneumoniae* (n = 554, 6.9%) and *Pseudomonas aeruginosa* (n = 506, 6.3%).

Signals created by WHONET-SaTScan

The WHONET-SaTScan analysis generated a total of 287 signals from 2012 to 2014 grouped into 90 'cluster summaries', among which some, overlapping in the spatial components of service/ward and resistance

Table 3

Comparison between *Acinetobacter baumannii* and *Klebsiella pneumoniae* alerts detected by San Filippo Neri's CPCIO and WHONET-SaTScan systems, Italy, 2012–2014

Year	Organism	Detected by the CPCIO				Detected by WHONET-SaTScan				Agreement between the two systems (%)
		Number of alerts	Date of activation	Ward	Number of cases[a]	Number of alerts	Start date	Ward	Number of cases	
2012	*Acinetobacter baumannii* MDR	1	14 Aug	ICU	≥3	0	NA	NA	NA	50
	Klebsiella pneumoniae MDR	1	11 Sep	ICU	≥3	1	22 Sep	OUT	2	
2013	*Acinetobacter baumannii* XDR	1	27 Jun	ICU	≥3	1	20 May	NSW	13	100
	Klebsiella pneumoniae MDR	1	11 Oct	ICU	≥3	1	6 May	ICU	6	
	Klebsiella pneumoniae KPC	1	27 Nov	ICU	≥3	1	3 Aug	ICU	3	
2014	*Klebsiella pneumoniae* KPC	1	Apr[b]	ICU	ND	0	NA	NA	NA	NA

CPCIO: Commissione Prevenzione e Controllo delle Infezioni Ospedaliere (hospital infections control team); ICU: intensive care unit; KPC: *Klebsiella pneumoniae* carbapenem-resistant; MDR: multidrug resistant; NA: not applicable; ND: no data; NSW: no specific ward; OUT: outpatient ward; XDR: extensive drug-resistant.

[a] The number of cases detected by the CPCIO is at least three to trigger the activation of control response as per outbreak definition (see text for details).

[b] The exact date of activation was not available at the time of this study and only an unofficial report from 2014 was available.

phenotype, were further merged manually into 71 final clusters. Table 2 shows the summary characteristics of the final 71 clusters. Of these: 18 were caused by *E. coli* strains mostly fully susceptible to all antibiotics except for three, one of which being an extended-spectrum beta-lactamase (ESBL) strain; 13 by *E. faecalis* with different combinations of resistance phenotypes; seven by *K. pneumoniae*, one of which with resistance to four different classes of antibiotics and one in the intensive care unit (ICU) caused by a carbapenem-resistant strain; four by *P. aeruginosa*, one in ICU by a possible extensive drug-resistant (XDR) strain; three by *S. aureus*, one being a meticillin-resistant *S. aureus* (MRSA) strain; two by *A. baumannii,* one of which involving 13 cases of an XDR organism over two months; lastly, two by *Enterococcus faecium* including one by an MDR strain and the other including two cases of a vancomycin-resistant (VRE) strain in a neonatology ward.

Comparison of alerts generated by WHONET-SaTScan with the hospital response system

In order to assess the validity of the method we compared the signal alerts generated by our analysis with the 'EpiD' procedure activated by the CPCIO. The total number of potential outbreaks detected by the WHONET-SaTScan system per year was higher than the number of activated 'EpiD' (respectively, including *C. difficile* in 'EpiD', 17 vs 4 in 2012, 42 vs 6 in 2013 and 12 vs 4 in 2014). Table 3 summarises the comparison between the two systems, by year. In 2012, of two alerts detected by CPCIO (i.e. two activated 'EpiD'), only the one involving *K. pneumoniae* is possibly in common between the two systems. However, this cluster was detected by WHONET-SaTScan in a different ward (outpatient) than by the CPCIO (which found the cluster in the ICU) and at a later time. As the CPCIO detected the *K. pneumoniae* cluster 11 days earlier, this outbreak was probably contained as result of the activation of the 'Epid' procedure. In 2013, three outbreaks were detected with a 100% agreement between the CPCIO and WHONET-SaTScan; one of these outbreaks involved *A. baumannii* in a cluster of long duration, which lasted from 20 May 2013 to 1 August 2013 with a recurrence interval of 2 years. This large outbreak, however, included a smaller signal outbreak clustered by service and resistance between 20 May 2013 and 25 June 2013 in the ICU with recurrence interval of 2.75 years (more rare) probably corresponding to the same signal that activated a response within the hospital. The signal of this cluster as generated by the WHONET-SaTScan is shown in the Figure. The other two outbreaks were caused by *K. pneumoniae* and seem to have activated the 'EpiD' procedure only months after the start of the outbreak, according to WHONET-SaTScan. In 2014, there was no official report from the hospital at the time of the study, and the alerts we could obtain were only from

Table 4

Cluster alerts detected by WHONET-SaTScan in San Filippo Neri Hospital considered relevant by the hospital's infection control system (CPCIO) and critical characteristics of the alerts for the evaluation, Italy, 2012–2014 (n = 18 alerts)

