



Contents lists available at [ScienceDirect](#)

Virus Research

journal homepage: www.elsevier.com/locate/virusres



Middle East respiratory syndrome: An emerging coronavirus infection tracked by the crowd

Ian M. Mackay*, Katherine E. Arden

Queensland Paediatric Infectious Diseases Laboratory, Queensland Children's Medical Research Institute, The University of Queensland, Brisbane, Australia

ARTICLE INFO

Article history:
Available online xxx

Keywords:
MERS-CoV
MERS
Camel
Zoonosis
Healthcare worker
Emerging infectious disease

ABSTRACT

In 2012 in Jordan, infection by a novel coronavirus (CoV) caused the first known cases of Middle East respiratory syndrome (MERS). MERS-CoV sequences have since been found in a bat and the virus appears to be enzootic among dromedary camels across the Arabian Peninsula and in parts of Africa. The majority of human cases have occurred in the Kingdom of Saudi Arabia (KSA). In humans, the etiologic agent, MERS-CoV, has been detected in severe, mild and influenza-like illness and in those without any obvious signs or symptoms of disease. MERS is often a lower respiratory tract disease associated with fever, cough, breathing difficulties, pneumonia that can progress to acute respiratory distress syndrome, multiorgan failure and death among more than a third of those infected. Severe disease is usually found in older males and comorbidities are frequently present in cases of MERS. Compared to SARS, MERS progresses more rapidly to respiratory failure and acute kidney injury, is more often observed as severe disease in patients with underlying illnesses and is more often fatal. MERS-CoV has a broader tropism than SARS-CoV, rapidly triggers cellular damage, employs a different receptor and induces a delayed proinflammatory response in cells. Most human cases have been linked to lapses in infection prevention and control in healthcare settings, with a fifth of virus detections reported among healthcare workers. This review sets out what is currently known about MERS and the MERS-CoV, summarises the new phenomenon of crowd-sourced epidemiology and lists some of the many questions that remain unanswered, nearly three years after the first reported case.

© 2015 Elsevier B.V. All rights reserved.

1. Brief history of the localised epidemic.

The world was made aware of a newly discovered coronavirus *via* an email from Dr. Ali Mohamed Zaki, an Egyptian virologist working at the Dr. Soliman Fakeeh Hospital in Jeddah in The Kingdom of Saudi Arabia (KSA). The email was published on the website of the professional emerging diseases (ProMED) network on 20-September-2014 (ProMED, 2014). That first case was a 60 year old man from Bisha in the KSA and, thanks to the email, the rapid discovery of a second case of the virus, this time in an ill patient from Qatar, was transferred to the United Kingdom for care (Fig. 1) (Bermingham et al., 2012). As of 20th January 2015, there have been 969 detections of viral RNA or virus-specific antibodies reported publicly, 955 confirmed by the World Health Organization (WHO), with over a third of the positive people dying

($n=351$, 37%; data from public sources including the WHO and Ministries of Health). First known as novel coronavirus (nCoV), the following two to three years were a slow discovery process revealing a virus that appears well established among dromedary camels (DC; *Camelus dromedarius*) across the Arabian Peninsula and parts of Africa. From infected DCs, the virus is thought to infrequently infect exposed humans. Concern was raised early on that patenting of the first viral isolate would lead to restricted access to the virus and to viral diagnostics (Sciencemag, 2014). However, sensitive, validated reverse transcriptase real-time polymerase chain reaction (RT-rtPCR)-based diagnostics were available (Abdel-Moneim, 2014) almost immediately. Virus was also made freely available subject to routine biosafety considerations, supporting many of the research findings described herein. In search of an animal host, bats were implicated in August 2013 (Memish et al., 2013a) but in that same month a DC link was reported (Reusken et al., 2013c) and that link has matured into a verifiable association. In humans, overt disease was finally given the name Middle East respiratory syndrome and the acronym MERS. From these animal-to-human spillover events, the MERS coronavirus (MERS-CoV; see Section 3 for variation in naming) spread sporadically among people, causing

* Corresponding author at: Level 7, SASVRC, Building C18, Back Road off Bramston Terrace, Royal Brisbane and Women's Hospital, Herston Q4029, Australia. Tel.: +61 7 3636 1619.

E-mail address: ian.mackay@uq.edu.au (I.M. Mackay).

