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Seroepidemiology of Middle East respiratory syndrome (MERS) coronavirus in Saudi Arabia (1993) and Australia (2014) and characterisation of assay specificity

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The pseudoparticle virus neutralisation test (ppNT) and a conventional microneutralisation (MN) assay are specific for detecting antibodies to Middle East respiratory syndrome coronavirus (MERS-CoV) when used in seroepidemiological studies in animals. Genetically diverse MERS-CoV appear antigenically similar in MN tests. We confirm that MERS-CoV was circulating in dromedaries in Saudi Arabia in 1993. Preliminary data suggest that feral Australian dromedaries may be free of MERS-CoV but larger confirmatory studies are needed.

Introduction

Middle East respiratory syndrome (MERS) is an emerging respiratory disease of global public health concern. As of 9 May 2014, 536 confirmed human cases have been reported to the World Health Organization (WHO) with 145 deaths [1]. The current epidemiology of MERS is one of zoonotic transmission, sometimes followed by chains of limited human-to-human transmission for limited periods of time within families or healthcare facilities. This is reminiscent of the emergence of severe acute respiratory syndrome (SARS) in late 2002 [2]. It is therefore critically important to identify the sources of zoonotic transmission, so that evidence-based interventions to minimise such infections can be implemented. Such an approach has for example been used to minimise the human health risk from highly pathogenic avian influenza A(H5N1) and SARS [3,4].

Seroepidemiology is an invaluable tool in such investigations. Many seroepidemiological studies on domestic livestock have reported high MERS seroprevalence in dromedary camels in the Arabian Peninsula and Africa [5-8]. The detection of MERS coronavirus (MERS-CoV) by reverse transcription-polymerase chain reaction (RT-PCR) and virus isolation in such animals supports these seroepidemiological findings and the contention that dromedary camels are a natural host for MERS-CoV [9-11]. But it is not clear if dromedaries are the main source of human infection.

We had previously reported a MERS-CoV pseudoparticle neutralisation test (ppNT) that can be used to detect antibody to MERS-CoV without the need for Biosafety Level-3 (BSL-3) containment that is required for conventional MERS-CoV microneutralisation (MN) tests [6]. In this study, we systematically investigate potential cross-reactions that may confound the use of these two assays in seroepidemiological studies in animals. Sera obtained from dromedary camels in Australia (2014) and different provinces of Saudi Arabia (1993) are included in this study.

Methods

Viruses

MERS-CoV EMC strain was provided by Dr Ron Fouchier, Erasmus Medical Centre, Rotterdam. The virus strains dromedary MERS-CoV Al-Hasa KFU-HKU13 2013 (Al-Hasa 13) and dromedary MERS-CoV Egypt NRCE-HKU270 2013 (Egypt 270) were isolated in our laboratory as previously described [10,12]. The viruses were cultured and titrated in Vero cells (ATCC CCL-81).

Table 1

Cross-neutralisation antibody titres for Middle East respiratory syndrome coronavirus (MERS-CoV) and bovine coronavirus (BCoV) in antisera raised against different coronaviruses

Genus	Antisera – BEI-Resources catalogue number is provided for sera obtained from BEI-Resources	Homologous Ab titre by ELISA unless otherwise specified	MERS-CoV MN titre	MERS-CoV ppNT titre	BCoV MN titre
Alpha-coronavirus	Gnotobiotic pig antiserum to porcine respiratory coronavirus – NR-460	1:1,200[a]	‹1:10	‹1:10	‹1:10
	Guinea pig antiserum to feline infectious peritonitis virus – NR-2518	1:2,000[a]	‹1:10	‹1:10	‹1:10
	Guinea pig antiserum to canine coronavirus – NR-2727	1:4,094[b]	‹1:10	‹1:10	‹1:10
	Gnotobiotic pig antiserum to porcine transmissible gastroenteritis virus – NR-458	1:1,400[a]	‹1:10	‹1:10	‹1:10
Beta-coronavirus	Guinea pig anti-SARS-CoV – NR-10361	1:2,560	‹1:10	‹1:10	‹1:10
	Rabbit antiserum for SARS-CoV S protein (zero titre) – NRC-769	‹1:10	‹1:10	‹1:10	‹1:10
	Rabbit antiserum for SARS-CoV S protein (low titre) – NRC-770	1:80	‹1:10	‹1:10	‹1:10
	Rabbit antiserum for SARS-CoV S protein (medium titre) – NRC-771	1:160	‹1:10	‹1:10	‹1:10
	Rabbit antiserum for SARS-CoV S protein (high titre) – NRC-772	1:640	‹1:10	‹1:10	‹1:10
	Mouse hepatitis virus (JHM strain) hyper-immunised mouse dam 1	1:1,778[c] neutralisation titre	‹1:10	‹1:10	‹1:10
	Mouse hepatitis virus (JHM strain) hyper-immunised mouse dam 2	1:363[c] neutralisation titre	‹1:10	‹1:10	‹1:10
	Mouse hepatitis virus (A59 strain) infected mouse	1:1,000[c] neutralisation titre	‹1:10	‹1:10	‹1:10
	BCoV antisera from guinea pig	1:20,480[b]	‹1:10	‹1:10	1:160
	BCoV antisera from germfree bovine calf – NR-456	1:10,000[a]	‹1:10	‹1:10	1:40
	BCoV antisera from germfree bovine calf	1:580[b] neutralisation titre	‹1:10	‹1:10	1:640
Gamma-coronavirus	Guinea pig antiserum to infectious bronchitis virus – NR-2515	1:50,000[a]	‹1:10	‹1:10	‹1:10

Ab: Antibody; ELISA: enzyme-linked immunosorbent assay; MN: microneutralisation test; ppNT: pseudoparticle neutralisation test; SARS-CoV: severe acute respiratory syndrome coronavirus.

Except if otherwise specified, antibody titres are obtained as part of this study. All homologous antibody titres are ELISA titres except for antisera to mouse hepatitis virus and one BCoV antiserum from germfree bovine calf, which are neutralising antibody titres.

[a] Homologous antibody titre data obtained from BEI-Resources.

[b] Homologous antibody titre data obtained from Linda Saif.

[c] Homologous antibody titre data obtained from Stanly Perlman.

Sera

Immune sera specific for alpha-coronaviruses (porcine respiratory coronavirus, feline infectious peritonitis virus, canine coronavirus and porcine transmissible gastroenteritis virus), beta-coronaviruses (mouse hepatitis virus strains JHM and A59, SARS coronavirus, bovine coronavirus (BCoV)) and gamma-coronavirus (infectious bronchitis virus) were obtained from BEI-Resources (animal CoV reagents supplied to BEI by Dr Linda Saif (http://www.beiresources.org/About/BEIResources.aspx) or generated by Dr Linda Saif or Dr Stanley Perlman, as indicated in Table 1). The homologous antibody titres to the immunising virus were also obtained from the respective sources supplying these antisera (Table 1).

Sera from 25 adult (≥2 year-old) dromedary camels were collected in 2014 in Australia, 17 being from feral camels from central Australia gathered and transported to an abattoir in Caboolture, Queensland, while the other eight sera originated from a camel farm in Coominya, Queensland. Dromedary sera from Egypt were collected from abattoirs in Egypt in 2014. Archived dromedary sera collected in 1993 from Al Hasa, Eastern Province (n=27), As Sulayyil, Ar Riyad province (n=30), Hafar Al-Batin, Eastern Province (n=45) and Medina, Al Medinah province (n=29) were retrieved from the serum archive at the Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, Saudi Arabia. Paired acute and convalescent sera from three dromedary calves (‹2 years-old), which had RT-PCR confirmed MERS-CoV infection in a dromedary farm in Al-Hasa, Saudi Arabia in December 2013 are included in this study. The epidemiological and virological data on these three animals as well as the serological responses to MERS-CoV have been reported previously [12].

Serological tests

The methods for the ppNT and MN neutralisation test for MERS-CoV, and for the MN test for BCoV have been previously reported [6,13]. We used serial two-fold dilutions of heat inactivated (56°C for 30 minutes) sera with an entry dilution of 1:10. Titres of ≥1:40 are reported as positive and those 1:10–1:20 regarded as indeterminate.

Middle East respiratory syndrome coronavirus spike pseudoparticle neutralisation test (ppNT)

A codon optimised spike gene was designed based on MERS-CoV genome sequence (GenBank accession number: JX869059.1), synthesised in Genecust (Luxembourg) and subcloned into pcDNA3.1+ vector to generate pcDNA-S. To produce human immunodeficiency virus (HIV)/MERS spike pseudoparticles, 10 µg pNL Luc E- R- and 10 µg pcDNA-S were co-transfected into 4×10^6 293T cells. Supernatants of transfected cells were harvested 48h later and quantified for HIV p24 viral protein using a p24 enzyme-linked immunosorbent assay (ELISA) Kit (Cell Biolabs, INC, San Diego, CA, USA) [6].

HIV/MERS pseudoparticles containing 5ng HIV p24 was used to infect Vero E6 cells (ATCC CRL-1586) in a single well (96 well plate format; 1×10^4 cells/well). Infected cells were lysed in 20 µl lysis buffer and 100 µl of luciferase substrate at two days post-infection (Promega Corporation, Madison, WI, USA). Luciferase activity was measured in a Microbeta luminometer (PerkinElmer, Waltham, MA, USA). For the ppNT assay, HIV/MERS pseudoparticles (5ng of p24) were pre-incubated with serially diluted sera for 30 min at 4°C and then added to cells in triplicate. Residual virus infection of the cells was assayed at two days post-infection, as described above. The highest serum dilution giving a 90% reduction of luciferase activity was regarded as the ppNT antibody titre.

Microneutralisation (MN) tests

MERS-CoV (strain: EMC) and BCoV (ATCC BRCV-OK-0514-2) were used. Vero cells (ATCC CCL-81) were used for MERS-CoV and HRT-18G cells (obtained from ATCC) for BCoV. Serum dilutions were mixed with equal volumes of 200 tissue culture infective dose $(TCID)_{50}$ of virus and incubated for one hour at 37°C. The virus–serum mixture was then added in quadruplicate to cell monolayers in 96-well microtitre plates. After one hour of adsorption, the virus–serum mixture was removed and 150µl of fresh culture medium was added to each well and the plates incubated at 37°C in 5% CO2 in a humidified incubator. A virus back-titration was performed without immune serum to assess input virus dose. Cytopathic effect (CPE) was read at three days post-infection for MERS-CoV and four days post-infection for BCoV. The highest serum dilution that completely protected the cells from CPE in half of the wells was defined as the neutralising antibody titre. Positive and negative control sera were included in each assay [13].

Table 2

Serological reactions to Middle East respiratory syndrome coronavirus (MERS-CoV) and bovine coronavirus (BCoV) in selected sera collected from dromedary camels in Egypt (2014), Australia (2014) and Saudi Arabia (1993)

Location of serum collection (year)	Serum identity number	MERS-CoV MN	MERS-CoV ppNT	BCoV MN
Egypt (2014)	E2	1:320	1:640	1:40
	E4	1:160	1:320	1:40
	E5	1:40	1:160	1:40
	E6	1:40	1:160	1:40
	E7	1:80	1:320	‹1:10
	E8	1:40	1:80	1:40
	E9	1:320	1:640	1:160
Australia (2014)[a]	A1	‹1:10	‹1:10	‹1:10
	A2	‹1:10	‹1:10	1:160
	A3	‹1:10	‹1:10	1:160
	A4	‹1:10	‹1:10	1:160
	A5	‹1:10	‹1:10	1:320
	A6	‹1:10	‹1:10	1:320
	A13	‹1:10	‹1:10	1:320
	A24	‹1:10	‹1:10	1:160
Saudi Arabia (1993)[b]	S1	1:320	1:1,280	1:80
	S2	1:320	1:2,560	1:40
	S3	1:640	1:1,280	1:160
	S4	›1:1,280	›1:5,120	1:160
	S5	1:40	1:160	‹1:10
	S7	1:80	1:80	‹1:10
	S8	1:640	1:1,280	1:320
	S9	‹1:10	‹1:10	‹1:10

MN: microneutralisation test; ppNT: pseudoparticle neutralisation test.

[a] Results for eight sera selected from 25 are shown.

[b] Results from eight sera selected from 131 are shown.

Results

We tested immune sera to a range of animal alpha-, beta- and gamma- coronaviruses and found no cross-reaction to MERS-CoV in either the MERS-CoV ppNT or MN assays (Table 1). Specifically, we demonstrated that bovine calf and guinea pig immune sera to BCoV do not cross-react in the MERS-CoV ppNT or MN assays.

Of the archived dromedary sera collected in 1993, 26 of 27 sera from Al Hasa, 22 of 30 sera from As Sulayyil, 43 of 45 sera from Hafar Al-Batin and 27 of 29 sera from Medina had detectable (≥1:40) ppNT antibody titres to MERS-CoV, with antibody titres ranging from 1:40 to ≥1:5,120. Data from representative sera are shown in

Table 3

Comparative antibody titres of dromedary camel sera to different isolates of Middle East respiratory syndrome coronavirus (MERS-CoV) and to an isolate of bovine coronavirus (BCoV)

Dromedary camel sera	Reciprocal microneutralisation (MN) antibody titres			
	MERS-CoV			BCoV
	Al-Hasa 13/2013	Egypt 270/2013	EMC/2012	
Calf pre-infection[a]	<1:10	<1:10	<1:10	<1:10
Calf post-infection[a]	1:80	1:40	1:80	<1:10
Adult 1, Saudi Arabia[b]	1:640	1:320	1:320	1:80
Adult 2, Saudi Arabia[b]	1:640	1:640	1:640	1:40
Adult 1, Egypt[b]	1:640	1:320	1:640	<1:10
Adult 2, Egypt[b]	1:640	1:640	1:1,280	1:40

MN: microneutralisation; ppNT: pseudoparticle neutralisation test.

[a] Acute and convalescent serum from a dromedary calf infected with Al-Hasa 13/2013 MERS-CoV (described in reference [12]. Note that titres in reference [12] were ppNT titres and the ppNT assay is more sensitive than MN assays).

[b] Adult sera were selected dromedary camel sera from Saudi Arabia and Egypt known to be seropositive to MERS-CoV.

Table 2. Many, but not all of the MERS-CoV antibody positive sera were also positive for BCoV antibody in MN tests.

Sixteen of the 25 dromedary sera collected in Australia in 2014 had BCoV antibody titres ranging from 1:40 –1:320 but none of them had any antibody reactivity to MERS-CoV in either ppNT or MN assays. Representative results are shown in Table 2.

Comparative MN tests were carried out using a clade B dromedary MERS-CoV isolate from Al Hasa (Al-Hasa 13), a clade A human MERS-CoV isolate from Saudi Arabia (EMC) and a genetically divergent MERS-CoV isolate from Egypt (Egypt 270) using an acute and convalescent serum from the dromedary calf 13 from which Al-Hasa 13 MERS-CoV was isolated [12]. Sera from two other adult dromedaries from Saudi Arabia and two from Egypt were included. All three MERS-CoV were neutralised to comparable titres by the convalescent sera from calf 13 and the four adult dromedaries. The paired sera from calf 13 did not show an antibody response to BCoV (Table 3) and two other calves (numbers 15 and 19 (reported in reference [12]), which seroconverted to MERS-CoV also failed to seroconvert to BCoV (data not shown).

Discussion

Antisera to alpha-, beta- or gamma- coronaviruses (other than MERS-CoV) had high homologous antibody titres but failed to cross-react with MERS-CoV in MN or ppNT tests. Amongst the studied serum panel, the lack of cross-reaction with SARS coronavirus is of note since this virus is phylogenetically more closely related to MERS-CoV.