Organism[a]	Type of alert	Alert characteristics[b]	Dates of outbreak (start–end)	Recurrence interval (1/n years)	Observed cases	Observed/ expected case ratio	Time span in days	CPCIO evaluation
2012								
E. coli	Ward/res	Gen med Fully susceptible	5 Sep–22 Sep	1/1.14	18	2.51	19	Pat/res
P. aeruginosa	Ward/res	Vascular surgery CTX, SXT	12 Oct–22 Oct	1/1.25	4	7.02	11	Pat/res
E. aerogenes	Ward/res	Vascular surgery Fully susceptible	22 Oct–25 Oct	1/19.85	2	6.06	4	Ward
S. marcescens	Res	SXT	13 Nov–15 Nov	1/22.40	2	5.56	3	Pat/res
2013								
E. coli	Ward/res	Neuro-rehab	20 Apr–20 Apr	1/1.52	2	153.85	1	Ward
K. pneumoniae	Res	CTX, CAZ, CIP, GEN, TZP, SXT	6 May–6 Jun	1/23.69	6	46.15	32	Pat/res
E. faecium	Serv/res	Neonatology AMP, ERY, GEN, IPM, LVX, MFX, VAN	10 Jun–12 Jun	1/1.30	2	21.05	3	Pat/res/serv
S. aureus	Res	LVX, OXA, PEN	26 Apr–22 Jun	1/1.44	10	4.13	58	Pat/res
P. aeruginosa	Ward/res	ICU CTX, CAZ, IPM, MEM, TZP, SXT	15 Jun–1 Jul	1/1.44	3	23.08	17	Pat/res/ ward
S. marcescens	Serv/res	ICU AMK	19 May–15 Jul	1/2.11	3	4.76	58	Pat/res/serv
A. baumannii	Res	CTX, CAZ, CIP, GEN, IPM, MEM, SXT	20 May–1 Aug	1/2.00	13	3.56	74	Pat/res
K. pneumoniae	Serv/res	ICU AMK, CTX, CAZ, CIP, GEN, IPM, MEM, TZP, SXT	3 Aug–7 Aug	1/2.78	3	38.96	5	Pat/res/serv
P. aeruginosa	Serv	Interm care unit	26 Aug–27 Sep	1/4.13	4	9.52	33	Serv
K. pneumoniae	Serv	Interm care unit	5 Oct–18 Oct	1/1.25	3	15.79	14	Serv
S. aureus	Ward/res	Ortho-Trauma Fully susceptible	2 Dec–2 Dec	1/2.11	2	142.86	1	Pat/res/ ward
S. marcescens	Serv/res	Gen Med AMK	24 Dec–3 Jan 2014	1/1.37	2	9.09	11	Pat/res/serv
2014								
P. aeruginosa	Serv/res	Surgery CIP, IPM, SXT	31 Mar–31 Mar	1/2.49	2	105.26	1	Pat/res/serv
S. maltophila	Ward/res	ICU	14 Apr–28 May	1/3.26	2	4.35	45	Pat/res/ ward

Gen med: general medicine; ICU: intensive care unit; interm care unit: intermediate care unit; neuro-rehab: neuro-rehabilitation; ortho-trauma: orthopaedic trauma; pat: pathogen type; res: resistance; serv: service.

[a] The organisms are abbreviated as follows: *A. baumannii: Acinetobacter baumannii; E. aerogenes: Enterobacter aerogenes; E. coli: Escherichia coli; E. faecium: Enterococcus faecium; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; S. aureus: Staphylococcus aureus; S. maltophila: Stenotrophomonas maltophila; S. marcescens: Serratia marcescens*.

[b] Antibiotics listed in this column are abbreviated as follows: AMK: amikacin; AMP: ampicillin; CAZ: ceftazidime; CIP: ciprofloxacin; CTX: cefotaxime; ERY: erythromycin; GEN: gentamicin; IPM: imipenem; LVX: levofloxacin; MEM: meropenem; MFX: moxifloxacin; OXA: oxacillin; PEN: penicillin; SXT: trimethoprim/sulfamethoxazole; TZP: tazobactam; VAN: vancomycin.

incomplete reports. However, none of the CPCIO alerts was detected by WHONET-SaTScan.

Evaluation of the alerts generated by WHONET-SaTScan

To assess the benefit of WHONET-SaTScan we asked a member of the CPCIO to evaluate the alerts generated by this system. Table 4 shows the alerts considered worth knowing by the hospital and by type of crucial characteristic (pathogen type, resistance phenotype or location). Of the 71 clusters detected by the WHONET-SaTScan analysis, 18 were considered of importance, of which only three had been initially detected by the CPCIO. The majority of these clusters were deemed relevant for the hospital because of the combined characteristics of pathogen type, resistance phenotype and location (n = 8, 44%), but also for their location alone (n = 4, 22%). Of these 18 clusters, including the ones detected by the CPCIO, six were considered of low concern, eight of moderate concern and four of high concern. For the eight alerts of moderate concern only two of the four types of possible actions were activated (i.e. 1 – notification of other members of CPCIO and 4 – start response measures), while the four alerts of high concern would trigger all four types of action. Among the high concern alerts, one caused by an *E. faecium* VRE strain in June 2013 and one by a *P. aeruginosa* MDR/XDR strain in July 2013, occurred completely undetected by the CPCIO.

Discussion

Timeliness is one of the main attributes of a good surveillance system, representing the ability to take appropriate public health action based on urgency [21]. Electronic data systems for the collection and analysis of microbiology data are becoming essential tools for surveillance to guarantee reliability, timeliness and standardisation across different compartments [22]. The aim of this work is to show the utility of a new tool, the WHONET-SaTScan, for surveillance of AMR in healthcare settings, especially in a context in which national surveillance programmes facilitate automated routine data collection, as the case of the Italian MICRONET [23].

When compared with traditional surveillance methods, the automated system used in this study showed a discrepancy in detected signals, as previously observed in other studies [15,24]. The higher number of signals produced by WHONET-SaTScan could be due to methodological differences compared to the CPCIO approach. WHONET-SaTScan generates a list of statistically significant signals, using an arbitrary choice for the cut-off value of significance (the recurrence interval), that affects sensitivity and specificity of the method, therefore meaning that statistically significant signals could not be necessarily indicative of a real outbreak or vice versa. Furthermore, the space-time permutation statistics cannot distinguish underlying fluctuations of local population sizes or temporal variations of detection frequency, leading to biased p-values [14]. In contrast, the CPCIO's method is based on the classic definition of outbreak based on the '3 by 5' rule, irrespective of the baseline incidence of the organism or the specific resistance phenotypes. In this case, its sensitivity is determined by the complexity of the case definition and personal interpretation, particularly in case of complex resistance phenotypes, while its specificity can be affected by baseline incidence. As a consequence, detection of clusters could be either delayed or even missing, especially if cases are spread throughout the hospital or, alternatively, infection control responses could be triggered when not needed, drawing staff and resources from the hospital and causing unnecessary distress to patients. On the other hand, traditional methods allow case-by-case interpretations based on personal experience and hospital background, identifying clusters not statistically but epidemiologically significant, like for example the cluster of *A. baumannii* in 2012, detected only by the CPCIO. Lack of information on the evolution of outbreaks after activation of the 'EpiD' procedure in the CPCIO reports, besides providing no indication on the efficacy of the measures adopted, interferes with the comparison between extent of outbreaks, as clusters detected by WHONET-SaTScan may result in higher case numbers and longer time spans.