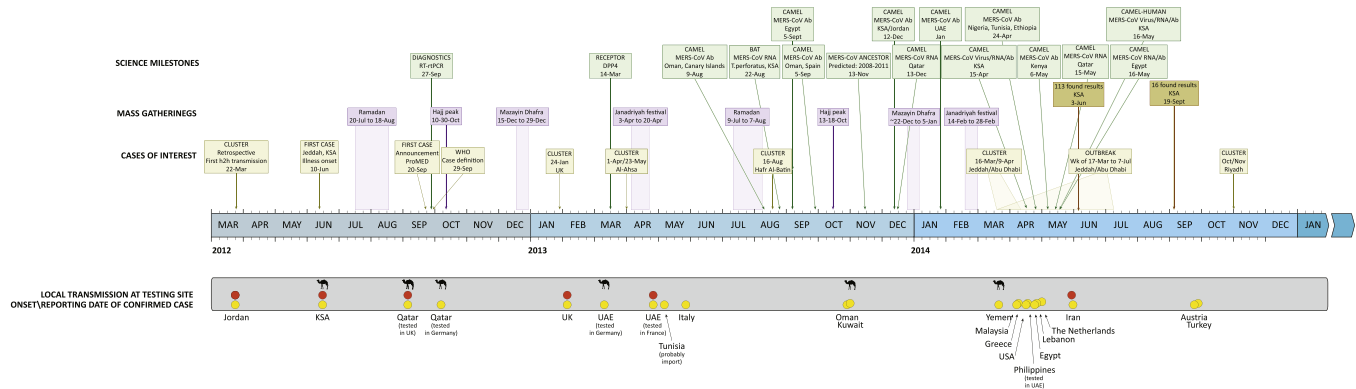


Fig. 1. A timeline of key scientific milestones, cases of interest and mass gatherings of relevance to the potential spread of MERS-CoV among humans and from animals to humans. A yellow circle indicates when a country reported a laboratory confirmed detection and an orange circle denotes ensuing local transmission. Mention of DC contact prior to disease is marked by a black camel icon.

more severe disease among older males with underlying diseases. The proportion of infected people who are confirmed to have died from MERS-CoV infection is much higher than for severe acute respiratory syndrome (SARS)-CoV, influenza virus or many other pathogens. The spread of MERS-CoV among humans has often been associated with outbreaks in hospitals, which in 2012–2014 usually commenced in March (Mackay, 2014; Maltezos and Tsiodras, 2014). This spread may be linked to some seasonal environmental changes, change in host animal behaviour, or perhaps simple coincidence between season and successive hospital outbreaks. Approximately a fifth of all cases to date have involved health-care workers (HCWs), spiking alongside periods of increased total case numbers. Social media, blogs and the mainstream media have kept close tabs on the spread of MERS-CoV to 23 countries in Europe, Asia and the United States of America (USA; Fig. 2), mostly with an origin in the KSA from where 88% of viral detections have occurred. Twitter in particular has provided a global forum through specific hashtags like #MERS and the Arabic hashtag #فئوروك or #Coruna. An engaged world has helped understand how the virus has affected the KSA and its neighbouring countries and allowed outsiders to view science musings take shape, collaborations form, local news and commentary trend and new results be discussed in real time. Social media provides new avenues for scientists to express experienced opinion, to more widely communicate their

research and to engage with public health entities, the public themselves and the mainstream media. This degree of engagement was not possible in 2002/2003 when the SARS global outbreak began its rise to 8100 human cases including 770 deaths (proportion of fatal cases, or PFC, of 9.5%). The ubiquity of social media appears to have changed what the public expects from a State when it communicates about new or existing infectious disease outbreaks and epidemics, and how quickly they expect that to occur.

2. Middle East respiratory syndrome (MERS)

Patients with MERS often present themselves to a hospital with systemic and lower respiratory tract (LRT) signs and symptoms of disease which usually include fever, chills or rigors, dry or productive cough, shortness of breath (dyspnea) and one or more comorbidities including diabetes (prevalent in the KSA), chronic kidney disease including renal failure, chronic heart disease and heart failure, recent surgery, hypertension, chronic lung disease, asthma, obesity, smoking, malignant disease or steroid use (Arabi et al., 2014; Assiri et al., 2013a; Hijawi et al., 2013; Zaki et al., 2012). MERS-CoV may be identified in patients with severe hypoxaemic respiratory failure and extrapulmonary organ dysfunction which can precede death in over a third of infections (Arabi et al., 2014; Assiri et al., 2013a; Hijawi et al., 2013; Zaki et al., 2012).