Many dromedary camel sera have antibodies to both MERS-CoV and BCoV and it is important to establish whether this represents separate infections with the two viruses or serological cross-reactions. Some previous studies have addressed this problem by testing for multiple viruses in parallel and demonstrating some sera with MERS-CoV reactivity in the absence of BCoV (or closely related human coronavirus OC43) reactivity [5,13-16]. In the present study, the lack of MERS-CoV ppNT or MN antibody reactivity in BCoV immune bovine calf or guinea pig sera (Table 1) confirms the specificity of these two serological assays to discriminate between these two viruses. However, dromedaries have unusual single heavy chain immunoglobulins [17] and it is conceivable that these single-chain Ig sera may have unusually broad cross-reactivity, although there is no direct evidence for this hypothesis. The observation that 18 of 25 dromedary sera from Australia have antibodies to BCoV (titres up to 1:320) without any cross-reactivity to MERS-CoV in the ppNT and MN assays is an important confirmation that these assays discriminate between the two viruses in dromedaries as well. Finally, we had three acute and convalescent sera from dromedary calves, which had RT-PCR confirmed MERS-CoV infection and they showed significant (more than four-fold) increases in antibody to MERS-CoV without any change in titre to BCoV. Collectively, these data conclusively demonstrate that ppNT or MN positive antibody titres to MERS-CoV in any animal species are strongly suggestive of MERS-CoV infection. This does not exclude the hypothetical possibility that a hitherto unknown coronavirus more closely related to, but distinct from MERS-CoV, may give cross-reactive antibodies in serosurveillance studies.

Some closely related coronaviruses are antigenically diverse and show limited cross-reactivity in serological assays, as has been reported, for example, for two serotypes of feline coronaviruses [18]. Given that MERS-CoV from different geographical regions (Saudi Arabia and Egypt) are genetically diverse [10], the question arises as to whether the MERS-CoV ppNT and MN assays using one MERS-CoV will detect antibodies to these genetically diverse MERS-CoV viruses. We find that genetically diverse MERS-CoV strains (clade A EMC, clade B Al-Hasa 13 and genetically distant Egypt 270) give comparable MN antibody titres in a dromedary calf seroconverting to Al-Has 13 clade B virus. Similarly adult dromedaries from Saudi Arabia and Egypt each give comparable (within twofold) titres to all three MERS-CoV. The data provide a-priori evidence that a single MERS-CoV isolate is likely to be sufficiently representative for MERS-CoV seroepidemiological studies. It also suggests that genetically diverse MERS-CoV may be antigenically conserved.

Although we have not carried out studies using specific immune sera to exclude cross-reactivity to currently endemic human 229E, OC43, HKU-1 and NL63 coronaviruses, we have so far tested human sera from Egypt and Hong Kong by the MERS-CoV MN tests (n=1,343) and ppNT (n=394) [6,10] with negative results. Since these human coronaviruses are ubiquitous with high seroprevalence in human adults worldwide [19,20], it is very likely that antibodies to 229E, OC43, NL63 and HKU-1 do not cross-react with MERS-CoV in these assays.

Serological evidence of MERS-CoV in dromedaries has been previously reported in archived sera dating back over past decades [7,11,16]. Our data with serological assays that have been demonstrated to be free of cross-reaction with BCoV and other coronaviruses reconfirms that MERS-CoV was circulating in dromedaries in Saudi Arabia as early as 1993.

Although adult dromedaries in the Arabian peninsula and in North and East Africa (e.g. Egypt, Nigeria, Tunisia, Ethiopia, Kenya) have very high seroprevalence to MERS-CoV (>90%) [6,8,21], we found that the sera from adult dromedary camels in Australia were uniformly seronegative. Given the small number of sera tested in this study, a larger seroepidemiological study would be needed to confirm that Australia is indeed MERS-CoV free. On the other hand, the BCoV-like virus so common in the Middle East is also prevalent in Australia. Dromedaries were imported into Australia between 1840 and 1907 to serve as means of transport but are now largely found as feral animals [22]. The dromedary population in Australia is now estimated to be around 450,000 (Al Jassim – data not shown).

We conclude that the MERS-CoV ppNT and MN tests reported here do not detect cross-reactive antibodies to other animal coronaviruses including the BCoV-like virus that is common in dromedaries. Thus these two serological assays can be used with confidence in seroepidemiological studies to identify animal species that may serve as reservoirs or vectors of MERS-CoV. We also confirm that MERS-CoV or a very closely related virus has been circulating in dromedaries in Saudi Arabia for at least two decades.

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Conflict of interest

None declared.

Authors' contributions

MH Hemida, RAM Jassim, G Kayali, MA Ali and A Alnaeem carried out field studies in Saudi Arabia, Australia and Egypt to collect the clinical specimens and epidemiological data. RAPM Perera helped plan the study, carried out the serological testing and analysed the data. P Wang and LY Siu developed the MERS-CoV pseudoparticle neutralisation test. DKW Chu isolated and genetically characterised the viruses used in this study. L Saif and S Perlman generated the coronavirus sera used in this study. Y Guan and LLM Poon contributed advice in study design and data analysis. M Peiris conceived and planned the study, analysed the data and wrote the manuscript. All authors critically reviewed the data and the manuscript.

References

1. World Health Organization (WHO). Middle East respiratory syndrome coronavirus (MERS-CoV) summary and literature update – as of 9 May 2014. Geneva:WHO. [Accessed 30 May 2014]. Available from: http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_Update_09_May_2014.pdf
2. Xu RH, He JF, Evans MR, Peng GW, Field HE, Yu DW, et al. Epidemiologic clues to SARS origin in China. Emerg Infect Dis. 2004;10(6):1030-7. http://dx.doi.org/10.3201/eid1006.030852
3. Sims LD, Peiris M. One health: the Hong Kong experience with avian influenza. Curr Top Microbiol Immunol. 2013;365:281-98. http://dx.doi.org/10.1007/82_2012_254
4. Peiris JSM, Guan Y, Yuen KY. Severe acute respiratory syndrome. Nat Med. 2004;10(12 Suppl):S88-97. http://dx.doi.org/10.1038/nm1143
5. Reusken CB, Haagmans BL, Müller MA, Gutierrez C, Godeke GJ, Meyer B, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. Lancet Infect Dis. 2013;13(10):859-66. http://dx.doi.org/10.1016/S1473-3099(13)70164-6
6. Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, et al. Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. Euro Surveill. 2013;18(36):pii=20574.
7. Alexandersen S, Kobinger GP, Soule G, Wernery U. Middle East respiratory syndrome coronavirus antibody reactors among camels in Dubai, United Arab Emirates, in 2005. Transbound Emerg Dis. 2014;61(2):105-8. http://dx.doi.org/10.1111/tbed.12212
8. Reusken CBEM, Messadi L, Feyisa A, Ularamu H, Godeke GJ, Danmarwa A, et al. Geographic distribution of MERS coronavirus among dromedary camels, Africa. Emerg Infect Dis. 2014. [Epub ahead of print]. http://dx.doi.org/10.3201/eid2007.140590
9. Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis. 2014;14(2):140-5. http://dx.doi.org/10.1016/S1473-3099(13)70690-X
10. Chu DKW, Poon LLM, Gomaa MM, Shehata MM, Perera RA, Zeid DA et al. MERS coronaviruses in dromedary camels, Egypt. Emerg Infect Dis. 2014;20(6):1049-53. http://dx.doi.org/10.3201/eid2006.140299
11. Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, Burbelo PD, et al. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. MBio. 2014;5(2):e00884-14. http://dx.doi.org/10.1128/mBio.01002-14
12. Hemida MG, Chu DKW, Poon LLM, Perera RAPM, Alhammadi MA, Ng H-Y, et al. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis. 2014 Jul. [Epub ahead of print]. http://wwwnc.cdc.gov/eid/article/20/7/14-0571_article.htm
13. Hemida MG, Perera RA, Wang P, Alhammadi MA, Siu LY, Li M, et al. Middle East Respiratory Syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010 to 2013. Euro Surveill. 2013;18(50):pii=20659.
14. Reusken CB, Ababneh M, Raj VS, Meyer B, Eljarah A, Abutarbush S, et al. Middle East Respiratory Syndrome

coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, June to September 2013. Euro Surveill. 2013;18(50):pii=20662.

15. Memish ZA, Cotten M, Meyer B, Watson SJ, Alsahafi AJ, Al Rabeeah AA, et al. Human Infection with MERS Coronavirus after Exposure to Infected Camels, Saudi Arabia, 2013. Emerg Infect Dis. 2014;20(6):1012-5. http://dx.doi.org/10.3201/eid2006.140402
16. Meyer B, Müller MA, Corman VM, Reusken CB, Ritz D, Godeke GJ, et al. Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. Emerg Infect Dis. 2014;20(4):552-9. http://dx.doi.org/10.3201/eid2004.131746
17. Wesolowski J, Alzogaray V, Reyelt J, Unger M, Juarez K, Urrutia M, et al. Single domain antibodies: promising experimental and therapeutic tools in infection and immunity. Med Microbiol Immunol. 2009;198(3):157-74. http://dx.doi.org/10.1007/s00430-009-0116-7
18. Horzinek MC, Lutz H, Pedersen NC. Antigenic relationships among homologous structural polypeptides of porcine, feline, and canine coronaviruses. Infect Immun. 1982;37(3):1148-55.
19. Shao X, Guo X, Esper F, Weibel C, Kahn JS. Seroepidemiology of group I human coronaviruses in children. J Clin Virol. 2007;40(3):207-13. http://dx.doi.org/10.1016/j.jcv.2007.08.007
20. Chan CM, Tse H, Wong SS, Woo PC, Lau SK, Chen L, et al. Examination of seroprevalence of coronavirus HKU1 infection with S protein-based ELISA and neutralization assay against viral spike pseudotyped virus. J Clin Virol. 2009;45(1):54-60. http://dx.doi.org/10.1016/j.jcv.2009.02.011
21. Corman VM, Jores J, Meyer B, Younan M, Liljander A, Said MY, et al. Antibodies against MERS coronavirus in dromedary camels, Kenya, 1992–2013. Emerg Infect Dis. 2014. [Epub ahead of print]. http://dx.doi.org/10.3201/eid2008.140596
22. Australian Government, Department of Sustainability, Environment, Water, Population and Communities. National Feral Camel Action Plan. Camberra: Natural Resource Management Ministerial Council. Available from: http://www.environment.gov.au/system/files/resources/2060c7a8-088f-415d-94c8-5d0d657614e8/files/feral-camel-action-plan.pdf

Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014

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Antibodies to Middle East respiratory syndrome coronavirus (MERS-CoV) were detected in serum and milk collected according to local customs from 33 camels in Qatar, April 2014. At one location, evidence for active virus shedding in nasal secretions and/or faeces was observed for 7/12 camels; viral RNA was detected in milk of five of these seven camels. The presence of MERS-CoV RNA in milk of camels actively shedding the virus warrants measures to prevent putative food-borne transmission of MERS-CoV.

In April 2014, serum, nasal swabs and rectal swabs were taken from 33 milking dromedary camels at two locations in Qatar (Al Shahaniya and Dukhan), areas with known Middle East respiratory syndrome coronavirus (MERS-CoV) circulation in camels [1] and data not shown. In addition, milk was collected from these animals according to local customs. Serum samples and milk were tested for the presence of MERS-CoV-specific antibodies by protein microarray, with confirmation by virus neutralisation. Swabs and milk were tested for the presence of MERS-CoV RNA by real-time reverse transcription (RT)-PCR testing for multiple genomic targets. Antibodies to MERS-CoV were detected in serum and milk from all camels at both locations. At the Dukhan location, none of the 21 animals tested was actively shedding viral RNA from the nose and/or in faeces and no evidence for the presence of MERS-CoV RNA in milk was observed. At the Al Shahaniya location, evidence for active virus shedding was observed for seven of the 12 camels tested. Viral RNA was detected in milk of five of the seven camels with active virus shedding.

Background

In 2012, MERS-CoV was identified in patients with severe respiratory illness in the Middle East [2]. As of 11 June 2014, a total of 683 cases including 204 deaths have been reported to the World Health Organization (WHO) [3]. All cases have had an epidemiological link to the Middle East, with confirmed cases in Iran, Jordan, Kuwait, Lebanon, Oman, Saudi Arabia, Qatar, United Arab Emirates and Yemen. Human-to-human transmission seems limited to family and healthcare settings and is assumed to have contributed to the recent upsurge of cases [4]. Overall, however, a large proportion of cases of MERS-CoV infection is community acquired, with suspected zoonotic transmission, although the extent thereof remains to be determined [5]. Dromedary camels (*Camelus dromedarius*) are the prime suspects to serve as an animal reservoir for MERS-CoV, although alternative sources remain possible [6-11].

In August 2013, dromedary camels were implicated for the first time as a possible source of the virus leading to human infection on the basis of the presence of MERS-CoV neutralising antibodies in dromedaries from Oman and the Canary Islands of Spain [6]. Since then, MERS-CoV-specific antibodies have been detected in camels across the Middle East and in several African countries [7-9]. Analysis of an outbreak of MERS-CoV infection

Table

Middle East respiratory syndrome coronavirus (MERS-CoV) analysis of serum, nasal and rectal swabs and milk of dairy dromedary camels, Al Shahaniya, Qatar, April 2014 (n=12)

Barn number	Camel dam[a] number	Age camel dam (years)	Age calf (months)	Real-time reverse transcription-PCR[b]							Serology			
				Nasal swab	Rectal swab	Whole milk	Milk fat	Skimmed milk	Cell pellet	Milk total[c]	Serum[d]	Milk[d]	Serum[e]	Milk[e]
1	1	8	3	–	–	–	–	–	–	–	+	+	≥1,280	80
	2	7	4	+	–	+	–	–	–	+	+	+	640	Eq.
	3	10	4	–	+	–	–	–	–	–	+	+	≥1,280	80
	4	8	5	+	–	–	–	–	–	–	+	+	≥1,280	Eq.
	5	7	6	+	+	+	–	+	+	+	+	+	≥1,280	40
	6	9	7	+	–	+	–	+	+	+	+	+	≥1,280	40
	7	9	7	+	–	+	–	+	+	+	+	+	≥1,280	40
2	8	10	5	–	+	+	–	+	+	+	+	+	≥1,280	40
	9	8	3	–	–	–	–	–	–	–	+	+	≥1,280	40
3	10	15	8	–	–	–	–	–	–	–	+	+	≥1,280	NT
	11	12	5	–	–	–	–	–	–	–	+	+	≥1,280	20
	12	10	7	–	–	–	–	–	–	–	+	+	≥1,280	80
Total number positive	NA	NA	NA	5	3	5	0	4	4	5	12	12	12	9

Eq.: equivocal (titre between ≥5 and <20); NA: not applicable; NT: not tested due to lack of sample.
A dash represents that the test was negative.

[a] A dam is the femal parent of a livestock animal.
[b] A sample is considered PCR positive for MERS-CoV when >2 targets (*UpE*, *Orf1a* and/or *N*) are reactive.
[c] Summary results of whole milk, milk fat, skimmed milk and cell pellet.
[d] Serology based on MERS-CoV S1 protein-microarray. Cut-off value 4,000 relative mean fluorescent intensity.
[e] Serology based on MERS-CoV neutralisation assay. Starting dilution 1:5. Neutralising antibody titres are shown.

in humans associated with a barn in Qatar in October 2013 found dromedaries and humans to be infected with nearly identical strains of MERS-CoV [1] and the virus was isolated from dromedaries shortly after [10]. Further support for camels as a reservoir came from a study in Saudi Arabia that found widespread circulation of different genetic variants of MERS-CoV in camels, and antibodies in samples taken since the early 90s [11].