The WHONET-SaTScan system showed some advantages compared to the CPCIO's. The '3 by 5' rule applied to a single ward at the time, in particular to critical care units, seems to be restrictive when compared with the WHONET-SaTScan ability to include groups of wards together or cover the whole hospital simultaneously. In this study, the 'EpiD' activated by the CPCIO occurred mainly in the ICU, while the clusters detected by WHONET-SaTScan were more homogeneously distributed throughout the hospital. The evaluation by the CPCIO coordinator showed that the main factor to trigger a response was the organism resistance profile, followed by pathogen type, location and source of specimen. The WHONET-SaTScan analysis allows for the investigation of clusters according to a specific resistance profile in combination to a specific location ('resistance/ward' and 'resistance/service'), useful when an outbreak is occurring in a critical care ward. In addition, within the same analysis WHONET-SaTScan identifies clusters of susceptible strains, otherwise neglected due to a higher focus on resistance. Such clusters could be, in fact, of great interest to the infection control team for their routes of transmission and to the medical team in terms of pathogen characteristics and for offering different therapeutic options.

This study is not exempt of limitations and bias. Its retrospective nature undermines the efficacy of the WHONET-SaTScan system in the 'field'. If conducted in real-time, it would have detected two clusters of MDR *K. pneumoniae* on average 126 days (95% confidence interval (CI): 66–186; n = 2), i.e. four months earlier, than the standard hospital control system, plus additional ones (two outbreaks of *E. faecium* VRE

strain and *P. aeruginosa* MDR/XDR) that had occurred unnoticed within the hospital. The possibility to investigate prospectively the list of statistically significant alerts in combination with the clinical and epidemiological expertise of the hospital control team would provide a better evaluation of its benefits. Moreover, the inclusion of *C. difficile*, at the time not included in the list of organisms in the WHONET-SatScan analysis, would have better met the needs of the facility under investigation.

Reporting bias occurs as a consequence of selective reporting and control within the hospital due to a combination of resource availability, therapeutic choices and background prevalence data. For example, because of the endemic distribution in Italy of MRSA or ESBLs and the lack of appropriate resources for a prompt and effective intervention, the hospital adopted the policy of not reporting alerts triggered by these organisms. Again, the choice of antibiotics routinely used would reflect the panel of antibiotics tested and included in the configuration of WHONET, thus generating a list of alerts biased by the hospital policy on testing and reporting microbiology data. Lack of representativeness is another limitation of this study, as SFNH has in place an official procedure for infection control and a regular collection of standardised microbiology data, which most likely does not reflect the situation of other hospitals in Italy, a country with high between-hospital and regional variation.

Nevertheless, this work represents the first application of the WHONET-SaTScan system in a healthcare facility in Italy with the potential to be applied to other hospitals, extended to multiple hospitals in the same area or region or even on a larger scale to the whole national territory. Although the WHONET software is implemented within the surveillance systems of other European countries [25-27], this pilot study represents the first example of its application to the detection of clusters of resistant pathogens within a national surveillance system in Europe.

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Conflict of interest

None declared.

Authors' contributions

Alessandra Natale, led all data analysis and writing of the manuscript. John Stelling, project co-supervisor, gave strong guidance on data analysis and use of the WHONET-SatScan software plus overall revision of the work. Marcello Meledandri, kindly filled the questionnaire on the comparison analysis between WHONET-SaTScan data and the hospital infection control system, and provided access to the CPCIO reports. Louisa Messenger, provided overall ideas. Fortunato D'ancona, project supervisor, provided facilities, MICRONET data access and supervision on data analysis, plus strong guidance on the writing of the manuscript.

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Cross-sectional surveillance of Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels and other mammals in Egypt, August 2015 to January 2016

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A cross-sectional study was conducted in Egypt to determine the prevalence of Middle East respiratory syndrome coronavirus (MERS-CoV) in imported and resident camels and bats, as well as to assess possible transmission of the virus to domestic ruminants and equines. A total of 1,031 sera, 1,078 nasal swabs, 13 rectal swabs, and 38 milk samples were collected from 1,078 camels in different types of sites. In addition, 145 domestic animals and 109 bats were sampled. Overall, of 1,031 serologically-tested camels, 871 (84.5%) had MERS-CoV neutralising antibodies. Seroprevalence was significantly higher in imported (614/692; 88.7%) than resident camels (257/339; 5.8%) ($p<0.05$). Camels from Sudan (543/594; 91.4%) had a higher seroprevalence than those from East Africa (71/98; 72.4%) ($p<0.05$). Sampling site and age were also associated with MERS-CoV seroprevalence ($p<0.05$). All tested samples from domestic animals and bats were negative for MERS-CoV antibodies except one sheep sample which showed a 1:640 titre. Of 1,078 camels, 41 (3.8%) were positive for MERS-CoV genetic material. Sequences obtained were not found to cluster with clade A or B MERS-CoV sequences and were genetically diverse. The presence of neutralising antibodies in one sheep apparently in contact with seropositive camels calls for further studies on domestic animals in contact with camels.