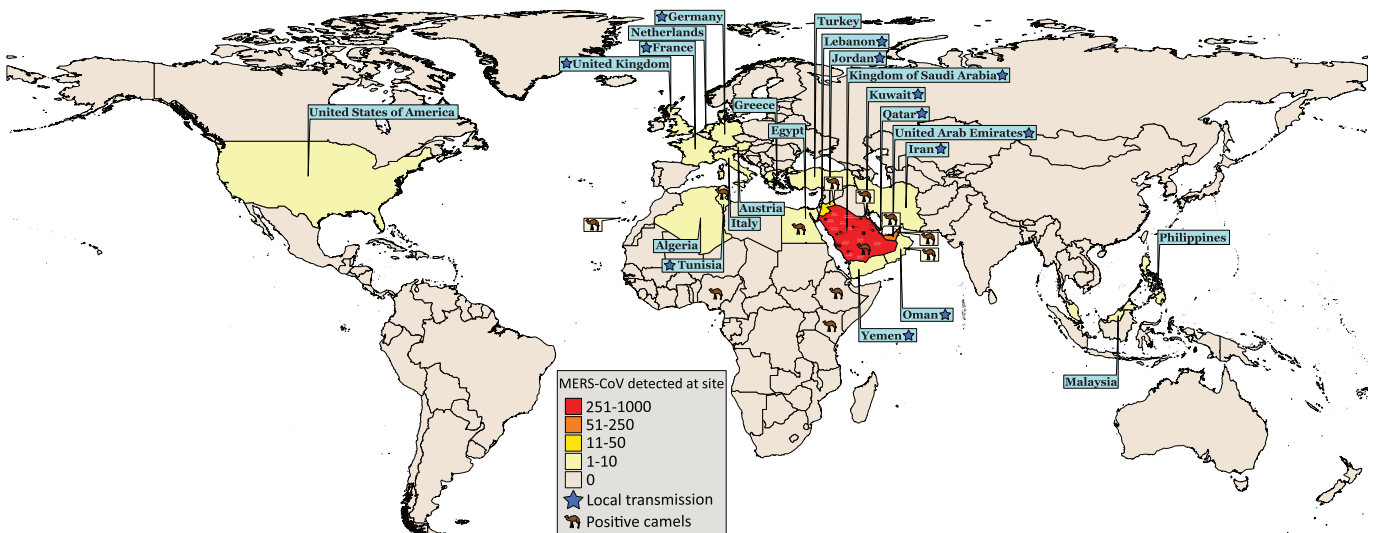


Fig. 2. The 23 countries in which MERS-CoV has been identified and a guide as to the number of cases at each location. Local transmission is highlighted (blue star) as are countries with DCs that contain antibodies reactive with MERS-CoV, viral RNA or infectious virus (camel icon). Correct as of the 20th January, 2015.

Extrapulmonary disease manifestations include circulatory, renal, hepatic and hematologic dysfunction. Gastrointestinal symptoms have been seen in 20–33% of cases (Assiri et al., 2013a; Mailles et al., 2013; Memish et al., 2013b; Zumla and Memish, 2014), manifesting as diarrhea, vomiting and abdominal pain. Gastrointestinal symptoms were not seen at all in one family cluster (Omrani et al., 2013) nor among symptomatic children in another (Memish et al., 2014a). On occasion, fever and gastrointestinal upset may form a prodrome, after which symptoms decline to be are later followed by more severe systemic and respiratory signs and symptoms (Kraaij-Dirkzwager et al., 2014; Mailles et al., 2013). Rarely, MERS-CoV has been detected in a person with fever but no respiratory or gastrointestinal symptoms (Memish et al., 2014a). The extent to which infection by other gastrointestinal pathogens affect this variability is unknown.

Chest radiography of MERS patients, as distinct from MERS-CoV positive people with a subclinical infection, reveal infiltrates consistent with acute viral pneumonia (Assiri et al., 2013a; Devi et al., 2014; Tsioupras et al., 2014; Zaki et al., 2012). Only on rare occasions have studies described upper respiratory tract (URT) signs and symptoms. In one example, approximately 15–25% of cases presented with rhinorrhoea and/or sore throat (2014e; Memish et al., 2013b; Payne et al., 2014; Zumla and Memish, 2014). To date, MERS has been an opportunistic disease. Severe MERS has been defined by admission to an intensive care unit; use of extracorporeal membrane oxygenation (ECMO), mechanical ventilation or vasopressors; cases being reported as critical or severe; a fatal outcome (Arabi et al., 2014). The relative speed of disease progression may relate to MERS-CoV reaching earlier peak viral loads and infecting different cells than the SARS-CoV (Drosten, 2013). Nonetheless, it is also apparent that MERS is not restricted to those with comorbidities. With increased laboratory testing, particularly of contacts of confirmed MERS cases, a number of MERS-CoV positive individuals without comorbidities have been detected who experience a mild illness or no symptoms at all (Al-Tawfiq and Memish, 2014). This demonstrates that MERS, like most respiratory viruses, is associated with a wide spectrum of symptoms and degrees of severity.

The mean incubation period in a study of 47 cases was 5.2 days, with 95% of cases having shown symptoms within 12.4 days (Assiri et al., 2013a). In a smaller study the incubation period ranged between one and nine days, with 13–14 days between when illness began in one person and subsequently spread to another (Memish et al., 2013b). The length and nature of the prodrome is undefined to date. The first WHO case definition (World Health Organization, 2014a) defined probable cases of MERS based on the presence of febrile illness, cough, requirement for hospitalization with suspicion of LRT involvement and included roles for contact with a probable or confirmed case or for travel or residence within the Arabian peninsula. If strictly adhered to, only the severe syndrome would meet the case definition and be subject to laboratory testing, which was the paradigm early on (Assiri et al., 2013a). From July 2013, the revised WHO case definition included the importance of seeking out and understanding the role of asymptomatic cases (World Health Organization, 2014d). Apart from reports from the WHO and KSA Ministry of Health, asymptomatic or subclinical cases of MERS-CoV infection have also been documented in the scientific literature (Drosten et al., 2014b; Memish et al., 2014b). In one such case, a HCW shed virus for 42 days in the absence of disease (Al-Gethamy et al., 2014).