Although camels are suspected to be the primary source of MERS-CoV leading to human infection, the routes of direct or indirect zoonotic transmission remain unknown. A possible route might be food-borne transmission through consumption of raw camel milk or undercooked meat. Here we report on our investigations into virus shedding of milking camels, in relation to the presence of MERS-CoV RNA in milk, as a first assessment of a potential role of consumption of raw camel milk in MERS-CoV transmission.

Analysis of dromedary serum, milk, nasal and rectal swabs

Sample collection

In April 2014, serum, nasal swabs, rectal swabs and milk were collected from 12 dromedary camels in three barns at the Al Shahaniya barn complex and 21 dromedary camels from a milking herd in the Dukhan area, Qatar. The milking camels at the barns at Al Shahaniya were kept together with racing camels that have regular contact with camels outside the barn at practice and racing events. Barn 1 held 22 racing and nine milking camels. Barn 2 held 18 racing and four milking camels, while Barn 3 held 15 racing and three milking camels. Each milking camel (dam) had their calf present. The age range of the calves was three to eight months (Table).

The herd in the Dukhan area was in a secluded area far from other animals. The age range of the calves was three to seven months. Both locations had known circulation of MERS-CoV in dromedaries at the end of 2013/beginning of 2014 [1] and data not shown.

Figure

Milking camels according to local customs, Al Shahaniya barn complex, Qatar, April 2014

Milk production is triggered by the calf: the calf is then set aside and the milk is collected.
Photographs by E. Farag.

No samples were collected from the calves. Serum and swabs from the dams were collected wearing a disposable gown, gloves, goggles and FFP2 mask, as described [1]. Milk was collected according to local customs as follows: dromedary calves were not weaned after delivery but kept at the farm in paddocks adjacent to their dams throughout lactation. Dams were reunited with their calf to trigger milk production. Once milk production was initiated, the milk samples were collected by the camel owner or handler according to regional customs. No specific hygienic precautions were taken (Figure). All samples were stored at −80°C until shipment to the Netherlands on dry ice. All sera and swabs were shipped in agreement with Dutch import regulations for animal samples from foot-and-mouth disease-endemic regions and stored and handled in a biosafety level 3 laboratory until inactivation by incubation for 4 hours at 56 °C or addition of lysis buffer, respectively.

Sample testing

Total nucleic acids from swabs were isolated using an automated MagNAPure 96 extraction with the total nucleic acid isolation kit (Roche, Mannheim, Germany). Swabs were tested for MERS-CoV RNA by internally controlled real-time RT-PCR targeting *UpE* and *N* genes, as described [1,12]. Initial observations of reduced nucleic acid recovery when whole milk was extracted using routine protocols for clinical samples triggered us to test milk fractions, besides whole milk, for putative increase of sensitivity [13,14]. Total RNA was manually extracted from whole milk, skimmed milk, cellular pellet and cream components of milk samples using the High Pure RNA isolation kit (Roche, Mannheim, Germany). Extracts of whole milk and milk fractions were tested for MERS-CoV RNA by internally controlled real-time RT-PCR targeting *Orf1A* and *UpE* genes, as described [1, 12]. According to international consensus, samples were considered positive for MERS-CoV RNA when at least two different targets were reactive [15].

At Al Shahaniya, seven of the 12 camels tested were actively shedding viral RNA from the nose (n=5) and/or faeces (n=3) with threshold cycle (Ct) values ranging between 23.0 and 29.7. Overall, milk obtained from five of the seven virus-shedding animals demonstrated presence of MERS-CoV RNA (Table) with Ct values ranging from 29.2 to 37.9. Sequence analysis of the PCR products from the milk fraction with the highest viral load confirmed the presence of MERS-CoV (data not shown).

At the Dukhan location, none of the 21 animals tested was actively shedding viral RNA and no evidence for the presence of MERS-CoV RNA in milk was obtained (data not shown). Milk fractions of bulk milk collected from dairy dromedaries in the Netherlands tested negative for MERS-CoV RNA (data not shown).

Serum and milk samples were tested for the presence of IgG antibodies reacting with MERS-CoV (residues 1–747), severe acute respiratory syndrome (SARS)-CoV (residues 1–676) and human coronavirus (HCoV)-OC43 (residues 1–760) spike domain S1 antigens using extensively validated protein-microarray technology, as described [6,16-18]. HCoV-OC43 S1 was used as proxy for bovine CoV (BCoV), which is known to circulate commonly in dromedaries [19,20]. All serum and milk samples from Al Shahaniya and the Dukhan location had MERS-CoV S1 binding antibodies (Table and data not shown).

Confirmation of array results from Al Shahaniya was done by MERS-CoV neutralisation assays, as described [6]. Neutralising antibody titres varied between 640 and ≥1,280 for serum and between 10 and 80 for milk with 9 out of 11 having titres fourfold above the starting dilution of 1:5 (Table). Control serum (n=3) and bulk milk collected from dairy dromedaries in the Netherlands were negative (data not shown). All serum and milk samples from both locations in Qatar and the Netherlands reacted with HCoV-OC43 S1 confirming common circulation of BCoV in camelids. All samples tested negative for SARS-CoV (data not shown).

To gain insight into possible faecal contamination of the milk samples, the samples were analysed for the presence of *Escherichia coli* by a quantitative PCR based on the *E. coli uidA* gene, with a limit of quantification of $<10^3$ genome copies per ml [21]. The presence of *E. coli* was not consistently detected in repeated testing (data not shown).

Discussion

Raw milk from dromedaries has been consumed by humans for thousands of years and is thought to have healing properties when consumed 'hot', directly out of the udder [22]. Nowadays, dromedaries are still an important source of milk in rural areas of arid countries such as Qatar and other countries in the Middle East and parts of Africa [23]. Food-borne transmission is a putative route of zoonotic transmission of MERS-CoV that needs further investigation. Recent data demonstrated that MERS-CoV experimentally introduced into camel milk can survive for up to 72 hours at 4 °C and 22 °C and it has been suggested that consumption of MERS-CoV-containing milk might result in introduction of the virus into the oral cavity and subsequent infection of the lower respiratory tract [24].

Here, we detected the presence of MERS-CoV RNA in five milk samples collected from seven animals shedding MERS-CoV from the nose and/or feces at Al Shahaniya. Although shedding of infectious virus in ruminant milk and infection of humans due to the consumption of raw milk have been described for several viruses [25,26], it cannot be concluded from our data that this holds true for MERS-CoV as well. The milk samples were collected according to local customs in which camel udders are not normally cleaned before milking and hygienic conditions are such that udders and milk can be contaminated with nasal secretions or faeces from the camel, saliva of the calves, which are allowed to suckle prior to milking to initiate the milk flow, or dirt from the bowl or the hands of the milker. Additional studies under controlled hygienic conditions are ongoing to determine whether MERS-CoV replicates in the udder or could be introduced as contaminant during the milking process.

It remains to be seen if the results reflect the presence of infectious virus in the milk samples. The RNA loads in the milk samples were too low to attempt virus isolation; we have observed that samples containing MERS-CoV RNA with Ct values >30 in general do not contain infectious virus particles. Experiments aiming at determining the amount of infectious virus present in milk samples such as those collected in our study should be conducted locally, avoiding detrimental effects of shipment and freeze-thaw cycles on virus viability. In addition, the presence of substantial levels of MERS-CoV neutralising antibodies in the milk samples might neutralise any infectious virus present during in vitro testing, which may differ from the in vivo situation, particularly if the virus is resistant to gastric juice and passage of infectious virus through the stomach occurs [27]. Nevertheless, it can be concluded that the presence of MERS-CoV RNA in raw milk as consumed locally might represent a source for zoonotic transmission of MERS-CoV and prudence is called for. Munster et al. showed that heat treatment (30 minutes at 63 °C) of MERS-CoV-containing camel milk reduced levels of infectious virus below detection level [24]. Boiling milk before consumption could be an easy, achievable local measure to prevent transmission and to preserve consumption of camel milk.

An interesting observation is the difference in virus shedding between the herds at Al Shahaniya and Dukhan (7/12 and 0/21, respectively) although virus circulation had been detected in the Dukhan location earlier (data not shown). While the current study provides only a snapshot, it suggests that herd management practices may influence virus circulation. In addition, the nasal and/or faecal shedding of MERS-CoV by animals with high levels of neutralising antibodies suggests that the presence of antibodies does not confer sterilising immunity.

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Conflict of interest

None declared.

Authors' contributions

CR: coordination of the study in the Netherlands, assisted in designing the study, analysed data, wrote manuscript.

EF: coordination of the study in Qatar, assisted in designing the study, read and revised manuscript. MJ: protocol development, performed laboratory testing, analysed data, read and revised manuscript. GJG: performed laboratory testing, analysed data, read and revised manuscript. AES: field work Qatar, read and revised manuscript. SP: performed laboratory testing, analysed data, read and revised manuscript. VSR: performed laboratory testing, analysed data, read and revised manuscript. KM: field work Qatar, read and revised manuscript. HAM: performed laboratory testing, analysed data, read and revised manuscript. HG: read and revised manuscript. FAH: read and revised manuscript. AI: field work Qatar, read and revised manuscript. BJB: design antigen production, provided antigens, read and revised the manuscript. HAR: read and revised manuscript. SKP: read and revised manuscript. MAT: read and revised manuscript. SAM: read and revised manuscript. MAH: overall coordination collaboration Qatar-the Netherlands, assisted in designing the study, read and revised manuscript. BH: data analysis, assisted in designing the study, read and revised manuscript. MK: overall coordination collaboration Qatar-the Netherlands; assisted in designing the study, data analysis, read and revised manuscript.

References

1. Haagmans BL, Al Dhahiry SH, Reusken CB, Raj VS, Galiano M, Myers R, et al. Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. Lancet Infect Dis. 2014;14(2):140-5. http://dx.doi.org/10.1016/S1473-3099(13)70690-X
2. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-20. http://dx.doi.org/10.1056/NEJMoa1211721
3. World Health Organization (WHO). Middle East respiratory syndrome coronavirus (MERS-CoV) – update. Geneva: WHO; 2014. [Accessed 12 Jun 2014]. Available from: http://www.who.int/csr/don/2014_06_11_mers/en/
4. European Centre for Disease Prevention and Contro (ECDC). Severe respiratory disease associated with Middle East respiratory syndrome coronavirus (MERS-CoV) – tenth update, 31 May 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/RRA-Middle-East-respiratory-syndrome-coronavirus-update10.pdf
5. World Health Organization (WHO). Middle East respiratory syndrome coronavirus (MERS-CoV). summary and literature update–as of 9 May 2014. Geneva: WHO; 2014. [Accessed 29 May 2014]. Available from: http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_Update_09_May_2014.pdf
6. Reusken CB, Haagmans BL, Müller MA, Gutierrez C, Godeke GJ, Meyer B, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. Lancet Infect Dis. 2013;13(10):859-66. http://dx.doi.org/10.1016/S1473-3099(13)70164-6
7. Reusken CBEM, Messasi L, Feyisa A, Ularamu H, Godeke GJ, Danmarwa A, et al. Geographic distribution of MERS Coronavirus among dromedary camels, Africa. Emerg Infect Dis. 2014;20(8). Forthcoming. http://dx.doi.org/10.3201/eid2008.140590
8. Corman VM, Jores J, Meyer B, Younan M, Liljander A, Said M, et al. MERS coronavirus anibodies in dromedary camels from Kenya, 1992-2013. Emerg Infect Dis. 2014;20(8). Forthcoming. http://dx.doi.org/10.3201/eid2008.140596
9. Hemida MG, Perera RA, Wang P, Alhammadi MA, Siu LY, Li M, et al. Middle East Respiratory Syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010 to 2013. Euro Surveill. 2013;18(50):pii=20659.
10. Raj VS, Farag E, Reusken CB, Lamers MM, Pas SD, Voermans J, et al. Isolation of MERS coronavirus from a dromedary camel, Qatar, 2014 Emerg Infect Dis. 2014;20(8). Forthcoming. http://dx.doi.org/10.3201/eid2008.140663
11. Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, de Wit E, et al. Middle East respiratory syndrome coronavirus infection in dromedary camels in saudi arabia. MBio. 2014;5(2):e00884-14. http://dx.doi.org/10.1128/mBio.01002-14
12. Corman VM, Eckerle I, Bleicker T, Zaki A, Landt O, Eschbach-Bludau M, et al. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Euro Surveill. 2012;17(39):pii=20285.
13. Lemay DG, Ballard OA, Hughes MA, Morrow AL, Horseman ND, Nommsen-Rivers LA. RNA sequencing of the human milk fat layer transcriptome reveals distinct gene expression profiles at three stages of lactation. PLoS One. 2013;8(7):e67531. http://dx.doi.org/10.1371/journal.pone.0067531
14. Blackwell JH, Hyde JL. Effect of heat on foot-and-mouth disease virus (FMDV) in the components of milk from FMDV-infected cows. J Hyg (Lond). 1976;77(1):77-83. http://dx.doi.org/10.1017/S0022172400055534
15. World Health Organization (WHO). Revised interim case definition for reporting to WHO – Middle East respiratory syndrome coronavirus (MERS-CoV). Geneva: WHO; 3 Jul 2013. [Accessed 12 Jun 2014]. Available from: http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/
16. Reusken CB, Ababneh M, Raj VS, Meyer B, Eljarah A, Abutarbush S, et al. Middle East Respiratory Syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, June to September 2013. Euro Surveill. 2013;18(50):pii=20662.
17. Meyer B, Müller MA, Corman VM, Reusken CB, Ritz D, Godeke GJ, et al. Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. Emerg Infect Dis. 2014;20(4):552-9. http://dx.doi.org/10.3201/eid2004.131746
18. Reusken C, Mou H, Godeke GJ, van der Hoek L, Meyer B, Muller MA, et al. Specific serology for emerging human coronaviruses by protein microarray. Euro Surveill. 2013;18(14):pii=20441.
19. Woo PC, Lau SK, Wernery U, Wong EY, Tsang AK, Johnson B, et al. Novel betacoronavirus in dromedaries of the Middle East. Emerg Infect Dis. 2014;20(4):560-72. http://dx.doi.org/10.3201/eid2004.131769
20. Wünschmann A, Frank R, Pomeroy K, Kapil S. Enteric coronavirus infection in a juvenile dromedary (Camelus dromedarius). J Vet Diagn Invest. 2002;14(5):441-4. http://dx.doi.org/10.1177/104063870201400518
21. Frahm E, Obst U. Application of the fluorogenic probe technique (TaqMan PCR) to the detection of Enterococcus spp. and Escherichia coli in water samples. J Microbiol Methods. 2003;52(1):123-31. http://dx.doi.org/10.1016/S0167-7012(02)00150-1
22. Yagil R. Camels and camel milk. Animal Production and Health Paper 26. Rome, Food and Agriculture Organization of the United Nations; 1982. Available from: http://www.fao.org/docrep/003/x6528e/x6528e00.HTM
23. Food and Agriculture Organization of the United Nations (FAO). FAOSTAT 2012. Rome: FAO. [Accessed 4 Aug 2013]. Available from: http://faostat3.fao.org/faostat-gateway/go/to/home/E
24. van Doremalen N, Bushmaker T, Karesh WB, Munster VJ. Stability of Middle East respiratoy syndrome coronavirus in milk. Emerg Infect Dis. 2014;20(7). http://dx.doi.org/10.3201/eid2007.140500
25. Donaldson AI. Risks of spreading foot and mouth disease through milk and dairy products. Rev Sci Tech. 1997;16(1):117-24.
26. Dörrbecker B, Dobler G, Spiegel M, Hufert FT. Tick-borne encephalitis virus and the immune response of the mammalian host. Travel Med Infect Dis. 2010;8(4):213-22. http://dx.doi.org/10.1016/j.tmaid.2010.05.010
27. Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. Antiviral Res. 2003;57(1-2):129-46. http://dx.doi.org/10.1016/S0166-3542(02)00206-1

Assessment of the Middle East respiratory syndrome coronavirus (MERS-CoV) epidemic in the Middle East and risk of international spread using a novel maximum likelihood analysis approach

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The emergence of the novel Middle East (ME) respiratory syndrome coronavirus (MERS-CoV) has raised global public health concerns regarding the current situation and its future evolution. Here we propose an integrative maximum likelihood analysis of both cluster data in the ME and importations in a set of European countries to assess the transmission scenario and incidence of sporadic infections. Our approach is based on a spatial-transmission model integrating mobility data worldwide and allows for variations in the zoonotic/environmental transmission and under-ascertainment. Maximum likelihood estimates for the ME, considering outbreak data up to 31 August 2013, indicate the occurrence of a subcritical epidemic with a reproductive number *R* of 0.50 (95% confidence interval (CI): 0.30–0.77) associated with a daily rate of sporadic introductions p_{sp} of 0.28 (95% CI: 0.12–0.85). Infections in the ME appear to be mainly dominated by zoonotic/environmental transmissions, with possible under-ascertainment (ratio of estimated to observed (0.116) sporadic cases equal to 2.41, 95% CI: 1.03–7.32). No time evolution of the situation emerges. Analyses of flight passenger data from ME countries indicate areas at high risk of importation. While dismissing an immediate threat for global health security, this analysis provides a baseline scenario for future reference and updates, suggests reinforced surveillance to limit under-ascertainment, and calls for alertness in high importation risk areas worldwide.