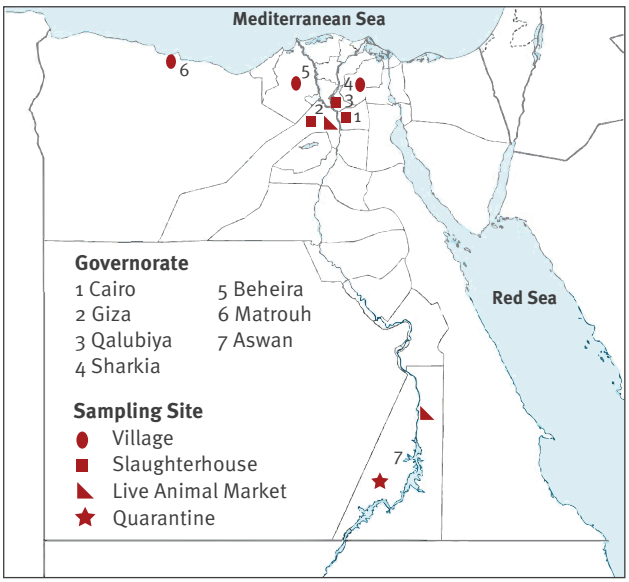
Introduction

Since the first human case of Middle East respiratory syndrome coronavirus (MERS-CoV) in Saudi Arabia, in 2012, the World Health Organization (WHO) was notified of 1,698 laboratory-confirmed human cases and at least 609 human deaths from 26 countries as of March 2016 [1]. Primary infections have originated from countries within the Arabian Peninsula, but travel-associated cases and some secondary and nosocomial transmissions have been reported in other countries. A recent study in 2016 found antibodies against MERS-CoV in human serum in Kenya [2]. Available data from serological and molecular studies suggest that the primary source of MERS-CoV infection for many in the Arabian Peninsula appears to be dromedary camels [3-5]. Bats are also incriminated in the origins of many known mammalian coronaviruses including severe acute respiratory syndrome (SARS) [6,7]. The close relationship of MERS-CoV genome sequences and sequences of bat coronaviruses suggests that bats may be a reservoir for MERS-CoV [8]. Moreover, bat cell lines display the MERS-CoV specific receptor, dipeptidyl peptidase 4 (DPP4), and can be infected under experimental conditions [9]. Previous epidemiological studies to investigate the presence of MERS-CoV in bats found a close relationship between characterised sequences generated from bat faecal samples, and previously characterised MERS-CoV sequences [10-12].

A retrospective serological study conducted on 189 archived dromedary camels sera originating from main camel-exporting countries, Sudan and Somalia, in the period from 1983 to 1997, showed the presence of MERS-CoV neutralising antibodies in 81% of total

Figure 1

Site map of the collected samples from dromedary camels and domestic animals in Egypt, August 2015–January 2016 (n =1,223 animals[a])



[a] In addition to 1,078 camels. a total of 145 domestic animals were sampled and included cattle (n=35), sheep (n=51), goats (n=36), donkeys (n=15), buffaloes (n = 4) and horses (n = 4).

samples suggesting long-term MERS-CoV circulation among camels [13]. Dromedaries from African countries (Egypt, Ethiopia, Kenya, Nigeria, Sudan, and Tunisia) and the Arabian Peninsula (Jordan, Oman, Qatar, Saudi Arabia, and United Arab Emirates) have high rates of MERS-CoV antibody seropositivity [14-20]. Dromedary camels are part of the culture of millions of people in Middle Eastern countries where camel milk and meat are consumed. Most dromedary camels traded in the Middle East are bred in East African countries, primarily in Ethiopia, Kenya, Somalia, and Sudan [21]. During the last 5 to 6 years (2010 to 2015), over 1.2 million camels were imported to Egypt, nearly 70% from Sudan and the rest from the African Horn, mainly Ethiopia [22].

Serological investigations carried out on camels in Egypt, revealed high levels of antibodies against MERS-CoV [17,23]. Furthermore, MERS-CoV was detected virologically in specimens collected from abattoirs in the country [23]. The objectives of this study were to determine the prevalence of MERS-CoV in imported and resident camels and investigate the prevalence of the virus among other domestic animals in Egypt.

Methods

Study animals and sampling strategy

A total of 1,176 sera and 1,223 nasal swabs, were collected from 1,223 animals including 1,078 dromedary camels (339 resident and 739 imported) and 145 other domestic animals (cattle, n=35; sheep, n=51; goats, n=36; donkeys, n=15; and buffalo and horses, n = 4 each) from different sampling sites (quarantine posts, live animal markets, slaughterhouses and villages) from seven governorates of Egypt (Figure 1) between August 2015 and January 2016.

Milk samples (3–5mL; n=38) and rectal swabs (in 1mL viral transport media; n=13) were also sampled from resident camels in a village located in the Matrouh governorate.

In addition, 109 throat swabs and 91 sera were collected from 24 fruit bats (*Rousettous aegyptiacus)* and 85 insectivorous bats (*Pipistrellus deserti*, n=28; *Nycteris thebaica*, n=30; *Taphozous perforates*, n=27) from Abo Rawash, Giza governorate, and included in the study.

A multistage sampling strategy involving a combination of simple stratified (for sex and age) and systematic sampling was employed to obtain samples from camels. Origin of camels was identified at the place of quarantine in Egypt, or from information obtained from the owners. Camels less than two years of age were considered young while those over two years-old were considered adult. Since the majority of the imported camels were adult male, purposive sampling was employed to include female adult camels particularly in the resident camels. Sampling procedures were approved by the Ethics Committee of the National Research Centre, Egypt.

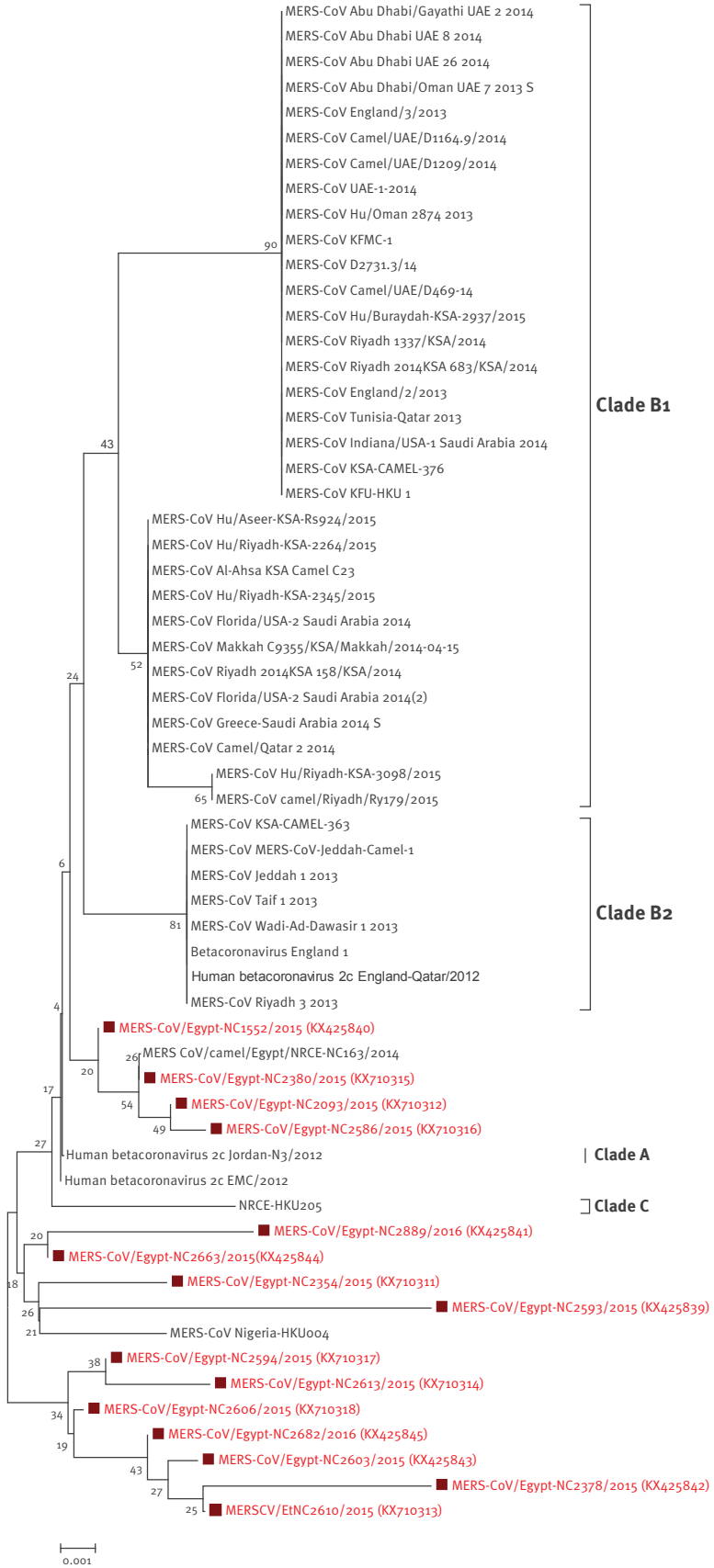
The nasal, throat, rectal swabs and milk were analysed using molecular virological techniques.