MERS can progress to an acute respiratory distress syndrome (ARDS) requiring external ventilation and then to multiorgan failure (Devi et al., 2014; Reuss et al., 2014; Zaki et al., 2012) similar to severe influenza and SARS cases (Zaki et al., 2012). Acute renal failure can occur in MERS patients, doing so sooner than it did among SARS patients (Eckerle et al., 2013). Progressive impairment of renal function and acute kidney injury can start 9–12 days after

symptom onset among MERS patients, compared to a median of 20 days for SARS patients (Chu et al., 2005; Eckerle et al., 2013; Zaki et al., 2012). This may be due to direct infection of renal tissue by MERS-CoV (Arabi et al., 2014; Zaki et al., 2012). Haematologic changes in MERS cases include thrombocytopenia (Assiri et al., 2013a; Drosten et al., 2013; Omrani et al., 2013) and lymphocytosis (Assiri et al., 2013a) or lymphopenia (Assiri et al., 2013a; Omrani et al., 2013) on admission (Assiri et al., 2013a). Monocyte numbers are often normal (Assiri et al., 2013a) while neutrophils may be raised (Zaki et al., 2012) or normal (Assiri et al., 2013a).

As a group, children have rarely been reported to be positive for the virus. Between 1st September 2012 and 2nd December 2013, 11 paediatric cases (2–16 years of age; median 13-years) were identified in total; nine were asymptomatic (72%) and one died (Memish et al., 2014a). In Amman, Jordan, 1005 samples from hospitalised children under the age of 2-years with fever and/or respiratory signs and symptoms were tested but none were positive for MERS-CoV RNA, despite being collected at a similar time to the first known outbreak of MERS-CoV in the neighbouring town of Al-Zarqa (Khuri-Bulos et al., 2013). A second trimester stillbirth occurred in a pregnant woman during an acute respiratory illness and while not RT-rtPCR positive, the mother subsequently developed antibodies to MERS-CoV, suggestive of recent infection (Payne et al., 2014). Her exposure history to a MERS-CoV RT-rtPCR positive relative and an antibody-reactive husband, her incubation period and her symptom history met the WHO criteria for being a probable MERS-CoV case (Payne et al., 2014).

3. The Middle East respiratory syndrome coronavirus (MERS-CoV)

The virus associated with MERS was initially identified as the “novel coronavirus” or nCoV; a problematic choice given that other novel coronaviruses could be discovered and were being discovered with regularity prior to and since the identification of MERS-CoV. When the first genome of a human variant was sequenced it was named human betacoronavirus 2c EMC (subsequently referred to here as EMC/2012), with the implication that it was a human not animal coronavirus. There were also variants named England-Qatar, Jordan-N3 and England 1. Ten months after its discovery, the coronavirus study group assembled an international consensus and the virus was renamed and given the acronym of MERS-CoV (de Groot et al., 2013).

3.1. The viral genome

MERS-CoV is a putative member of a new species (van Boheemen et al., 2012) within the order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*, genus *Betacoronavirus*, subgroup 2c (Raj et al., 2014b). The first full sequence defined a single-stranded, positive sense, 30,119 nucleotide (nt) long genome (Fig. 3) (van Boheemen et al., 2012; Zaki et al., 2012). Based on analysis of 42 complete sequences, the genome is predicted to be evolving at 1.12×10^{-3} substitutions per site (Cotten et al., 2014). This permitted a predictive calculation of the time to most recent viral ancestor (tMRCA) for most of the variants, which suggested MERS-CoV first appeared around March 2012 (ranging from December 2011 to July 2012) (Cotten et al., 2014). Comparison of the first open reading frame's (ORF 1ab) amino acid sequence to that from its closest betacoronavirus relatives, *Tylonycteris* bat HKU4 and *Pipistrellus* bat HKU5, found there was less than 80% identity which supported the conclusion that MERS-CoV was a novel and distinct virus. This genomic region is a key taxonomic identifier of CoV species. MERS-CoV is predicted to encode at least ten open reading frames bracketed by

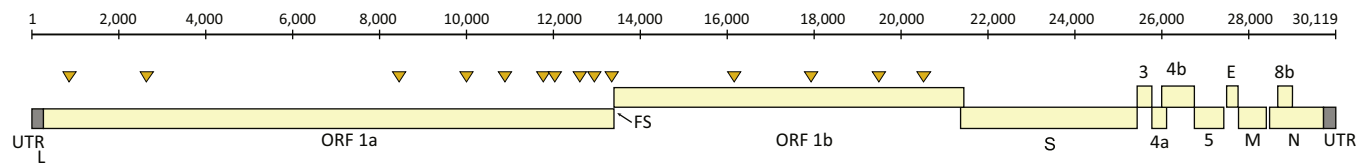


Fig. 3. Schematic of MERS-CoV genome (EMC/2012 variant isolated from sputum of a 60-year old man from Bisha, KSA). Open reading frames are indicated as yellow rectangles bracketed by terminal untranslated regions (UTR; grey rectangles). The 5'UTR includes the predicted leader (L) transcription-regulatory sequence. FS=frame-shift. Predicted papain-like proteinase cleavage sites are indicated with orange arrows resulting in ~16 cleavage non-structural protein products (based on (van Boheemen et al., 2012)). The genome is drawn to scale using Geneious v6.1.6 and annotated using Adobe Illustrator. MERS-CoV EMC/2012 sequence GenBank accession number JX869059 (Zaki et al., 2012).