Introduction

As of 31 August 2013, a total of 108 laboratory-confirmed cases of human infection with the Middle East respiratory syndrome coronavirus (MERS-CoV) have been reported to the World Health Organization (WHO) [1]. Since the first identification of the virus in 2012 [2], a rapid coordinated response has been put in place to confront the novel emerging epidemic. This response consisted in the enhancement of surveillance systems, the provision of updated information on the epidemic situation, technical guidance for the clinical management of probable infections [3-7], and the search for the possible virus reservoir [8,9]. There are still many uncertainties about various aspects of the outbreak, including its full geographical extent, a possible extension of an initial virus reservoir to other hosts, the transmission path of the infection to humans and the associated risk. All these aspects call for heightened surveillance, enhanced investigations and the development and application of epidemiological methods to assess the epidemic situation and determine the potential of the virus to spread in humans and to circulate at a global scale.

In such a situation, statistical, mathematical and computational methods allow estimating key epidemiological parameters from available data, under various assumptions and accounting for the many uncertainties. The reproductive number *R*, i.e. the average number of secondary cases generated by a primary case, is a key summary measure of the transmissibility of an emerging infection. A first estimation of the MERS-CoV reproductive number was based on the analysis of cluster-size data with assumed cluster partition in terms of transmission trees, highlighting the similarity of the current MERS-CoV situation to the pre-epidemic stage of the severe acute respiratory syndrome (SARS) outbreak [10]. Aside from the

Table 1

Number of clusters of a given size, depending on the total number of cases of Middle East respiratory syndrome coronavirus considered

Size of cluster (number of cases)	Number of clusters[a]			
	Baseline (all laboratory-confirmed cases in the ME region[b] as of 31 August 2013)	All laboratory-confirmed cases in the ME region[b] as of 31 August 2013, and the probable cases in the Jordan April 2012 outbreak	All laboratory-confirmed cases worldwide as of 31 August 2013	All laboratory confirmed-cases in ME region[b] as of 31 May 2013
1[c]	42	42	44	16
2	8	7	10	2
3	2	2	4	1
5	2	2	2	0
10	0	1	0	0
22	1	1	1	1
Total number of cases	96	104	108	45

ME: Middle East.

[a] Unless otherwise specified.
[b] Including Jordan, Qatar, Saudi Arabia, and United Arab Emirates.
[c] Sporadic case.

transmissibility, an additional important characteristic of the epidemic remains unknown i.e. the incidence of infection. Observed cases may indeed only represent a proportion of the current epidemic, with a majority of infections going undetected because of mild illness or asymptomatic infection. This aspect also has further relevant implications for the correct estimation of other important overall statistics (e.g. the severity of the disease) and of the risk of importation of cases from affected areas to other locations of the world. Limited data may also hide important changes in the virus transmissibility related, for example, to viral adaptations to humans that may alter its pandemic potential, thus presenting an additional challenge for the assessment of the epidemic situation.

To fill the gaps in current knowledge, we present here an innovative integrative maximum likelihood approach to describe the epidemic in the Middle East (ME) region, comprising Jordan, Qatar, Saudi Arabia and the United Arab Emirates. We synthesise evidence from multiple sources of information: sizes of clusters of cases, traffic data, and imported cases outside the region. The methods used account for the limited information available and reporting inaccuracies. Our aim is to complete early findings on the MERS-CoV epidemic by focusing on the virus transmissibility from human-to-human, its possible changes in time, and the expected number of cases in the ME region. Based on international travel flows, the public health threat for other geographical locations not yet affected by the virus is also assessed.

Methods

Analytic overview

The integrative approach we use is based on a combined maximum likelihood analysis to jointly estimate the reproductive number R and the daily rate p_{sp} of sporadic introduction of the virus in the population through zoonotic/environmental transmissions [11]. The integrative approach builds on two aspects of the currently reported outbreak – the distribution of cluster sizes, providing information on R (Method 1), and the number of imported cases in countries out of the source region providing information on R and p_{sp} based on the fit of a stochastic spatial metapopulation model integrating aviation data worldwide (Method 2).

Method 1

We considered laboratory-confirmed cases reported to WHO as of 31 August 2013 in the ME region including Jordan, Qatar, Saudi Arabia, and United Arab Emirates [1] (Table 1). This region is also referred to as the source region in this study. The cases were distributed into clusters according to information found in WHO reports. Using this dataset as baseline data, we estimated the reproductive number R using the cluster sizes distribution. Several distributions can be used that correspond to different hypotheses regarding the number of secondary cases distribution (offspring distribution) [12]. In particular we considered a Poisson offspring distribution accounting for no overdispersion around a common mean [13] and a geometric offspring distribution assuming a constant rate of transmission during an exponentially distributed infectious period [14].

As cluster sizes may be biased downwards by incomplete observation, we allowed for uncertainty by assuming that each case in a cluster would go unobserved with probability p_{cl} during investigation (p_{cl} =0 representing no missed cases). This corresponds to the following distribution for reported cluster sizes:

$$P(O = k|R, p_{cl}, O \geq 1) = \frac{\sum_{j \geq k} P(S=j|R)\binom{j}{k} p_{cl}^{j-k}(1-p_{cl})^k}{1-P(O=0|R,p_{cl})}, \quad (1)$$

where O is the observed size of the cluster, S its real size

$$(O \leq S), P(O = 0|R, p_{cl}) = \sum_{j \geq 1} P(S = j|R) p_{cl}^{j}, \text{ and } P(S|R)$$

is the offspring distribution discussed above. Eventually, the likelihood was computed as

$$\mathcal{L}_1(R, p_{cl}; \{o_i\}) = \left(\sum_i o_i\right)! \prod_i \frac{1}{o_i!} P(O_i = o_i|R, p_{cl}, O \geq 1)^{o_i}$$

over a bi-dimensional grid of {R, p_{cl} } values.

We performed a sensitivity analysis by considering: (i) the addition, to the baseline data, of the complete Jordan cluster including eight more cases (cluster size=10, Table 1) identified through a retrospective serology study carried out on 124 individuals [4,15]; (ii) all laboratory-confirmed cases (n=108) reported worldwide to WHO as of 31 August 2013 [1]; (iii) laboratory-confirmed cases in the ME region up to 31 May, 2013 [1]. The corresponding cluster size data are reported in Table 1.

Method 2

Due to a large concern around the ongoing outbreak and enhanced surveillance following the WHO guidelines for patients returning from the affected area, the detection of probable cases imported in countries out of the ME region is expected to be more complete than in the region itself where primary cases may have gone undetected. Another source of information to estimate the reproductive number R, discounting possible notification/surveillance biases in the source region, is therefore provided by the importation of cases in newly affected countries [16,17]. As a basis for our estimation model, we use a method already employed for the estimation of the seasonal transmission potential of the 2009 influenza A(H1N1)pdm09 virus, which was based on the calibration of a global epidemic and mobility model (GLEAM) [18,19] to the chronology data of the 2009 pandemic [17]. We modified and extended this method by accounting for the different transmission scenarios in the ME region. The method concurrently allows the estimation of the incidence of infection from sporadic cases in the region, and therefore provides a measure of possible under-ascertainment of cases.

GLEAM is based on a spatially structured metapopulation approach comprising 3,362 subpopulations in 220 countries in the world coupled through mobility connections. The model is informed with high-resolution demographic data for six billion individuals and multiscale mobility data including the full air traffic database from the International Air Transport Association (IATA) and short-range ground mobility obtained from national commuting data [19]. The infection dynamics takes place within each subpopulation and assumes a modified susceptible, exposed, infectious, recovered individuals (SEIR) compartmentalisation [20] to account for different transmission scenarios in the ME region [11]: (*i*) introduction of sporadic infections from zoonotic/environmental transmission with a daily rate p_{sp} and a uniform spatial distribution in the source region; (*ii*) modified human-to-human transmissibility with respect to standard homogeneous mixing in all subpopulations of the model to allow for large variations in the number of secondary cases produced by a given primary case. Epidemiological parameters for the compartmental model were based on the estimates obtained from the analysis of the outbreak data including 22 cases at a healthcare facility in Al-Ahsa in Saudi Arabia [6], namely average latency period of 5.2 days and generation time of 7.6 days.

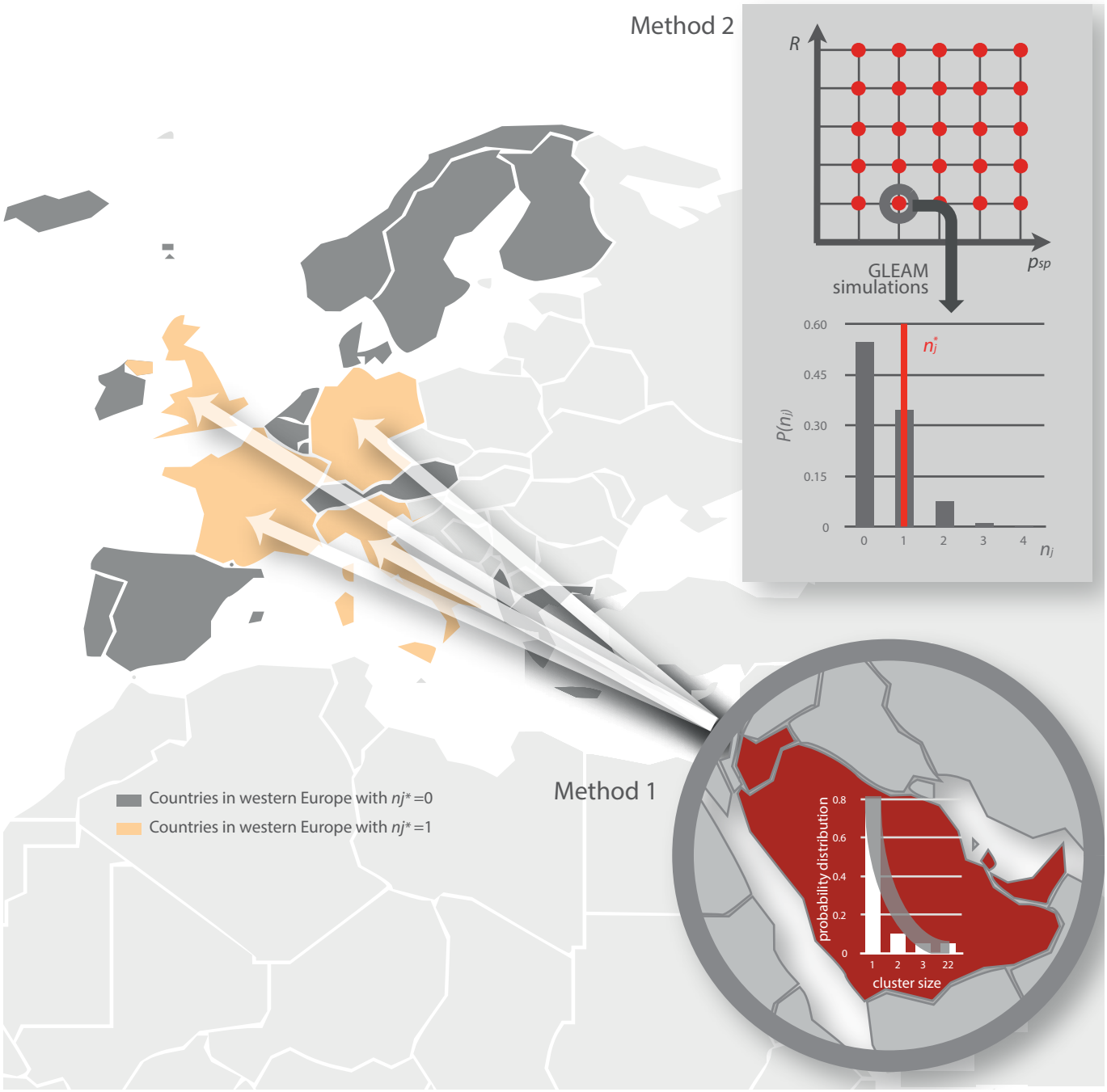
The daily rate p_{sp} of sporadic cases emergence in the ME region and the reproductive number R are the free parameters of the model. For each set of values of these two parameters, GLEAM allows the generation of stochastic numerical realisations of the MERS-CoV outbreak simulating the local epidemic in the source region and the possibility of international dissemination through mobility processes entirely based on real data. We thus generate with a Monte Carlo procedure the probability distribution $P_i(n_i)$ of the number n_i of imported MERS-CoV cases in country i out of the source region as of 31 August 2013 (4×10^3 stochastic realisations for each point (R, p_{sp}) of the space of parameters). Being all independent importation events, we can define a likelihood function

$$\mathcal{L}_2(R, p_{sp}; \{n_j^*\}) = \prod_j P_j(n_j^*)$$

where n_j* is the empirically observed number of imported cases per country (see schematic example in Figure 1). We further restrict our analysis to certain western European countries including Austria, Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Italy, Liechtenstein, Luxemburg, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, and the United Kingdom, to focus on an area where respiratory diseases surveillance is homogeneous and with a high sensitivity to detect importations. We disregard other possible sources of heterogeneity. These data consisted therefore in: n_j*=1 for France, Germany, Italy and the United Kingdom, and n_j*=0 for all other western European countries j considered in the analysis (Figure 1). We estimated the log-likelihood over a bi-dimensional grid of (R, p_{sp}) values and used bivariate linear interpolation over a refined grid.