Serological testing

Serum microneutralisation assay was conducted as described [17], using Vero-E6 cell monolayers. Briefly, twofold serial dilutions of 200µL heat-inactivated sera (56°C for 30 min) were made, starting with a dilution of 1:10. The serum dilutions were mixed with equal volumes of 200 tissue culture infectious dose (TCID50) of dromedary MERS-CoV Egypt NRCE-HKU270 (Egypt 270). After 1 hour of incubation at 37°C, 35 µL of the virus–serum mixture were added in quadruplicate to Vero-E6 cell monolayers in 96-well microtitre plates. After 1 hour of adsorption, an additional 150 µL of culture medium were added to each well. The plates were then incubated for three more days at 37°C in 5% CO2 in a humidified incubator. Virus back-titration was performed without immune serum to assess input virus dose. Cytopathic effect (CPE) was read at 3 days post infection. The highest serum dilution that completely protected the cells from CPE in half of the wells was taken as the neutralising antibody titre and was estimated using the Reed–Muench method. Positive cut off points was set at values greater or equal to 1:20 serum dilution points.

Figure 2

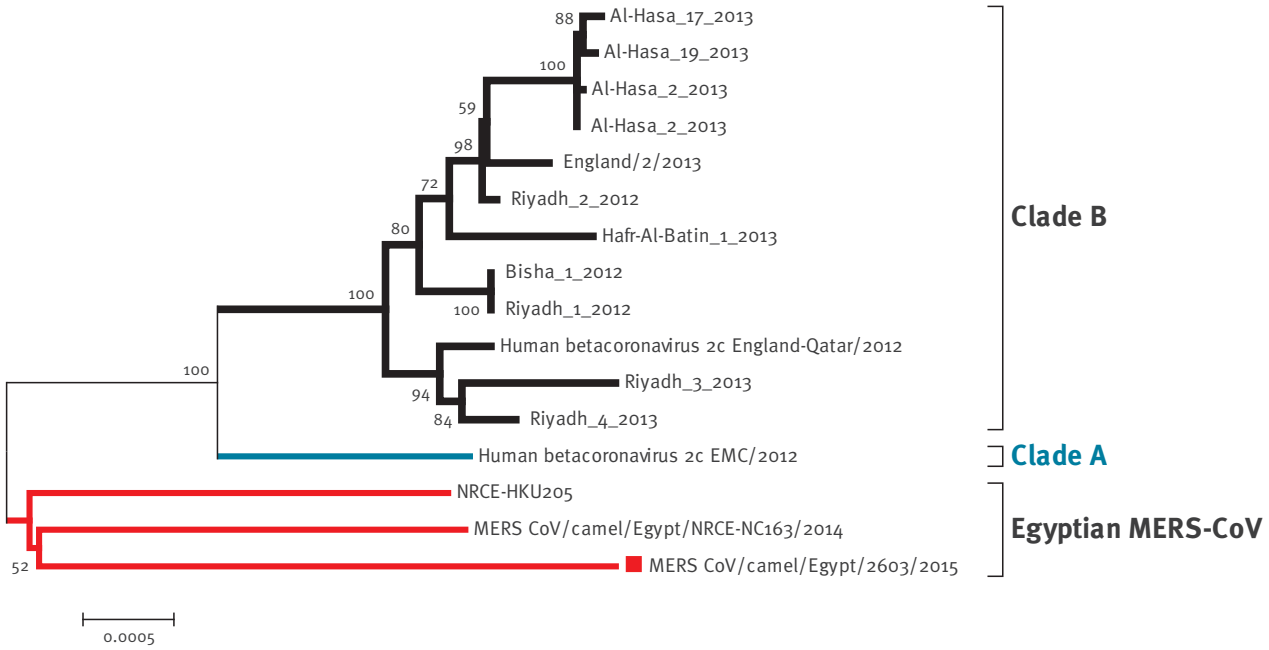
Phylogenic analysis of partial MERS-CoV spike sequences retrieved from dromedary camels residing in or imported to Egypt from Sudan between August 2015 and January 2016



Representative viruses from clades A, B and C are indicated and marked with vertical bar. Phylogenetic analysis was done using the neighbour-joining algorithm with the Kimura two-parameter model. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The reliability of phylogenetic inference at each branch node was estimated by the bootstrap method with 1,000 replications; evolutionary analysis was conducted in MEGA 6.06. Viruses sequenced for this study are marked with red squares.