5' and 3' untranslated regions (Raj et al., 2014b). Structural proteins include the spike (S), envelope (E), membrane (M) and nucleocapsid (N) (van Boheemen et al., 2012). Nonstructural proteins (nsps) from the products of ORF1a and ORF1b have been predicted, following identification of conserved domains and after comparative analysis with other coronaviral proteins. The nsps include a papain-like protease (PLpro; nsp4 (Kilianski et al., 2013; Lin et al., 2014) transmembrane domains (nsp4, nsp6), a 3C-like protease (3CLpro; nsp5 (Kilianski et al., 2013)), an RNA-dependent RNA polymerase (RdRp; nsp12), a helicase (nsp13) and an exonuclease (nsp14) (van Boheemen et al., 2012).

Complete genome deduction using deep sequencing methods has been the predominant tool for genome analysis during the emergence of MERS-CoV (Cotten et al., 2013a,b, 2014) the first time it's use has been so pervasive for the study of a viral outbreak with global reach. While the error rate can be higher than for traditional Sanger sequencing, the near-complete genomic length covered by just a single run (e.g. 90% of the MERS-CoV EMC/2012 genome) and the depth of coverage at each nucleotide is such that deep sequencing corrects for erroneous nucleotide (Raj et al., 2014a; van Boheemen et al., 2012). Subgenomic sequencing, a mainstay of viral genotyping and molecular epidemiology to this point, has been used rarely for MERS-CoV identification or confirmation, despite assays having been suggested early on Corman et al. (2012b). Such an approach is simpler, more accessible to a wider range of laboratories and faster. It's utility has since been demonstrated using molecular assays to amplify and sequence a 615 nucleotide long fragment of the spike S2 domain gene fragment (Smits et al., 2015). This assay agreed with the results generated by the sequencing of full genomes and defined additional sequence groupings within an existing MERS-CoV clade. With the addition of more genomes over time from both humans and from DCs, two clades have become apparent; A and B. Clade A contains only human-derived MERS-CoV genomes (Fig. 4) (Cotten et al., 2013b).

3.2. Genomic variability and molecular epidemiology

To date, the MERS-CoV genomes collected from samples spanning just two years are genetically very similar to each other. An alignment of 56 complete or near-complete MERS-CoV genomes sampled from 2012 to 2014 differed by 0–0.38%. For comparison, an alignment of 31 complete HCoV-NL63 genomes from samples collected between 1983 and 2009 shows they diverge by 0.5% at the nucleotide level (data not shown; theoretically equates to 145nt for a 27,553nt genome). There is as yet no study which attaches clinical relevance to the clades or smaller groupings of MERS-CoV nor any of the genomic variation noted to date (Drosten et al., 2015). It is interesting that Clade A contains only the African green monkey kidney (Vero; innate immune deficient cells) cell-culture passaged EMC/2012 variant and two variants of the Jordan-N3 variant from 2012, but no camel-derived MERS-CoV genomes (Cotten et al., 2013b). When the MERS-CoV genome of the variant from Bisha was re-sequenced directly from the original URT sample, the

comparison of trimmed genomes (EMC/2012 vs. Bisha_1) revealed 115 nucleotide differences (0.38% difference) resolving Bisha.1 into Clade B (Cotten et al., 2013b). This is unusual because when a Jordan-N3 virus was intentionally serially passed through Vero or MRC5 cell culture (Jordan-N3/2012 MG167), only 2nt changes occurred within the entire coding region of the resultant sequence, despite eight passages (Frey et al., 2014). For comparison, after three passages through Vero cell culture, no genetic changes were found in a DC MERS-CoV variant of Qatar_2.2014 (Raj et al., 2014a).

A very divergent MERS-CoV variant originated from an Egyptian DC likely imported from Sudan was identified as NRCE-HKU205|Nile|2013. It constructs a lineage outside the current clades, perhaps comprising the first occupant of Clade C (Corman et al., 2014a; Cotten et al., 2013b; Smits et al., 2015). This lineage may represent additional diversity of MERS-CoV variants remaining to be discovered in DC from outside the Arabian peninsula. A virus sequenced from a *Neoromicia capensis* bat was more closely related to MERS-CoV than previous bat sequences had been, providing a link between human, camel and bat viruses as members of the same CoV species (Corman et al., 2014a). Despite usually comprising $\leq 1\%$ of the total genome, *in silico* comparison shows that viral genetic changes among variants permit geographic tracking of the spread of variants and identification that Riyadh, in particular, harbours a wide range of MERS-CoV variants (Fig. 4) (Cotten et al., 2013b). This process of molecular epidemiology can also imply some physical direction to the movement of MERS-CoV around the region and over time (Cotten et al., 2014).