Figure 1

Maximum likelihood approach integrating two methods, to estimate the daily rate of sporadic cases of Middle East respiratory syndrome coronavirus infection and the reproductive number



GLEAM: global epidemic and mobility model; p_{sp}: daily rate of sporadic introduction of the virus in the population through zoonotic/environmental transmissions; R: reproductive number.

In this maximum likelihood approach, the source region used comprises Jordan, Qatar, Saudi Arabia, and United Arab Emirates.

Method 1 (bottom circle) is based on the maximum likelihood analysis of cluster size distribution obtained from laboratory-confirmed cases in the source region (countries in red in the zoomed area).

Method 2 (top panel) is based on the maximum likelihood analysis on data on case importations in certain western European countries, as schematically indicated on the map (countries included are: Austria, Belgium, Denmark , Finland, France, Germany, Iceland, Ireland, Italy, Liechtenstein, Luxemburg, the Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, and the United Kingdom). For each point in the parameter space (R, p_{sp}) we run 4,000 stochastic GLEAM simulations from the same initial conditions and parameterised as described in the main text. With each run providing the simulated number of imported cases n_j for a given country j, we can compare the resulting simulated probability distribution of n_j with the observed value n_j* for that country as of 31 August 2013, and compute a likelihood function for all western European countries included in the analysis.

Table 2

Best estimate values for the reproductive number R and the daily rate p_{sp} of emergence of sporadic cases of Middle East respiratory syndrome coronavirus due to zoonotic or environmental transmissions

Analysis	Data	R (95% CI)	p_{sp} (95% CI)
Baseline	Baseline data: all laboratory-confirmed cases in the ME region[a] up to 31 Aug 2013	0.50 (0.30–0.77)	0.28 (0.12–0.85)
Sensitivity	All laboratory-confirmed cases in the ME region[b] as of 31 Aug 2013, and the probable cases in the Jordan Apr 2012 outbreak	0.65 (0.34–0.80)	0.28 (0.12–0.83)
	All laboratory-confirmed cases reported worldwide to WHO as of 31 Aug 2013	0.50 (0.31–0.77)	0.28 (0.12–0.85)
	All laboratory-confirmed cases reported in an extended source region including ME region and neighbouring countries[b]	0.50 (0.31–0.76)	0.14 (0.05–0.38)[c]
	Data considering a restricted source region limited to Saudi Arabia	0.60 (0.30–0.76)	4.73 (2.32–15.37)[c]
	Data considering all laboratory-confirmed cases in ME region[a] as of 31 May 2013	0.54 (0.34–0.90)	0.43 (0.12–0.95)
Alternative baseline scenario	Baseline data considering a Poisson offspring distribution	0.69 (0.34–0.79)	0.28 (0.12–0.71)

CI: confidence interval; ME: Middle East; WHO: World Health Organization.

Results for the baseline and for the scenarios of the sensitivity analysis are obtained assuming a geometric offspring distribution for the analysis of cluster data and for the best estimate of the uncertainty parameter, p_{cl}. The last row of the Table refers to the baseline scenario considering a Poisson offspring distribution for the analysis of cluster data.

[a] Jordan, Qatar, Saudi Arabia and the United Arab Emirates.

[b] Bahrain, Iraq, Iran, Israel, Kuwait, Lebanon, Oman, Palestine, Syria, and Yemen in addition to the ME region.

[c] To be comparable with the other estimates, this value has been rescaled to take into account the change of population size of the source region; it thus represents the daily rate of sporadic cases scaled to the ME region.

Given the unknown geographical extension of the source of the MERS-CoV infection in the ME and its reservoir, we performed a sensitivity analysis by considering: (i) an extended definition of source region to neighbouring countries, thus additionally including Bahrain, Iraq, Iran, Israel, Kuwait, Lebanon, Oman, Palestine, Syria, and Yemen (extended source region), based on travel recommendations [21]; (ii) a restricted source region localised only in Saudi Arabia, i.e. the country in the ME that reported the largest number of cases; (iii) a variation in the time of the initial emergence of the virus with transmission to humans, assuming that sporadic cases may be introduced up to two months before the known initial cases (Jordan cluster, April 2012 [4]), to allow for lack of identification or detection prior to the Jordan cluster.

Integrative approach combining Methods 1 and 2

Methods 1 and 2 were jointly combined in the following integrated likelihood function:

$$\mathcal{L}(R, p_{sp}, p_{cl} | \{o_i\}, \{n_j^*\}) = \mathcal{L}_1(R, p_{cl} | \{o_i\}) \mathcal{L}_2(R, p_{sp} | \{n_j^*\})$$

owing to the independence of the two observed processes (cluster sizes and importations). Maximum likelihood estimates were computed over the three-dimensional (3D) grid (R, p_{sp}, p_{cl}) and the deviance

$$D(R, p_{sp}) = -2\left(\log \mathcal{L}(R, p_{sp}) - \max(\log \mathcal{L})\right)$$

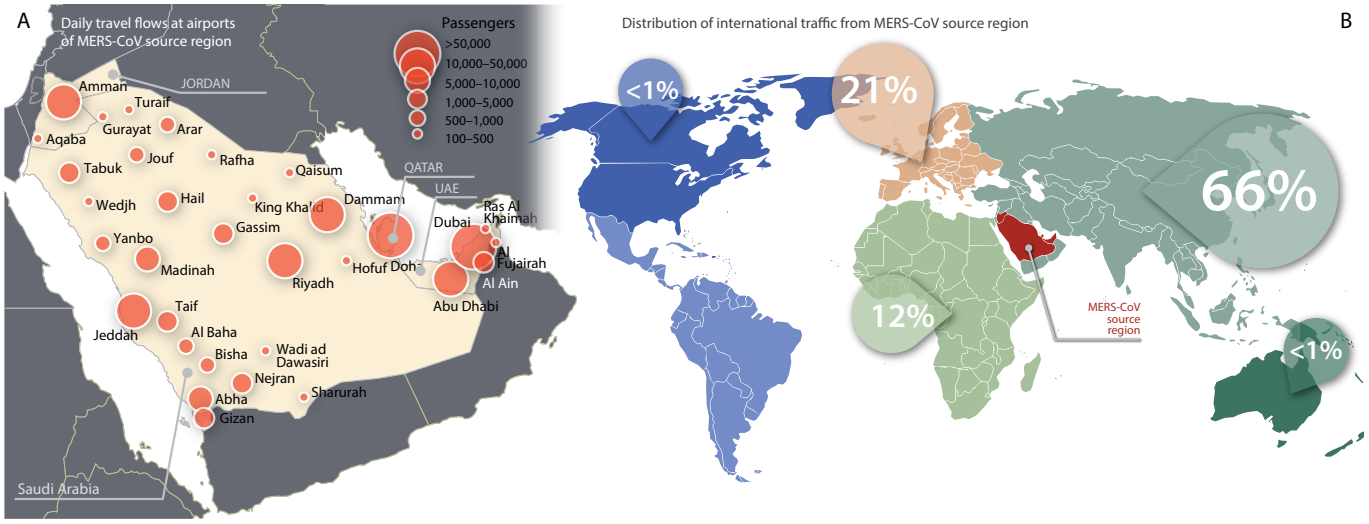
was used to measure distance from the best fit. Associated confidence intervals were obtained by profiling the deviance in the 3D space [22]. It is important to note that such estimates cannot be derived from the maximum likelihood analysis of each Method considered separately, nor conditionally one to the other, and the full computation of

$$\mathcal{L}(R, p_{sp}, p_{cl} | \{o_i\}, \{n_j^*\})$$

needs to be considered. In this respect, our integrative approach represents a substantial advance with respect to prior work based on the analysis of cluster data only [10].

Figure 2

Air traffic capacity in the Middle East respiratory syndrome coronavirus source region considered in this study and international destinations from this region



MERS-CoV: Middle East respiratory syndrome coronavirus; UAE: United Arab Emirates.

Airports in the MERS-CoV source region (Jordan, Qatar, Saudi Arabia, UAE) are represented with a circle proportional to the daily traffic they handle (panel A). Their international traffic, out of the region, is broken down by continent of destination (panel B). Breakdown by country for the first 20 countries with highest traffic from the ME region: India (11.7%), Bahrain (8.7%), Pakistan (8.6%), United Kingdom (8.4%), Oman (5.8%), Egypt (5.2%), Kuwait (4.3%), Iran (3.6%), Germany (3.5%), Lebanon (2.9%), Bangladesh (2.8%), Thailand (2.5%), Sri Lanka (2.3%), Singapore (2.1%), Syria (2.0%), France (2.0%), Kenya (1.6%), Italy (1.5%), Malaysia (1.4%), Switzerland (1.4%). Statistics are based on the 2002 International Air Transport Association (IATA) air traffic data for direct flights integrated into the global epidemic and mobility model (GLEAM) [19] after accounting for traffic growth in the period from 2002 to 2011 [23].

Air traffic data analysis

We additionally analysed the air traffic data integrated into GLEAM (direct flights from reference [19] accounting for traffic growth [23]) to evaluate the traffic capacity of the airports in the ME region and to assess the importation risk of the countries belonging to other areas of the world than the western European countries previously considered.

Results

The integrated analysis based on outbreak data up to 31 August 2013 led to a R value equal to 0.50 (95% CI: 0.30–0.77) and daily rate p_{sp} of MERS-CoV introductions into the human population in the ME region equal to 0.28 (95% CI: 0.12–0.85) (Table 2). These best estimates were obtained considering a geometric offspring distribution, yielding higher maximum likelihood values in the analysis. The corresponding best estimate for uncertainty in the cluster distribution suggests a consistent fraction of cases missed in cluster investigations (p_{cl}=0.35; 95% CI: 0–0.85), but little impact of inaccuracies in reported cluster size on the estimates of the other parameters.

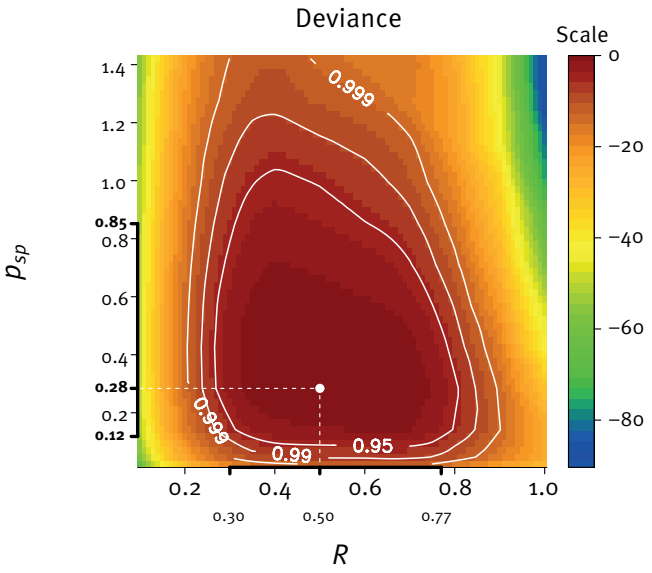
The estimated daily rate of sporadic cases in the ME region (p_{sp}=0.28; 95% CI: 0.12–0.85) can be compared to the observed value (p^*_{sp}=0.116), computed based on 60 sporadic cases reported between April 2012 and 31 August 2013, including 42 sporadic cases and 13 cluster index cases in the ME region (assuming that each cluster is originated by a single index case), as well as five laboratory-confirmed cases exported from the region (four to western Europe [1,3,5] and one to Tunisia [1]). This yields that the true number of cases from zoonotic/environmental transmission might be between 1.03- and 7.32-fold the observed number.

Our estimates are very robust against the addition of cases out of the ME region to the distribution of clusters sizes (R=0.50; 95% CI: 0.31–0.77; no change for p_{sp}). An increase in R, though with limited change in the associated confidence interval, is obtained if we include the full Jordan cluster of April 2012 by considering also cases retrospectively confirmed by serology (R=0.65; 95% CI: 0.34–0.80); no variations are obtained in the confidence interval of the estimated daily rate of sporadic cases in the region (Table 2).

Similar results for the reproductive number are obtained when we consider variations in the geographical definition of the MERS-CoV source region. Extending the source region considered here to neighbouring countries does not affect the estimated basic reproductive number and associated confidence interval (R=0.50; 95% CI: 0.31–0.76), but lowers the value of the daily rate of sporadic cases (p_{sp}=0.14; 95% CI: 0.05–0.38). If we assume that the source region is instead restricted to Saudi Arabia, a substantial increase in p_{sp} is obtained (4.73; 95% CI: 2.32–15.37), with increase

Figure 3

Heatmap of deviance values versus reproductive number R and daily rate of sporadic cases p_{sp}



Deviance was calculated as $D(R,p_{sp})$=-2($\log\mathcal{L}(R,p_{sp})$-max($\log\mathcal{L}$)) using the profiled log likelihood (for each pair (R,p_{sp}), the uncertainty parameter p_{cl} in cluster size distribution maximizing the log-likelihood $\mathcal{L}$ was chosen). Vertical and horizontal dashed lines show the maximum likelihood values for R and p_{sp}, respectively. Solid white curves contour the deviance-based confidence regions of levels 95%, 99%, and 99.9%. The 95% profiled confidence intervals for R and p_{sp} are highlighted in bold on the axes.

in the reproductive number and unaltered confidence interval (R=0.60; 95% CI: 0.30–0.76).

No variation in p_{sp} was observed in testing a different hypothesis on the offspring distribution, whereas an increase for the best estimate of R was found (0.69 when considering a Poisson offspring distribution vs. 0.50 in the baseline, with similar CIs, Table 2). Larger CIs, but no significant variation in the parameters' estimates, were observed by considering empirical data up to the end of May (Table 2).

Analyses of traffic data expose large traffic fluxes towards the continents of Asia, Europe and Africa (Figure 2) from the ME region. Of the 20 countries with highest traffic from the ME region, six were found to neighbour this region (Bahrain, Iran, Kuwait, Lebanon, Oman and Syria), seven were identified in south Asia (Bangladesh, India, Malaysia, Pakistan, Singapore, Sri Lanka, Thailand) and five, including Switzerland, were in Europe, and these comprised the four countries (France, Germany, Italy and the United Kingdom) reporting importation of cases from the affected area. Two countries (Egypt and Kenya) were also found in Africa.

Discussion

Results of our integrative modeling approach suggest the occurrence of a subcritical MERS-CoV epidemic in the ME region, as quantified by a reproductive number smaller than one. The outbreak is not able to generate a self-sustaining epidemic in humans, and sporadic cases from zoonotic/environmental transmission are expected to represent a large fraction of the total size of the epidemic.

The estimated CI for the reproductive number is found to be very stable across changes in the data interpretation. In all cases, considering data up to 31 August 2013, we found that it is highly unlikely (‹5% probability) to have a MERS-CoV outbreak with R above 0.80 or below 0.30. The variation of the best estimate from the baseline case (R=0.50) to the various scenarios explored as sensitivity analysis (up to R=0.69) is explained by the presence of a large region in the parameter space (R, p_{sp}) where the likelihood function shows small variation around its maximum value (darker red area in Figure 3). This is likely induced by the limited data available not allowing us to further narrow down the confidence intervals of the estimates.

The analysis based on the integration of two independent methods allows us to provide an estimate for the daily rate of introductions of MERS-CoV infections in the human population in the ME region, in addition to the estimate for the reproductive number. The estimated 95% CI in the baseline scenario (0.12–0.85) compared to the observed value (0.116) suggests a negligible to significant under-ascertainment rate for zoonotic/environmental transmissions (1.03–7.32 times the reported sporadic cases), indicating that notified sporadic cases likely represent a substantial proportion of the total, but improved surveillance in the region including serological surveys around cases is needed. Since evidence for mild illness, as well as for a wide spectrum of clinical disease, was observed [3,6], our findings are compatible with an under-ascertainment rate for zoonotic/environmental transmissions that may be due in a large part to a selection bias towards cases of more severe illness, where patients having mild illnesses or asymptomatic infections may go undetected [24].