Figure 3

Phylogenic analysis of a full MERS-CoV genome sequence retrieved from an imported dromedary camel from Sudan between August 2015 and January 2016



Representative viruses from the two major MERS-CoVs clades (A and B) are indicated and marked with vertical bar. Phylogenetic analysis was done using the neighbour-joining algorithm with the Kimura two-parameter model. The reliability of phylogenetic inference at each branch node was estimated by the bootstrap method with 1,000 replications. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analysis was conducted in MEGA 6.06. The virus sequenced for this study is marked by a red square.

Real-time reverse transcription-PCR

Real-time reverse transcription-PCR (rtRT-PCR) targeting upstream of the envelope protein gene (UpE) of MERS-CoV was used for screening [24]. Confirmation was made using the open reading frame (ORF) 1a, RNA-dependent RNA polymerase (RdRp) or nucleocapsid protein (N) gene, based on the recommendation of World Health Organization for MERS-CoV diagnosis [25]. Briefly, 5 µL of extracted RNA was subjected to rtRT-PCR using UpE primers described elsewhere [24]. The rtRT-PCR was performed using a Verso One Step rtRT-PCR Kit according to the manufacturer's protocol. All positive samples by the UpE assay regardless of cycle threshold (Ct) value were then confirmed by one of ORF1a, RdRp, or N gene RT-PCR assay as described previously [24,26]. PCR products were analysed by sequencing using the protocol available on the web (on line Technical Appendix: http://wwwnc.cdc.gov/eid/article/20/6/14-0299-techapp1.pdf).

Reverse transcription-PCR for MERS-CoV genotyping

A partial 640 bp fragment of the spike gene was amplified using 50-Fwd (5'-CCAATTTA-CGCCAGGATGAT-3') and 50-Rev (5'-AATAGAGGCGG AAATAGCAC-3') primers in the first round using one step RT-PCR kit (QIAGEN) and a total reaction volume of 25 µL including 5 µL of 5X reaction buffer, 1 µL dNTPs, 1 µL enzyme mix, 1.5 µL (10 pmol) forward primer, 1.5 µL (10 pmol) reverse primer, 10 µL ddH2O and 5 µL of sample RNA. Subsequent to thirty min at 50 °C and 95 °C for 15 min, the RT-PCR also comprised 45 cycles of 94 °C for 15 s, 55 °C for 30 s and 72 °C for 60 s followed by a final step of 72 °C for 10 min. The PCR product was then submitted to a second PCR round using the same primers as in the first round and Phusion High Fidelity PCR Master Mix Kit (Thermo Scientific). The PCR had a 25 µL reaction volume, with 12.5 µL of 2 X phusion master mix, 1.5 µL (10 pmol) forward primer, 1.5 µL (10 pmol) reverse primer, 7.5 µL H2O and 2 µL of the first round PCR product. The PCR cycler conditions were 98 °C for 30 s then 45 cycles (98 °C for 10 s, 55 °C for 30 s, 72 °C for 60 s), then 72 °C for 10 min. The final PCR product was gel purified and subsequently sequenced with the same primers at the Macrogen sequencing facility (Macrogen, South Korea). One positive imported sample (NC2603/2015) from Sudan was subjected to whole genome sequencing according to a previously published procedure [27]. The phylogenetic tree was constructed using MEGA6 programme [28].

Data management and analysis

Data collected from the study animals were coded and entered in a Microsoft excel sheet. All statistical analyses were performed using SPSS version 16 for windows. The association between MERS-CoV prevalence in camels and the study variables (sampling site, origin, age

Table 1

MERS-CoV surveillance test results in camels based on origin, Egypt, August 2015–January 2016 (n = 1,078 camels[a])

Camel origin	Microneutralisation test			CMLE OR[b] (95% CI)	P value (for OR)	P value (for hypothesis)	rtRT-PCR			P value (for hypothesis)
	Number tested	Number of camels positive	Per cent positive				Number tested	Number of camels positive	Per cent positive	
East Africa	98	71	72.4%	0.84 (0.51–1.41)	0.50	p<0.001 x2=53.24	115	4	3.5%	p<0.001 x2=15.246
Sudan	594	543	91.4%	3.39 (2.24–4.98)	<0.0001		623	35	5.6%	
Egypt (resident)	339	257	75.8%	1.00	Ref.		340	2	0.6%	
Total	**1,031**	**871**	**84.5%**	**NA**	**NA**	**NA**	**1,078**	**41**	**NA**	**NA**

CI: confidence interval; CMLE: conditional maximum likelihood estimate; MERS-CoV: Middle East respiratory syndrome coronavirus; NA: not applicable; OR: odds ratio; ref.: reference; rtRT-PCR: real-time reverse transcription PCR.

[a] Of 1,078 camels, a subset of 1,031 underwent serum testing for MERS-CoV antibodies by microneutralisation assays, while all were sampled for rtRT-PCR testing.

[b] CMLE OR is the conditional maximum likelihood estimate of the odds ratio based on Mid-P exact confidence interval.

and sex) were analysed by Pearson chi-squared test of independence. Statistical significance was considered at p- value less than 0.05.

Results

Serological analysis

Of the 1,031 camels, which were serologically tested, 871 (84.5%) had MERS-CoV neutralising antibodies in their sera (Table 1).

The seroprevalence was significantly higher in imported (614/692; 88.7%) than in resident camels (75.8%; Table 1) ($p<0.05$). Based on the area of origin, seroprevalence varied significantly among camels originating from East Africa, Sudan, and Egypt and was 72.4%, 91.4%, and 75.8%, respectively ($p<0.05$). Camels sampled from live animal markets, quarantine facilities, slaughterhouses, and villages had seroprevalence of 94.5%, 95.7%, 77%, and 75% respectively and the differences was significant ($p<0.05$ Table 2). Overall, adult camels had significantly higher seroprevalence (87.3%) than young camels (51.8%) ($p<0.001$). A significantly higher seropositivity was observed for camels from the live animal markets (OR=5.52; $p<0.0001$) and quarantine facilities (OR=7.25; $p<0.0001$) as compared with those from villages and the slaughterhouses.

Both male and female camels had a comparable ($p>0.05$) level of seroprevalence (85.1% and 82.7% respectively), and risk of seropositivity (Table 2). Tested samples from 126 ruminants (cattle, sheep, goats, and buffaloes) and 19 equines (donkeys and horses) were negative for neutralising MERS-CoV antibodies but one serum sample from a sheep had 1:640 neutralising titre. None of the 91 tested bats was positive for MERS-CoV neutralising antibodies.