When compared to Bisha_1.2012, most single nucleotide differences among variants were located in the last third of the genome, encompassing the S protein (Fig. 5) and accessory proteins (Cotten et al., 2013b). At least nine MERS-CoV genomes harbour amino acid substitutions in the ribosome binding domain (RBD) of the spike protein and codons 158 (N-terminal region), 460 (RBD), 1020 (in heptad repeat 1), 1202 and 1208 bear investigation as markers of adaptive change (Cotten et al., 2014; Raj et al., 2014a). Studies are needed to determine whether there any functional outcomes on virus replication and transmission due to these and future changes (Cotten et al., 2014). An early *in vitro* analysis did not find differences in shedding, replication or immune escape among viruses isolated up to May 2014 (Drosten et al., 2015). The location and crystal structure of the RBD was described in several reports from mid-2013 (Chen et al., 2013; Du et al., 2013b; Lu et al., 2013; Mou et al., 2013; Wang et al., 2013).

4. Molecular detection of MERS-CoV infection

Early diagnostic methods appeared within days of the ProMED email announcing the first MERS case. These included Vero and LLC-MK2 cell culture and several in-house RT-rtPCR assays (Fig. 6) (Corman et al., 2012a,b; Zaki et al., 2012). Antibody testing of human sera remains rare.

RT-rtPCR assays validated by Corman et al. were quickly recommended by the WHO having been shown to be sensitive