The integrative approach allows us to overcome scarce data availability on the outbreak that may limit the statistical power of each of the approaches if considered separately. The combination of the likelihood functions indeed enables solving possible degeneracies and providing point estimates and confidence intervals for both parameters, R and p_{sp}.

Our estimates for the reproductive number are consistent with the results of Breban et al. [10] – the only study to date reporting results on inter-human transmissibility – thus further confirming the robustness of our epidemic assessment. Our work presents however substantial differences in the methodology and in its achievable predictions as further discussed below.

One major difference is that our integrative approach allows the quantification of sporadic cases under-ascertainment through the estimate of the rate of introduction of sporadic cases in the ME region, combined with the estimate for the reproductive number. In the study from Breban et al. [10], daily introductions are calculated on the basis of the two assumed scenarios for the transmission trees, i.e. from the assumed number of index cases among the reported data. Our procedure instead makes no assumption on the completeness of reported data, or on the local transmission trees, and relies on alternative data sources (case importations) to estimate the number of sporadic cases in the region.

The cluster data analysis of Method 1 relies on the assumption that the final size of the cluster is observed and that each cluster is the result of human-to-human transmission starting from a single index case. The first hypothesis was almost met, since, as of 7 September 2013, only two cases reported after 31 August 2013 were later linked to the cases analysed here, with no impact on the reported estimates [25].

Considering the second hypothesis, while we allow for uncertainty in case detection in the close contact investigation, we do not consider the possibility of co-exposure of epidemiologically linked cases to the same source of zoonotic/environmental infection, differently from Breban et al. [10]. In view of the persisting large uncertainties regarding the virus path of infection to humans and with insufficient data from epidemiological investigations to reliably reconstruct transmission trees within clusters, we chose a worst-case assumption for the transmissibility of the virus. This may lead to overestimating the reproductive number, however not affecting our conclusion on the subcritical nature of the current MERS-CoV epidemic. In addition, such assumption does not affect the estimate of the size of the epidemic in the affected region (Method 2), as this is based on case importations assuming no knowledge on the local transmissions, independently of their type (whether human-to-human or zoonotic/environmental).

We considered the cluster analysis (Method 1) restricted to the ME region as we assumed a rather homogeneous implementation of control measures around cases that may be different from the one put in place in affected countries experiencing importation of cases, mainly due to the additional available knowledge of travel history associated to imported cases. The extension of the analysis to all MERS-CoV clusters of laboratory-confirmed cases reported to WHO did not alter our estimates. The integrative approach also relies on the assumptions of homogeneous mixing in the local populations and homogeneous travel behaviour informed by traffic data (regardless e.g. of travel frequencies of specific population classes), similarly to previous studies [16,17]. This is due to the lack of data characterising interactions between travellers and local population, and characterising the demographic profile of passengers.

The spatial component of our approach allows us to shed light on additional aspects of the epidemic. If we assume that the source of MERS-CoV infection is restricted to Saudi Arabia, where the majority of cases has been observed, our estimates indicate that a much larger number of sporadic cases in the area would be needed to sustain the observed importation of cases in the western European countries considered (4.73 daily introductions of sporadic cases vs. 0.28 in the baseline). The biggest airports, handling the vast majority of the international air traffic of the region, are indeed mainly localised in the United Arab Emirates, Qatar, and Jordan (panel A of Figure 3). This strongly reduces the traffic capacity of the ME region, as well as the corresponding likelihood of exporting cases out of the region, when the restricted hypothesis on the source region is considered. An analysis based on cluster data only would remain unchanged and would not be able to detect important variations in the epidemic size estimation. This result further indicates the relevance of air travel in the epidemic assessment and therefore the need for an integrative approach also based on mobility and space. It also implies that improving the knowledge of the geographical extent of the source region is critical, along with the identification of the virus' path of transmission to humans.

These results are obtained under the assumption of homogeneous sensitivity of the surveillance systems of the restricted set of countries in western Europe with case importations considered in Method 2. The further application of this approach to a wider range of countries or other outbreaks would require the assessment of the sensitivity of the surveillance systems of the affected countries.

Changes in time of countries' public health actions for surveillance and control of the MERS-CoV epidemic certainly occurred in response to the increasingly available emerging evidence and the higher awareness of the disease, however data are too scarce to provide estimates of the reproductive number as a function of time R(t). Here we assumed a constant R for the period under study, with the underlying assumption of a constant and homogeneous implementation of intervention measures in the region.

Other possible factors leading to variations of the current situation may be pathogen- and host-related. Evolution of the MERS-CoV virus to adapt to humans and reach sustainable and efficient human-to-human transmission represents a potential future scenario, as it happened for SARS [26]. Seasonality may also affect virus transmissibility. We tested for possible variations of R and p_{sp} estimates by comparing two different points in time (end of May and end of August 2013) and found no significant change, except for a reduction of the CIs following a larger dataset available for the estimation.

Given the available data, this result seems to indicate that no variation in the rate of introductions and in the transmissibility of the virus has occurred in the period from May to August 2013 that may point to differences in the transmission to humans or to viral adaptations to human hosts. Furthermore, the crude notification data from the affected countries do not provide any epidemiological evidence of a seasonality of the epidemic. On the other hand, if compared to the reported number of sporadic cases at the two dates, our p_{sp} estimates are in favour of an increase in sporadic case ascertainment as the estimated under-ascertainment rate has decreased from 2.2–17.5 considering cases up to 31 May to 1.03–7.32 up to 31 August. Further data to update the integrative approach will contribute to provide a continuous assessment of the outbreak in case an evolving situation is suspected.

Other events that are related to human movements and mixing may as well alter the assessed scenario. Vast international mass gatherings annually taking place in Saudi Arabia are known to bring large number of pilgrims to the affected area, with expected increased rates of local mixing that may favour the transmission of the virus, followed by a potential amplification of its international dissemination due to the return of pilgrims to their own countries [27]. The occurrence of these large scale events calls for additional studies in pilgrims' screening [28] next to enhanced local surveillance in the region and guidance to local authorities [29] that would help to assess and control possible changes in time in the virus transmission.

Air travel clearly represents the main mean for global spatial spread of infectious disease epidemics in the modern world, as it was previously experienced with SARS and the influenza A(H1N1)pdm09 pandemic [16,17,30-32]. Besides seasonal variations due to specific events (e.g. mass gatherings) or in/out flows of expatriates for seasonal jobs, a potential emerging pandemic in the ME area would constitute a very high risk for considerably rapid and wide international spread. The ME area indeed covers a central role in connecting different regions of the world and has witnessed a dramatic increase of traffic growth in the last decade (153% of relative increase in Saudi Arabia in the period from 2002 to 2011, 240% in Jordan, 408% in Qatar, 512% in the United Arab Emirates, against a global relative increase of 168%) [23]. Analysis of the air traffic data integrated into GLEAM suggests that other countries than the ones already affected are at high risk of MERS-CoV importation through infected passengers, in particular in the southern regions of Asia (Figure 2), similarly to reference [27]. Should the outbreak evolve in a self-sustained epidemic, such risk assessment analyses cannot rely on travel data only and would require the full integration of the air travel data with an epidemic model, as in GLEAM, to explicitly simulate the evolving epidemic, estimate importation likelihood [31] and provide predictions for future stages of the epidemic [17,33].

With a subcritical epidemic in the ME region associated with a large potential for international dissemination, priority for the epidemic control should be given to the identification of the transmission of infection to humans to limit sporadic cases, to the reduction of human-to-human transmission through rapid case identification and isolation, and to the enhancement of surveillance systems in those countries that are at a higher risk of importation because of travel flows to/from the affected area.

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Conflict of interest

None declared.

Authors' contributions

CaP and PYB did the analyses for Method 1. ChP and VC did the analyses for Method 2. ChP devised an updated version of GLEAM adapted to the MERS-CoV study and ran GLEAM simulations. ChP and CaP performed the integrative analysis. PYB and VC conceived and designed the study. All authors were involved in the interpretation of results and the preparation of the manuscript. VC drafted the manuscript. All authors provided comments and approved the final version of the manuscript.

References

1. World Health Organization (WHO), Global Alert and Response (GAR), Coronavirus infections. Geneva: WHO. [Accessed 11 Sep 2013]. Available from: http://www.who.int/csr/disease/coronavirus_infections/en/index.html
2. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012;367(19):1814-20. http://dx.doi.org/10.1056/NEJMoa1211721
3. Health Protection Agency (HPA) UK Novel Coronavirus Investigation team. Evidence of person-to-person transmission within a family cluster of novel coronavirus infections, United Kingdom, February 2013. Euro Surveill. 2013;18(11). pii: 20427.
4. Hijawi B, Abdallat M, Sayaydeh A, Alqasrawi S, Haddadin A, Jaarour N, et al. Novel coronavirus infections in Jordan, April 2012: epidemiological findings from a retrospective investigation. East Mediterr Health J. 2013; 19: S12-8.
5. Guery B, Poissy J, el Mansouf L, Sejourne C, Ettahar N, Lemaire X, et al. Clinical features and viral diagnosis of two cases of infection with Middle East respiratory syndrome coronavirus: a report of nosocomial transmission. Lancet. 2013; 381(9885):2265-72. http://dx.doi.org/10.1016/S0140-6736(13)60982-4
6. Assiri A, McGeer A, Perl TM, Price CS, Al Rabeaah AA, Cummings DA et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. N Engl J Med. 2013; 369(5):407-16. http://dx.doi.org/10.1056/NEJMoa1306742
7. Buchholz U, Müller MA, Nitsche A, Sanewski A, Wevering N, Bauer-Balci T, et al. Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October-November 2012. Euro Surveill. 2013;18(8). pii:20406.

8. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, Epstein JH, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis. 2013;19(11):1819-23. http://dx.doi.org/10.3201/eid1911.131172
9. Perera RA, Wang P, Gomaa MR, El-Shesheny R, Kandeil A, Bagato O, et al. Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. Euro Surveill. 2013; 18(36). pii:20574.
10. Breban R, Riou J, Fontanet A. Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. Lancet. 2013;382(9893):694-9. http://dx.doi.org/10.1016/S0140-6736(13)61492-0
11. European Centre for Disease Prevention and Control (ECDC). Rapid Risk Assessment: Severe respiratory disease associated with Middle East respiratory syndrome coronavirus (MERS-CoV). Stockholm: ECDC; 17 May 2013. Available from: http://www.ecdc.europa.eu/en/publications/publications/risk-assessment-middle-east-respiratory-syndrome-coronavirus-mers-cov-17-may-2013.pdf
12. Farrington CP, Kanaan MN, Gay NJ. Branching process models for surveillance of infectious diseases controlled by mass vaccination. Biostatistics. 2003;4(2):279-95. http://dx.doi.org/10.1093/biostatistics/4.2.279
13. De Serres G, Gay NJ, Farrington CP. Epidemiology of transmissible diseases after elimination. Am J Epidemiol. 2000; 151(11): 1039-48. http://dx.doi.org/10.1093/oxfordjournals.aje.a010145
14. Ferguson NM, Fraser C, Donnelly CA, Ghani AC, Anderson RM. Public health risk from the avian H5N1 influenza epidemic. Science. 2004;304(5673): 968-9. http://dx.doi.org/10.1126/science.1096898
15. ProMED-mail. MERS-COV - EASTERN MEDITERRANEAN (31): Jordan, retrospective case identification, WHO, request for information. Archive Number: 20130617.1777989. 17 Jun 2013. Available from: http://www.promedmail.org/direct.php?id=20130617.1777989
16. Fraser C, Donnelly CA, Cauchemez S, Hanage WP, Van Kerkhove MD, Hollingsworth TD, et al. Pandemic potential of a strain of influenza A(H1N1): early findings. Science. 2009; 324(5934): 1557-61. http://dx.doi.org/10.1126/science.1176062
17. Balcan D, Hu H, Gonçalves B, Bajardi P, Poletto C, Ramasco JJ, et al. Seasonal transmission potential and activity peaks of the new influenza A(H1N1): a Monte Carlo likelihood analysis based on human mobility. BMC Med. 2009;7: 45. http://dx.doi.org/10.1186/1741-7015-7-45
18. Colizza V, Barrat A, Barthelemy M, Valleron A-J, Vespignani A. Modeling the Worldwide Spread of Pandemic Influenza: Baseline Case and Containment Interventions, PLoS Med. 2007;4(1):e13. http://dx.doi.org/10.1371/journal.pmed.0040013
19. Balcan D, Colizza V, Gonçalves B, Hu H, Ramasco JJ, Vespignani A. Multiscale mobility networks and the spatial spreading of infectious diseases, Proc Natl Acad Sci USA. 2009;106(51):21484-9. http://dx.doi.org/10.1073/pnas.0906910106
20. Anderson RM, May RM. Infectious Diseases of Humans: Dynamics and Control. Oxford: Oxford University Press; 1992.
21. Center for Disease Control and Prevention (CDC). A Novel Coronavirus Called "MERS-CoV" in the Arabian Peninsula, updated July 26, 2013. Atlanta: CDC Available from: http://wwwnc.cdc.gov/travel/notices/watch/coronavirus-saudi-arabia-qatar#footnote
22. Venzon DJ, Moolgavkar SH A method for computing profile-likelihood based confidence intervals. Applied Statistics. 1988; 37: 87-94. http://dx.doi.org/10.2307/2347496
23. International Civil Aviation Organization and Civil Aviation Statistics of the World, statistics. Washington, DC: The World Bank. Available from: http://data.worldbank.org/indicator/IS.AIR.PSGR
24. World Health Organization (WHO), Global Alert and Response (GAR), MERS-CoV update – as of 26 June 2013. Geneva: WHO. Available from: http://www.who.int/csr/don/2013_06_26/en/index.html
25. World Health Organization (WHO), Global Alert and Response (GAR), Middle East respiratory syndrome coronavirus (MERS-CoV) - update 7 September 2013. Available from: http://www.who.int/csr/don/2013_09_07/en/
26. Holmes KV. Structural biology. Adaptation of SARS coronavirus to humans. Science. 2005; 309(5742): 1822–23. http://dx.doi.org/10.1126/science.1118817
27. Khan K, Sears J, Hu VW, Brownstein JS, Hay S, Kossowsky D, et al. Potential for the International Spread of Middle East Respiratory Syndrome in Association with Mass Gatherings in Saudi Arabia. PLOS Curr. 2013;5.
28. Gautret P, Charrel R, Belhouchat K, Drali T, Benkouiten S, Nougairede A, et al. Lack of nasal carriage of novel corona virus (HCoV-EMC) in French Hajj pilgrims returning from the Hajj 2012, despite a high rate of respiratory symptoms. Clin Microbiol Infect. 2013; 19(7):E315-7. http://dx.doi.org/10.1111/1469-0691.12174
29. World Health Organization (WHO), International travel and Health, World-travel advice on MERS-CoV for pilgrimages, 25 July 2013. Geneva: WHO. Available from: http://www.who.int/ith/updates/20130725/en/index.html
30. Hufnagel L, Brockmann D, Geisel T. Forecast and control of epidemics in a globalized world. Proc Natl Acad Sci USA. 2004; 101(42):15124. http://dx.doi.org/10.1073/pnas.0308344101
31. Colizza V, Barrat A, Barthelemy M, Vespignani A. Predictability and epidemic pathways in global outbreaks of infectious diseases: the SARS case study. BMC Med. 2007;5: 34. http://dx.doi.org/10.1186/1741-7015-5-34
32. Khan K, Arino J, Hu W, Raposo P, Sears J, Calderon F, et al. Spread of a Novel Influenza A (H1N1) Virus via Global Airline Transportation. N Engl J Med. 2009; 361(2): 212-4. http://dx.doi.org/10.1056/NEJMc0904559
33. Tizzoni M, Bajardi P, Poletto C, Ramasco JJ, Balcan D, Gonçalves B, et al. Real-time numerical forecast of global epidemic spreading: case study of 2009 A/H1N1pdm. BMC Med. 2012;10:165. http://dx.doi.org/10.1186/1741-7015-10-165

Epidemiological surveillance of colonising group B *Streptococcus* epidemiology in the Lisbon and Tagus Valley regions, Portugal (2005 to 2012): emergence of a new epidemic type IV/clonal complex 17 clone

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This study presents the serotype distribution and the antibiotic resistance profile of 953 colonising group B *Streptococcus* (GBS) recovered from women of child bearing age (15 to 49 years) between 2005 and 2012 in the Lisbon and Tagus Valley region, Portugal. Overall, serotypes Ia, II, III, and V were the most common, accounting 752 of the 953 isolates (about 80%). However, there were changes in GBS distribution, in particular in the two last years of the study. Of note, the proportion of serotype IV isolates increased from 1% (2/148) in 2006 to 20% (19/97) in 2012. Also, considerable proportions of serotype IV isolates from 2010 to 2012 were respectively resistant to erythromycin (9/43; 21%) or clindamycin (6/43; 14%). The identification of nine serotype IV isolates presenting a novel association with the clonal complex (CC) 17 lineage, involving a putative capsular switch, may accentuate their virulence potential and ecological success. Molecular analysis of this subgroup of isolates revealed the presence of *rib*, IS (insertion sequence) *861* and GBSi1 group II intron within the C5a peptidase gene (*scpB*) – laminin-binding protein gene (*lmb*) region, reflecting high clonality and a putative common origin. A close surveillance of the emergent type IV/CC17 isolates is crucial considering the potential impact over GBS treatment guidelines and capsular vaccine development.