Virus genomic detection

Of the 1,078 nasal samples from camels, 41 (3.8%) were positive for MERS-CoV using MERS-CoV PCR tests indicating the presence of active or passive viral infection. Of the 41 positive camels, four originated from East Africa, 35 from Sudan and the other two from the study sites in Egypt (Table 1). The confirmed PCR-positive MERS-CoV cases was significantly higher in females than males ($p<0.001$). All the 38 milk samples and 13 rectal swabs were negative for MERS-CoV. Similarly, the 145 nasal swabs from domestic ruminants and equines were negative for MERS-CoV. Throat swabs collected from 109 bats were negative for MERS-CoV.

Sequence analysis

A phylogenetic tree was compiled based on partial spike nucleotide sequences obtained from 15 strongly positive samples. The sequences were derived from one camel residing in Egypt as well as from camels imported from Sudan, which had been sampled in a slaughterhouse (n = 9) and live animal markets (n = 5). The tree suggested that sequences from camels investigated in Egypt formed separate groups from previously published sequences of MERS-CoV (Figure 2). Moreover, a phylogenetic analysis of full genomes showed that sequences from camels sampled in Egypt were genetically diverse and clustered neither with clades A or B (Figure 3).

Discussion

The present study demonstrated that most of the camels that were imported to Egypt were seropositive for MERS-CoV (88.7%; 614/692) and virus genetic materials was detected in 5.3% (39/738) of the imported camels. The origins of the camels were Sudan and East Africa. Surprisingly, no human cases of MERS CoV infection has been recorded among camel traders from these countries. This may be due to the lack of diagnostic tools and experience for virus detection or

Table 2

MERS-CoV surveillance test result in camels based on sampling site, age and sex, Egypt, August 2015–January 2016 (n = 1,078 camels[a])

Category	Microneutralisation test			CMLE OR[b] (95% CI)	P value (for odd ratio)	P value (for hypothesis)	rRT-PCR			P value (for hypothesis)
	Number tested	Number positive	Per cent positive				Number tested	Number positive	Per cent positive	
Sampling site										
Live animal market	289	273	94.5%	5.52 (3.20–9.96)	<0.0001	p<0.001 x2=67.47	290	9	3.1%	p<0.001 x2=31.97
Village/Egypt	339	256	75.8%	1.00	Ref.		340	2	0.6%	
Quarantine	164	157	95.7%	7.25 (3.42–17.42)	<0.0001		164	4	2.4%	
Slaughterhouse	239	184	77%	1.09 (0.73–1.61)	0.69		284	26	9.2%	
Total	1,031	871	84.5%	NA	NA	NA	1,078	41	3.8%	NA
Age										
Young	81	42	51.8%	1.00	Ref.	p<0.001 x2=71.39	82	2	2.4%	p=0.77 x2=0.53
Adult	950	829	87.3%	6.34 (3.93–10.24)	<0.0001		996	39	3.9%	
Sex										
Male	765	651	85.1%	1.19 (0.82–1.73)	0.35	p=0.38 x2=0.86	798	21	2.6%	p<0.001 x2=13.07
Female	266	220	82.7%	1.00	Ref.		280	20	7.1%	

CI: confidence interval; CMLE: conditional maximum likelihood estimate; MERS-CoV: Middle East respiratory syndrome coronavirus; NA: not applicable; OR: odds ratio; ref.: reference; rtRT-PCR: real-time reverse transcription PCR.

[a] Of 1,078 camels, a subset of 1,031 underwent serum sampling for MERS-CoV antibodies by microneutralisation assays, while all were sampled for rtRT-PCR testing.

[b] CMLE OR: Conditional maximum likelihood estimate OR based on Mid-P exact confidence interval.

maybe due to the rarity of virus transmission from camels to humans.

Data from experimental camel infections suggest that MERS-CoV is a mild respiratory infection in camels [29] and although camels previously sampled at abattoirs shed the virus, they did not have overt clinical symptoms [23]. Egypt imports large numbers of live camels each year to meet its animal protein demand. According to the Ministry of Agriculture, almost 70% of the imported camels during the past five years originated from the Sudan and the rest from East Africa, mainly Ethiopia. These imported camels are quarantined usually for 2–3 days at the point of entry before they gain entry for sale at live animal markets. The animals often travel long distances by trucks and may be moved from one live animal market to another. Transport stress and close vicinity of camels during transport may precipitate disease dissemination, particularly in animals with latent infection and carrier animals, while transmission may be facilitated spatio-temporally in the different markets. The high MERS-CoV seroprevalence both in resident and imported camels and the presence of active viral infection circulating in the country were indications that the virus may have become ubiquitous in Egypt. Inter-market movement and transport stress may partially explain the higher seropositivity and molecular analysis results in samples obtained from the live animal markets, quarantine facilities, and the slaughterhouses.

Testing of archived dromedary sera has revealed that MERS-CoV has been circulating for at least three decades and is not a newly emerged virus, but rather a virus that has only recently been discovered [3,13,15]. Results of study in Egypt published in 2014 showed that 93.6% of camels originating from Sudan were seropositive for MERS-CoV, a finding is consistent with the present study where 91.4% of camels imported from that country were seropositive [23].

Analysis of the results based on age showed that adult camels had higher seroprevalence of MERS-CoV antibodies (87.3%) compared with young camels (51.8%) ($p<0.05$). The variation might be due to the small number of young camels tested or the higher likelihood of exposure of adult camels. In addition, young camels have been more acutely infected in past studies and may have died rather than seroconverted [18]. Similar studies elsewhere also indicated a higher seroprevalence in adult than in juvenile camels [30]. Although the number of seropositive samples was comparable in female and male camels, the number of confirmed PCR positive MERS-CoV animals was significantly higher in females than males ($p<0.05$). There was however no significant difference in rtRT-PCR positive cases between the age groups.

Nucleotide sequencing of the amplicons from 15 of 41 PCR-positive samples for MERS-CoV genetic material, followed by phylogenetic analysis showed that the sequences recovered in the current study in Egypt were distinct from those in clade A and B. This was also the case for previously identified MERS-CoV sequences derived from camels in Egypt (e.g. MERS CoV/camel/Egypt/NRCE-NC163/2014) [31] which were distinct from MERS-CoV EMC/2012 isolate [23].