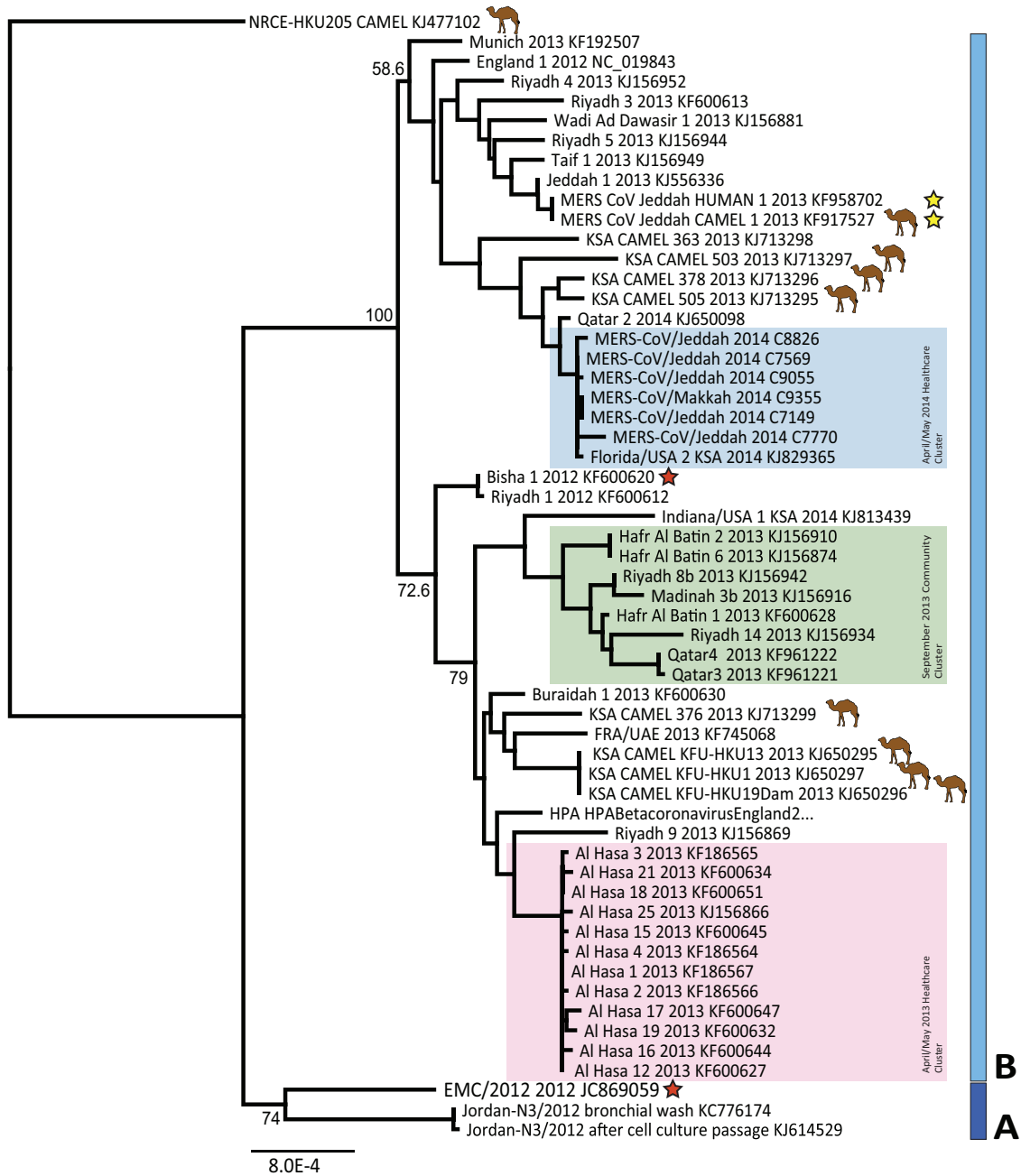


Fig. 4. The genetic relationship between all near-complete and complete MERS-CoV genome nucleotide sequences (downloaded from GenBank using the listed accession numbers; England2 was obtained from <http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/MERSCoV/respPartialgeneticsequenceofnovelcoronavirus/%5D>). This neighbour joining tree was created in MEGA v6 using an alignment of human and DC-derived MERS-CoV sequences (Geneious v6). The tree was rooted using NRCE-HKU205 CAMEL KJ477102. Clades are indicated next to dark (Clade A) or pale (Clade B) blue vertical bars. Camel icons denote genomes from DC. MERS-CoV sequences described as originating from the same patient either before (Bisha.1) or after (EMC/2012) passage in cell culture (Cotten et al., 2013b) are starred (red). Identical genomes from a human and one of his ill camels from Jeddah (Azhar et al., 2014a), the KSA are also starred (yellow). Healthcare or community outbreaks are boxed (blue, green, pink) and labelled.

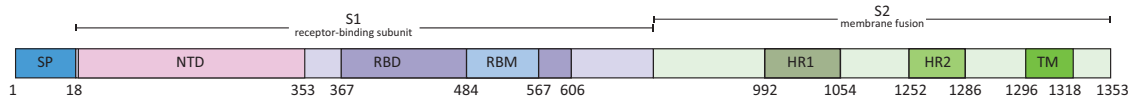


Fig. 5. Schematic representation of the MERS-CoV S protein. SP-signal peptide; NTD, N-terminal domain; RBD, receptor binding domain; RBM, receptor binding motif; HR, heptad repeat; TM, transmembrane domain; S1/S2, S Spike protein subunits separated after protease cleavage. Numbering from Lu et al. (2013) and Du et al. (2013a).

and specific (Corman et al., 2012a,b). The target sequences of the recommended screening assays remained conserved among genomes until at least mid-2014 when last checked by IMM (Fig. 6).

RT-rtPCR on the upstream E region (*upE*; Fig. 6) of the MERS-CoV genome was used to screen 5235 nasopharyngeal swabs from 3210 incoming (29th September to 9th October 2013) and 2025 outgoing (14th October to 26th October 2013) adult pilgrims who

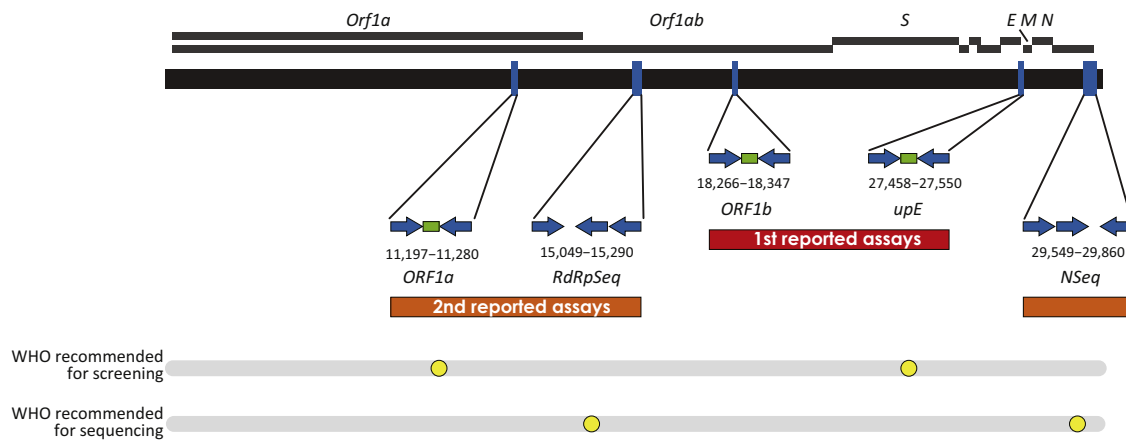


Fig. 6. A schematic depicting the location of primers (blue arrows indicate direction) and oligoprobes (green rectangles) for the earliest RT–rtPCR screening assays and conventional, semi-nested (three primers) RT–PCR confirmatory sequencing assays (Corman et al., 2012a,b). Publication order is noted by first [27th September 2012; red] and second [6th December 2012; orange] coloured rectangles, both by Corman et al. Those assays recommended by the WHO (World Health Organization, 2013b) are highlighted underneath by yellow dots.

performed the Hajj in 2013, with no cases being identified (Memish et al., 2014d). This may reflect the absence of MERS-CoV, no LRT testing (Memish et al., 2014d) or that 61% of pilgrims were arriving from countries without any known MERS-CoV circulation. Similarly, no MERS-CoV was identified during the 2012 in Hajj among the nasal swabs from 154 pilgrims tested by RT–rtPCR (Corman et al., 2012a) before leaving for and departing from, the KSA nor from 114 swabbed pilgrims with influenza-like illness in the KSA during the Hajj in 2013 (Barasheed et al., 2014; Gautret et al., 2013). Across a period of rapid case accumulation and intense screening in Jeddah during March to July 2014 (called the Jeddah–2014 outbreak hereafter), ~5000 samples were tested in a month yielding ~140 MERS-CoV detections (~3% prevalence) (MERS, 2014g). Among 5065 individuals sampled and tested across the KSA between 1st October 2012 and 30th September 2013, 108 (2.1%) detections were made using the *upE* and ORF 1a assays. This was a hospital-centric population which included hospitalised cases ($n=2908$), their families ($n=462$) and associated HCWs ($n=1695$) (Memish et al., 2014b). Among the detections, 19 were HCWs and 10 were family contacts (Memish et al., 2014b). During times of high MERS-CoV activity, its 2–3% prevalence is not very different from a more hospital-based prevalence for other HCoVs (Mackay et al., 2012). Given the proportion of deaths among those infected with MERS-CoV, it is not a virus that should reasonably be described as a “storm in a teacup”, however to date it has been given many “opportunities” for worldwide spread and it has not yet taken any of them.