Introduction

Streptococcus agalactiae, group B *Streptococcus* (GBS) is an opportunistic microbial agent of neonatal pneumonia, septicaemia and meningitis in human newborns [1]. GBS is also a significant cause of morbidity and mortality in non-pregnant adults, particularly those with underlying medical conditions and in the elderly [1]. Up to 36% of pregnant women are anogenitally colonised, although the carrier status is considered dynamic during pregnancy [1,2]. In newborns, maternal GBS carriage has been recognised as the major risk factor of early onset disease (EOD, <7 days of age), but bacteria can also be acquired through horizontal nosocomial transmission [1].

Classification of GBS serotype is based on 10 immunologically unique capsular polysaccharides (Ia, Ib, II-IX), whose prevalence varies according to geographical location, time of study and ethnicity [1,3]. Thus, the continuous monitoring of circulating GBS isolates is important in assessing changes in GBS serotype distribution, which is essential for the development of polysaccharide-based vaccines suitable for different geographical areas [4,5]. Serotypes Ia, II, III and V have been the most frequently described in European countries such as the Czech Republic, France, Germany, Greece, Ireland, Italy, the Netherlands, Portugal, Sweden, and the United Kingdom [6], as well as in the United States (US) [7], whereas serotypes VI and VIII, to date scarcely found in these countries, could frequently be identified in Japan [8]. With the exception of a study carried out in Abu Dhabi, United Arab Emirates, where serotype IV predominated among colonised pregnant women (15/57, 26% of the GBS isolates) [9], there are few reports among other countries worldwide of serotype IV as a predominant serotype both in cases of colonisation and infection [6-8,10-13].

Previous reports from Portugal, for the period from 2002 to 2007 [6,12], have shown a low and stable prevalence of serotype IV (6/269 (2%) and 3/100 (3%) among colonised women of reproductive age (15 to 49

years). The same scenario was observed among neonatal (2/64 cases; 3%) for the years 2000 to 2004 [12] and non-pregnant adult infections (2/225 cases; 1%) from 2001 to 2008 [14]. After 2010, reports from Brazil Ireland and the US [15-18] revealed an increased prevalence of serotype IV in colonisation and infection, suggesting the possibility that this serotype could be emerging as an important pathogen, as happened with serotype V during the 1990s [19].

In this report we describe the annual serotype distribution and the antimicrobial susceptibility of colonising GBS isolated in the Lisbon and Tagus Valley region in Portugal from 2005 to 2012, revealing the increasing frequency of serotype IV and a novel serotype IV clone defined by its clonal complex (CC)17 hypervirulent lineage, recently identified in Taiwan, France and the US [10,16,20].

Methods

Group B *Streptococcus* collection

A total of 953 non-redundant GBS carriage isolates recovered from rectovaginal specimens of healthy women in reproductive age (668 pregnant) were included in this study. GBS were isolated according to the US Centers for Diseases Control and Prevention (CDC) guidelines [21,22]. The Portuguese National Institute of Health and six tertiary hospitals (Maternidade Alfredo da Costa, Hospital Garcia de Orta, Hospital Dona Estefânia, Hospital CUF Descobertas, Hospital Fernando Fonseca and Hospital Distrital de Santarém) located in the Lisbon and Tagus Valley region, Portugal, participated in this survey between January 2005 and December 2012. GBS isolates were identified to the species level by standard criteria based on colony morphology, Gram staining, catalase test, and commercial group B *Streptococcus* latex-agglutination assays.

Capsular serotyping

All isolates were serotyped by slide agglutination using specific rabbit antisera against GBS polysaccharide antigens Ia, Ib, II to VIII (Essum AB) according to the instructions of the manufacturer. Non-serotypeable isolates were subjected to capsular (*cps*) genotyping, through the polymorphism analysis of *cpsD-cpsE-cpsF* region [6]. All serotype IV isolates were further confirmed through capsular genotyping. Non-typeable isolates after both serotyping and *cps* genotyping procedures were designated as NT.

Antimicrobial susceptibility profile

All GBS isolates were tested for penicillin G, erythromycin, clindamycin and vancomycin susceptibility by Epsilometer (E)-test, in accordance to the Clinical and Laboratory Standards Institute (CLSI) guidelines [23], to determine the minimum inhibitory concentration (MIC). The constitutive and inducible macrolide-lincosamide-streptogramin resistance phenotypes (cMLS$_B$ and iMLS$_B$, respectively) were identified by the double-disc diffusion method, as well the macrolide-specific efflux resistance phenotype (M) [6,23]. Macrolide resistance genes *ermTR*, *ermB* and *mefA* were also investigated by polymerase chain reaction (PCR) [6].

Considering that tetracycline is nowadays not recommended for the prophylaxis of GBS neonatal infection [21,22], this antibiotic was not tested by all laboratories involved in the present study; consequently, only a subset of 372/953 (39%) GBS isolates was tested for tetracycline by disc-diffusion in accordance to the CLSI guidelines [23].

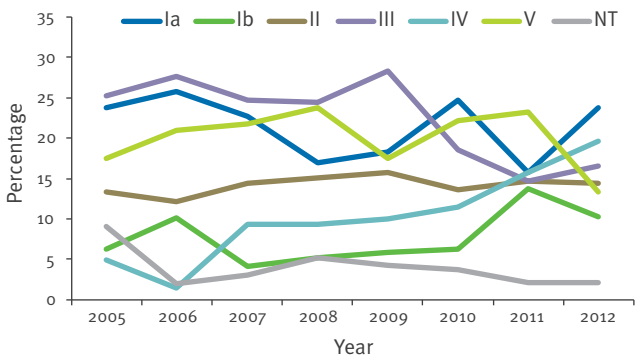
Molecular analysis of serotype IV isolates

In order to estimate the frequency of type IV isolates belonging to the sequence type (ST) 17 lineage, the presence of the *hvgA* gene (encoding a surface adhesin characteristic of the hypervirulent GBS CC17) was achieved by PCR, as described elsewhere [24]. Serotype IV *hvgA*-positive isolates were further subjected to multilocus sequence typing (MLST) analysis [25], including the partial sequencing (about 500 bp) of seven housekeeping loci. Alleles of all loci were examined through the GBS MLST database (http://pubmlst.org/sagalactiae/) providing an allelic profile or ST.

Serotype IV characterisation also included the study of the Alp family, a major streptococcal antigen, by using multiplex PCR for direct identification of the *alpha-C*, *rib*, *epsilon* and *alp2–alp4* genes [26]. The prevalence of mobile genetic elements (*MGEs*), IS (insertion sequence) *861*, IS*1381*, IS*1548* and GBSi1 group II intron within the C5a peptidase gene (*scpB*) – laminin-binding protein gene (*lmb*) region within type IV/CC17 isolates were also evaluated by PCR, as previously described [27,28].

Figure 1

Serotype distribution among group B *Streptococcus* colonising isolates (n=953) from women of reproductive age, Lisbon and Tagus Valley regions, Portugal, 2005–2012



NT: non-typeable.

Results

Annual distribution and frequency of serotypes

Among the 953 isolates analysed, serotypes III, Ia, and V were the most frequent ones during the whole study period (2005–2012) (222 (23%), 203 (21%), and 192 (20%), respectively), followed by serotypes II, IV, Ib and NT (135 (14%), 89 (9%), 72 (8%) and 40 (4%), respectively) (Figure 1). Serotypes VI to VIII were not found.

Variations in the distribution of GBS serotypes were observed, especially in 2011 and 2012, when the proportion of the serotypes III and V decreased whereas the proportion of serotypes IV and Ib increased. Indeed, a remarkable increase in serotype IV frequency has been observed, from 1% (2 of 148 isolates) in 2006 to 20% (19 of 97 isolates) in 2012 (20-fold), ranking this serotype as the second most detected in 2012 (Figure 1). In contrast, serotype II remained stable during the eight years study period, as its frequency ranged between 12% (n=116) and 16% (n=151).

Susceptibility to antimicrobials

Neither resistance nor reduced susceptibility to vancomycin or to penicillin G, a first-line antibiotic for the prophylaxis and treatment of GBS infections, were detected.

For the total isolates in the 2005 to 2012 period, the percentage of GBS isolates that were resistant to erythromycin ranged from 14% (21/148) in 2006 to 23% (22/95) in 2011, whereas the percentage of GBS isolates with resistance to clindamycin ranged from 6% (7/120) in 2009 to 18% (17/97) in 2012 (Figure 2). Of note, the higher resistance rates for both antibiotics respectively were observed in the two last years of the study (2011 and 2012) (Figure 2).

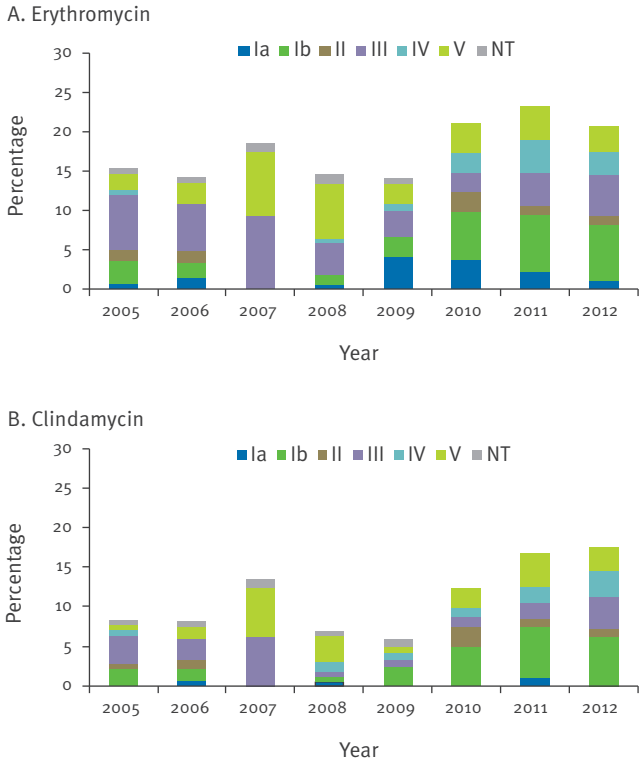
Among the 162/953 (17%) erythromycin-resistant isolates, 99/162 (61%) displayed the $cMLS_B$ phenotype, 56/162 (35%) had the $iMLS_B$, and the M phenotype accounted for 7/162 isolates (4%). All of the $cMLS_B$ and $iMLS_B$ resistance phenotypes were conferred by the presence of the *ermB* and *ermTR* genes, respectively, whereas the M phenotype was related to the presence of the *mefA* gene. Among the 372 GBS isolates tested for tetracycline, 306 (82%) were resistant to this antibiotic. Only 41/162 erythromycin-resistant GBS isolates were tested for tetracycline and all were resistant to the latter, which could be expected considering a putative horizontal gene transfer event involving the same conjugative transposon carrying both genetic resistance determinants [26]. We verified that the erythromycin (n=162) and clindamycin (n=98) resistant isolates involved multiple serotypes (Figure 2), despite the predominance of serotypes III and V from 2005 to 2008; however, the distribution profile remained very similar during the last three years (2010–2012), which could contradict the association between serotype III and macrolide resistance, previously demonstrated in Portugal and Spain [6,29]. This situation constitutes a new scenario involving other serotypes, namely Ib and IV (Figure 2). In fact, in 2007 none of the four isolated strains serotyped as Ib was resistant to macrolides, but during 2010 to 2012, 19/28 (68%) and 16/28 (57%) serotype Ib isolates were resistant to erythromycin and clindamycin, respectively; however, the number of Ib isolates was relatively low during this triennium. In 2006 and 2007, none of the 11 serotype IV isolates was resistant to erythromycin or clindamycin, whereas during 2010 to 2012, 9/43 (21%) and 6/43 (14%) serotype IV isolates were resistant to erythromycin and clindamycin, respectively.

Frequency of clonal complex 17 lineage in serotype IV isolates

Nine of 89 (10%) serotype IV isolates collected over the eight-year period belonged to the hypervirulent CC17 lineage, and all displayed ST291 (a single locus variant of ST17); these nine isolates were recovered in 2008 (n=3), 2009 (n=1), 2010 (n=1) and 2012 (n=4). Concerning their susceptibility to antimicrobials, with one exception (one isolate from 2012, which was co-resistant to clindamycin and erythromycin (MIC≥256 µg/ml)), the remaining eight isolates were fully susceptible to penicillin G, erythromycin, clindamycin, and

Figure 2

Percentage of the different group B *Streptococcus* serotypes among erythromycin (A) (n=162) and clindamycin (B) (n=98) resistant isolates, Lisbon and Tagus Valley regions, Portugal, 2005–2012



NT: non-typeable.

vancomycin. All displayed the GBS surface protein *rib* gene, the GBSi1 in the *scpB-lmb* intergenic region and the IS*861*. Excluding one serotype IV isolate from 2010, the insertion sequence IS*1381* was not detected.

Discussion

The GBS capsule has long been recognised as one of the most important virulence factors. Variations of the polysaccharide structure allow the antigenic distinction of 10 different serotypes [1,3]. It has been reported that predominating serotypes change over time, vary by geographical region and ethnic origin and can be associated with different diseases. The existence of several serotypes together with their differential distribution constitutes a major obstacle for the development of a global and effective GBS vaccine to prevent GBS neonatal infections [4].