All the 145 domestic animals (ruminants and equines) tested for MERS-CoV genetic materials were negative, in agreement with previous studies conducted in Jordan and Egypt [19]. Except one sheep, all domestic animals serologically tested were negative. Similarly, previous serological studies conducted on goats, sheep, and cows were all negative [19]. Also according to a prior report, 25 cows and eight buffalo from Egypt tested negative to MERS-CoV neutralising antibodies [17]. The seropositive sheep found in the current study was apparently in contact with seropositive camel herds in villages. This finding is significant and adds to the knowledge of host range of MERS-CoV. The DPP4 receptor for MERS-CoV has been found to be present in camel, goat, cow and sheep [32], and Reusken et al. [19] have earlier confirmed that six sheep reacted to MERS-CoV antigens but without neutralising antibodies [19]. Further and extensive studies on domestic animals especially in those in contact with camels are required to elucidate the possibility of MERS-CoV transmission from camels to such animals.

Whereas MERS CoV has been found in one bat sample in Saudi Arabia [5], all the 109 bats in the present study, were negative for MERS-CoV using both serology and molecular assays. Bats have been incriminated as the origin of many known mammalian coronaviruses including SARS [7]. A 190 nt RNA fragment of MERS-CoV was detected in a bat faecal sample [11]. However, since human–bat contact is limited, camels have been more implicated as a probable intermediate host [33].

In conclusion, the very high prevalence of MERS-CoV neutralising antibodies in both resident and imported camels indicates the widespread and ubiquitous presence of the virus in the country. A systematic longitudinal study, however, is needed to follow up imported camels from their country of origin until they reach the slaughterhouses to understand the epidemiology of the disease along the camel market chain. A separate study on resident camels is needed to understand the dynamics of infection in local camels as opposed to in imported camels. The very high seroprevalence detected in camels warrants the initiation of an active surveillance study on humans, particularly those that are at higher risks of exposure to MERS-CoV infections such as camel traders and abattoir workers.

*Authors' correction

The order of Dr Folorunso Oludayo Fasina's names was wrong in the authors' list, leading to abbreviation as FF Oludayo instead of FO Fasina. This was corrected on 17 March 2017 at the request of the author.

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Conflict of interest

None declared.

Authors' contributions

Mohamed Ali, Yilma Jobre and Abebe Wossene designed the study and wrote the article. Mahmoud Shehata, Ahmed El Sayed, Rabeh El-Shesheny, Ahmed Kandeil, Mokhtar Gomaa and Ahmed El-Taweel conducted the laboratory work. Basma Elsokary, Naglaa Hassan, Heba Sobhy and Ihab El Masry managed the field study. Juan Lubroth, Sophie VonDobschuetz, Emma Gardner and Subhash Morzaria funded the study and participated in study design. Gwenaelle Dauphin Fasina Folorunso Oludayo, Peter Daszak and Maureen Miller participated in the manuscript preparation and the analysis of data.

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Letter to the editor: Pre-exposure prophylaxis for HIV in Europe: The need for resistance surveillance

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To the editor: In a recent paper by Hauser et al. in this journal, a prevalence of 10.8% of transmitted drug-resistant viruses was reported among newly diagnosed HIV cases in Germany in 2013 and 2014 [1]. The authors conclude that genotypic resistance testing remains important for treatment as well as HIV prevention. We comment on the use of pre-exposure prophylaxis (PrEP) in relation to drug resistance in HIV infections and the need for European surveillance of drug resistance.

PrEP with tenofovir and emtricitabine prevents new HIV infections in persons at high risk of acquiring HIV [2]. In 2016, the European Commission approved emtricitabine/tenofovir disoproxil once per day for PrEP. France and Norway are the only two countries in Europe fully reimbursing PrEP but many more are considering implementing PrEP pilot projects in 2017 and 2018 [3]. PrEP is cost effective with the current drug prices [4,5] and a generic version of tenofovir and emtricitabine is expected in 2017 or 2018, which may reduce the costs and lead to more widespread use of PrEP in Europe.

PrEP use also poses some challenges as the included drugs are part of the recommended first and second line regimens to treat HIV-infected individuals. The resistance patterns that develop against either drug in a situation of therapy failure are well known: the primary mutation selected by tenofovir that causes a diminished treatment response is the K65R amino acid substitution in the reverse transcriptase. In addition, the presence of multiple thymidine-associated mutations (TAMs) selected by zidovudine, a previously frequently used drug in HIV treatment, can affect the effect of tenofovir on the virus. In individuals failing emtricitabine (or the commonly used lamivudine)-containing regimens, the amino acid changes M184I/V are frequently seen [6]. Viruses with these mutations can be transmitted, resulting in the failure of tenofovir/emtricitabine-based PrEP [7,8].

The use of PrEP by individuals infected with HIV but unaware of this can lead to the generation of resistant viruses in these individuals. Transmission to, or selection in, an HIV-positive person on PrEP carries the risk of forward transmission of these resistant virus to other individuals (both on and off PrEP).

Therefore, we recommend surveillance on national level as well European level. As mentioned by Hauser et al., Germany has a mandatory notification system of new HIV diagnoses, but this is not the case in all European countries [1]. In addition, baseline genotypic resistance testing is not routinely performed in all countries. We recommend the surveillance network *Strategy to Control Spread of HIV Drug Resistance* (SPREAD) to collect these data [9]. SPREAD is organised in 28 countries by the European Society of Antiviral Research (ESAR) and monitors drug-resistant viruses in newly diagnosed individuals [10]. SPREAD can add the use of PrEP in the baseline questionnaire and install a registry within the existing SPREAD database, collecting data on selection of resistant viruses and treatment and/or prophylaxis failure due to PrEP use. In this way, we hope that outbreaks of PrEP-resistant viruses will be identified in a timely manner.

In conclusion, as PrEP for HIV prevention is expected to be rolled out in European countries in the near future, and considering the informal use of PrEP in the community, we suggest including variables on PrEP use in the European surveillance SPREAD programme, increasing the proportion of baseline resistance testing in newly diagnosed HIV infections and installing a registry on the selection of resistant viruses and failure of PrEP within the existing SPREAD database.

Note

Disclaimer: This paper is the view of the authors and not necessarily that of the agencies that they represent.

Conflict of interest

None declared.

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