Due to its low prevalence in European countries and in the US, serotype IV was not selected for the development of capsular polysaccharide-based vaccines [4,5]. This situation has changed in the last decade, when some countries, including the US, saw the emergence of serotype IV among colonising and invasive GBS isolates [15,16]. This scenario may become risky if the emergence of serotype IV combines with antibiotic resistance, which was the case in our study where co-resistance to second-line macrolide antibiotics was observed in recent years (2010–2012). Corroborating our findings, resistance to macrolides and clindamycin has been described in the US [16] among invasive serotype IV isolates, predicting the emergence of serious problems for the intrapartum antibiotic prophylaxis in pregnant women allergic to penicillin. GBS serotype distribution changes and antibiotic resistance trends constitute emerging phenomena that emphasise the need for constant monitoring, in order to develop accurate GBS prevention strategies.

Another major concern is the association of serotype IV with the ST17 lineage identified in our study, supporting that previously described in a few other geographical regions, such as France, Taiwan and US [10,16,20]. It is worth noting that ST17 lineage was long considered as a homogeneous epidemic clone, almost exclusively composed by serotype III isolates, and characterised by its rapid global dissemination and successful adaptation to human neonates [30]. The origin of the novel association of CC17 with serotype IV can be due to an exchange of a 35.5 kb DNA segment containing the entire capsule operon, culminating in a type III to type IV capsular switch, as described by Bellais et al. [10]. This phenomenon predicts an important epidemiological success for this new clone. As both French and Portuguese type IV/CC17 GBS isolates were recently identified (after 2008), and as they share the same ST291, we could speculate on a common ancestor; however, this hypothesis needs further evaluation as this ST was also described among serotype IV invasive isolates from Minnesota, US [16].

In our study, the clonal origin hypothesis was evaluated through the screening of specific mobile genetic elements among our type IV/ST291 isolates, as their acquisition via recombination or horizontal transfer events are linked with the evolution and niche adaptation of bacterial species or particular clones. We verified that all type IV/ST291 isolates shared the same MGE profile composed by IS*861* and GBSi1 within the *scpB-lmb* intergenic region in the absence of IS*1381*. Only one variant carrying this latter IS has been identified in 2010. This MGE profile strongly correlates to the evolutionary scheme proposed by Héry-Arnaud et al. [28] for the ST17 lineage; however, the existence of type IV/ST291 variants, containing IS1381 or displaying antibiotic resistance, suggests differential evolutionary status from a common ancestor.

In conclusion, a novel epidemic GBS type IV/CC17 clone seems to be emerging through a putative clonal expansion among neonates and adults, as might have occurred since the 1960s with type III/ST17, an 'epidemic clone' with a rapid global dissemination and adaptation to human neonates [30].

The sudden increase of GBS serotype IV detection in different countries does not rely on the emergence of type IV/CC17 only, as other genetic lineages (such as CC1 and CC23) or different types of pulsed-field gel electrophoresis have been identified, constituting the majority of the serotype IV isolates [15-18,20]. A careful surveillance of GBS type IV/ST291 emergence is recommended, in order to define its host specificity, tropism, virulence potential and antibiotic resistance phenotype.

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Conflict of interest

None declared.

Authors' contributions

C. Florindo, V. Damião, I. Silvestre, C. Farinha, and members of the Group for the Prevention of Neonatal GBS Infection were involved in strain characterisation and data analysis. C. Florindo, M. J. Borrego, F. Martins-Pereira and I. Santos-Sanches were involved in the methodological design. Preparation of first draft: C. Florindo. Draft revision and approval: all.

References

1. Edwards MS, Nizet V. Group B streptococcal infections. In: Remington JS, Klein JO, Wilson CB, Nizet V, Maldonado YA, eds. Diseases of the fetus and newborn infant. 7th ed. Philadelphia: Elsevier; 2011:419-69. http://dx.doi.org/10.1016/B978-1-4160-6400-8.00012-2
2. Barcaite E, Bartusevicius A, Tameliene R, Kliucinskas M, Maleckiene L, Nadisauskiene R. Prevalence of maternal group B streptococcal colonisation in European countries. Acta Obstet Gynecol Scand. 2008;87(3):260-71. http://dx.doi.org/10.1080/00016340801908759
3. Slotved HC, Kong F, Lambertsen L, Sauer S, Gilbert GL. Serotype IX, a proposed new Streptococcus agalactiae serotype. J Clin Microbiol. 2007;45(9):2929-36. http://dx.doi.org/10.1128/JCM.00117-07
4. Johri AK, Paoletti LC, Glaser P, Dua M, Sharma PK, Grandi G et al. Group B Streptococcus: global incidence and vaccine development. Nat Rev Microbiol. 200;4(12):932-42.
5. Rodriguez-Granger J, Alvargonzalez JC, Berardi A, Berner R, Kunze M, Hufnagel M et al. Prevention of group B streptococcal neonatal disease revisited. The DEVANI European project. Eur J Clin Microbiol Infect Dis. 2012;31(9):2097-104. http://dx.doi.org/10.1007/s10096-012-1559-0
6. Florindo C, Viegas S, Paulino A, Rodrigues E, Gomes JP, Borrego MJ. Molecular characterization and antimicrobial susceptibility profiles in Streptococcus agalactiae colonizing strains: association of erythromycin resistance with subtype III-1 genetic clone family. Clin Microbiol Infect. 2010;16(9):1458-63. http://dx.doi.org/10.1111/j.1469-0691.2010.03106.x
7. Ippolito DL, James WA, Tinnemore D, Huang RR, Dehart MJ, Williams J et al. Group B streptococcus serotype prevalence in reproductive-age women at a tertiary care military medical center relative to global serotype distribution. BMC Infect Dis. 2010;10:336. http://dx.doi.org/10.1186/1471-2334-10-336
8. Lachenauer CS, Kasper DL, Shimada J, Ichiman Y, Ohtsuka H, Kaku M et al. Serotypes VI and VIII predominate among group B streptococci isolated from pregnant Japanese women. J Infect Dis. 1999; 179(4):1030–1033. http://dx.doi.org/10.1086/314666
9. Amin A, Abdulrazzaq YM, Uduman S. Group B streptococcal serotype distribution of isolates from colonized pregnant women at the time of delivery in United Arab Emirates. J Infect. 2002;45(1):42-6. http://dx.doi.org/10.1053/jinf.2001.0990
10. Bellais S, Six A, Fouet A, Longo M, Dmytruk N, Glaser P et al. Capsular switching in group B Streptococcus CC17 hypervirulent clone: a future challenge for polysaccharide vaccine development. J Infect Dis. 2012;206(11):1745-52. http://dx.doi.org/10.1093/infdis/jis605
11. Fluegge K, Wons J, Spellerberg B, Swoboda S, Siedler A, Hufnagel M et al. Genetic differences between invasive and noninvasive neonatal group B streptococcal isolates. Pediatr Infect Dis J. 2011;30(12):1027-31. http://dx.doi.org/10.1097/INF.0b013e31822a2a1f
12. Martins ER, Pessanha MA, Ramirez M, Melo-Cristino J; Portuguese Group for the Study of Streptococcal Infections. Analysis of group B streptococcal isolates from infants and pregnant women in Portugal revealing two lineages with enhanced invasiveness. J Clin Microbiol. 2007;45(10):3224-9. http://dx.doi.org/10.1128/JCM.01182-07
13. Figueira-Coelho J, Ramirez M, Salgado MJ, Melo-Cristino J. Streptococcus agalactiae in a large Portuguese teaching hospital: antimicrobial susceptibility, serotype distribution, and clonal analysis of macrolide-resistant isolates. Microb Drug Resist. 2004;10(1):31-6. http://dx.doi.org/10.1089/107662904323047772
14. Martins ER, Melo-Cristino J, Ramirez M; Portuguese Group for the Study of Streptococcal Infections. Dominance of serotype Ia among group B Streptococci causing invasive infections in nonpregnant adults in Portugal. J Clin Microbiol. 2012;50(4):1219-27. http://dx.doi.org/10.1128/JCM.05488-11
15. Diedrick MJ, Flores AE, Hillier SL, Creti R, Ferrieri P. Clonal analysis of colonizing group B Streptococcus, serotype IV, an emerging pathogen in the United States. J Clin Microbiol. 2010;48(9):3100-4. http://dx.doi.org/10.1128/JCM.00277-10
16. Ferrieri P, Lynfield R, Creti R, Flores AE. Serotype IV and invasive group B streptococcus disease in neonates, Minnesota, USA, 2000-2010. Emerg Infect Dis. 2013;19(4):551-8.
17. Kiely RA, Cotter L, Mollaghan AM, Cryan B, Coffey A, Lucey B. Emergence of group B Streptococcus serotype IV in women of child-bearing age in Ireland. Epidemiol Infect. 2011;139(2):236-8. http://dx.doi.org/10.1017/S0950268810001275
18. Palmeiro JK, Dalla-Costa LM, Fracalanzza SE, Botelho AC, da Silva Nogueira K, Scheffer MC et al. Phenotypic and genotypic characterization of group B streptococcal isolates in southern Brazil. J Clin Microbiol. 2010;48(12):4397-403. http://dx.doi.org/10.1128/JCM.00419-10
19. Elliott JA, Farmer KD, Facklam RR. Sudden increase in isolation of group B streptococci, serotype V, is not due to emergence of a new pulsed-field gel electrophoresis type. J Clin Microbiol. 1998;36(7):2115-6.
20. Tien N, Ho CM, Lin HJ, Shih MC, Ho MW, Lin HC. Multilocus sequence typing of invasive group B Streptococcus in central area of Taiwan. J Microbiol Immunol Infect. 2011;44(6):430-4. http://dx.doi.org/10.1016/j.jmii.2011.04.013
21. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. MMWR Recomm Rep 2002;51(RR-11):1-22.
22. Verani JR, McGee L, Schrag SJ; Division of Bacterial diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). MMWR Recomm Rep 2010;59(RR-10):1-36.
23. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Tenth Edition. M02-A10. Wayne: CLSI; 2010.
24. Lamy MC, Dramsi S, Billoët A, Réglier-Poupet H, Tazi A, Raymond J et al. Rapid detection of the highly virulent group B Streptococcus ST-17 clone. Microbes Infect. 2006;8(7):1714-22. http://dx.doi.org/10.1016/j.micinf.2006.02.008
25. Jones N, Bohnsack JF, Takahashi S, Oliver KA, Chan MS, Kunst F et al. Multilocus sequence typing system for group B streptococcus. J Clin Microbiol. 2003;41(6):2530-6. http://dx.doi.org/10.1128/JCM.41.6.2530-2536.2003
26. Gherardi G, Imperi M, Baldassarri L, Pataracchia M, Alfarone G, Recchia S. Molecular epidemiology and distribution of serotypes, surface proteins, and antibiotic resistance among group B streptococci in Italy. J Clin Microbiol. 2007;45(9):2909-16. http://dx.doi.org/10.1128/JCM.00999-07
27. Al Safadi R, Amor S, Hery-Arnaud G, Spellerberg B, Lanotte P, Mereghetti L et al. Enhanced expression of lmb gene encoding laminin-binding protein in Streptococcus agalactiae strains harboring IS1548 in *scpB*-lmb intergenic region. PLoS One. 2010;5(5):e10794. http://dx.doi.org/10.1371/journal.pone.0010794
28. Héry-Arnaud G, Bruant G, Lanotte P, Brun S, Rosenau A, van der Mee-Marquet N et al. Acquisition of insertion sequences and the GBSi1intron by Streptococcus agalactiae isolates correlates with the evolution of the species. J Bacteriol. 2005;187(17):6248-52. http://dx.doi.org/10.1128/JB.187.17.6248-6252.2005
29. Martins ER, Andreu A, Correia P, Juncosa T, Bosch J, Ramirez M et al. Group B streptococci causing neonatal infections in barcelona are a stable clonal population: 18-year surveillance. J Clin Microbiol. 2011;49(8):2911-18. http://dx.doi.org/10.1128/JCM.00271-11
30. Sørensen UB, Poulsen K, Ghezzo C, Margarit I, Kilian M. Emergence and global dissemination of host-specific Streptococcus agalactiae clones. MBio. 2010;1(3):1-9. http://dx.doi.org/10.1128/mBio.00178-10

The 2014 Hajj and Umrah – current recommendations

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This year, the Hajj – the annual pilgrimage to Mecca, Saudi Arabia – will take place approximately during 2–7 October 2014. Umrah is a similar pilgrimage, but it can be undertaken any time during the year. In 2014, it is expected that the Ramadan period, approximately between 28 June and 28 July, will attract the largest number of Umrah pilgrims.

The congregation of so many people from different parts of the world in crowded conditions within a confined area for a short period of time presents many public health challenges. About three million pilgrims attend the Hajj every year. They travel to Saudi Arabia from more than 180 different countries, of which some 45,000 travel from the European Union (EU) and European Economic Area (EEA).

As the reported number of human cases of Middle East respiratory syndrome coronavirus (MERS-CoV) infection in the Arabian Peninsula has increased rapidly in the past months, several organisations have issued travel advice specifically related to MERS-CoV, in addition to the general Hajj and Umrah travel advice published every year. On 3 June, the World Health Organization (WHO) published travel advice on MERS-CoV for pilgrimages and actions for countries to take in preparation for, during and after Umrah and Hajj [1].

The United States Centers for Disease Control and Prevention (CDC) published advice on what travellers can do to protect themselves from MERS-CoV on 4 June [2]. The CDC reiterates the WHO recommendations and makes further reference to the Saudi Arabian Ministry of Health recommendation [3] that some groups should postpone travel.

The European Centre for Disease Prevention and Control (ECDC) published an updated risk assessment on 31 May, which also contains recommendations for travellers [4].

References

1. World Health Organization (WHO). World - travel advice on MERS-CoV for pilgrimages. Geneva: WHO; Jun 2014. Available from: http://www.who.int/ith/updates/20140603/en/
2. Centers for Disease Control and Prevention (CDC). Hajj and Umrah in Saudi Arabia. Atlanta: CDC; Jun 2014. Available from: http://wwwnc.cdc.gov/travel/notices/alert/hajj-umrah-saudi-arabia
3. Memish ZA, Al Rabeeah AA. Health conditions for travellers to Saudi Arabia for the Umra and pilgrimage to Mecca (Hajj) - 2014. J Epidemiol Glob Health. 2014;4(2):73-5.
4. European Centre for Disease Prevention and Control (ECDC). Updated rapid risk assessment. Severe respiratory disease associated with Middle East respiratory syndrome coronavirus (MERS-CoV), Tenth update, 31 May 2014. Stockholm: ECDC; May 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/RRA-Middle-East-respiratory-syndrome-coronavirus-update10.pdf

Call for experts to join the Scientific Panels of the European Food Safety Authority – deadline extended to 7 July 2014

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The European Food Safety Authority (EFSA) is extending the deadline of its call for experts, launched in April 2014.

EFSA is looking for scientific experts for membership of its Scientific Committee and the following eight Scientific Panels: Additives and products or substances used in animal feed (FEEDAP), Animal health and welfare (AHAW), Biological hazards (BIOHAZ), Contaminants in the food chain (CONTAM), Dietetic products, nutrition and allergies (NDA), Genetically modified organisms (GMO), Plant health (PLH) and Plant protection products and their residues (PPR).

The current members of the Scientific Committee and Panels are serving a three-year term that is due to expire in mid-2015. The new members will be appointed for the following three-year period starting in July 2015.

Applications are invited on the EFSA website (http://www.efsa.europa.eu/en/scpanels/memberscall2011.htm) by 7 July 2014. Official notification of the extension of the call will be published shortly in the Official Journal of the European Union.