4.1. Specimen types and length of viral shedding

Data have shown one or more RT–rtPCR negative URT samples from clinically suspect MERS cases may be contradicted by further URT sampling or the preferred use of LRT samples (Birmingham et al., 2012; Omrani et al., 2013). Higher viral loads occur in the LRT compared to the URT, especially with increasing time from onset of symptoms (CDC, 2014; Drosten et al., 2013; Memish et al., 2013b). Since the majority of disease symptoms appear to have been manifesting as systemic and LRT disease, this may not be surprising (Assiri et al., 2013a). However at writing, no human data exist to define whether the virus replicates solely in the LRT, the URT, has a preference for one over the other, or replicates in other human tissues *in vivo*. Sampling of the URT has been frequently noted from the largest human MERS-CoV investigative studies (Gautret et al., 2013; Health Protection Agency (HPA) UK Novel Coronavirus

Investigation Team, 2013; Memish et al., 2014b,d) and, if noted, for other smaller investigative MERS-CoV testing (Kraaij-Dirkzwager et al., 2014; Memish et al., 2013b). In a macaque monkey model, MERS-CoV RNA was shed from both the URT and LRT (deWit et al., 2013). In a human case, throat swabs were positive for six days, and again after a gap (Kraaij-Dirkzwager et al., 2014). In another case, a 40-year old female HCW shed MERS-CoV RNA from the URT (Ziad A. Memish, personal communication) for at least 42-days between April and June 2014 (Al-Gethamy et al., 2014). She did not show signs of disease during her time shedding virus. In a study of MERS cases in an intensive care setting, three of 12 patients shed virus for 12–22 days (Arabi et al., 2014). Elsewhere, viral RNA was detected in human oronasal swabs for 16 days (Drosten et al., 2013) and in pharyngeal or endotracheal aspirates for 24 days (Spanakis et al., 2014). Over three quarters of MERS cases shed viral RNA in their lower respiratory tract specimens (tracheal aspirates and sputum) for at least 30 days while only 30% of contacts were still shedding RNA in their upper respiratory specimens (Memish et al., 2014e).

The LRT is a WHO-recommended sampling site for MERS-CoV RT–rtPCR testing, especially when collection of samples will be delayed by a week or more after symptom onset (World Health Organization, 2013b). Samples to test include bronchoalveolar lavage (BAL), tracheal/tracheobronchial aspirate, pleural fluid and sputum (CDC, 2014; World Health Organization, 2013b). Fresh samples yield better diagnostic results than refrigerated material (Drosten et al., 2013) and if delays of ≥ 72 h are likely, samples (except for blood) should be frozen at -70°C (CDC, 2014). Lung biopsy or autopsy tissues can also be tested if available (World Health Organization, 2013b). From the URT, which is a less invasive and convenient sampling site, a combined nose and throat swab or a nasopharyngeal aspirate is recommended (CDC, 2014). Paired sera, collected two to three weeks apart are preferable for serological testing while a single sample is preferred if collected two weeks after onset of disease (World Health Organization, 2013b).

Urine has been found to contain MERS-CoV RNA 12 and 13 days after symptom onset and stool samples were RT–rtPCR positive up to 16 days after onset (Drosten et al., 2013; Kraaij-Dirkzwager et al., 2014); both sample types should be considered (CDC, 2014; World Health Organization, 2013b). In two cases that arrived in the Netherlands, urine was RT–rtPCR negative but faeces was weakly positive while sera were RT–rtPCR positive for five days or more (Kraaij-Dirkzwager et al., 2014). MERS-CoV viral RNA detection in serum has proven a useful retrospective source of PCR template when respiratory samples were not available (Hijawi et al., 2013). RNAemia may also correlate with disease severity; signs of virus

cleared from serum in one recovered human case while lingering until the death of another (Faure et al., 2014).

In a study of different sample types (64 nasopharyngeal [NPA], 30 tracheal aspirates, 13 sputa and 3 BAL), tracheal aspirates and BAL returned the best viral load values followed by NPA and sputum, which generally equated with whole genome sequencing success (Memish et al., 2014c). This represents the only study of the effect of sample type on molecular analysis and it confirmed both the importance of LRT sampling for whole genome sequencing, while noting that 57% of samples from 112 distinct patients in the KSA in fact originated from the URT (Memish et al., 2014c).

4.2. MERS-CoV and other viruses and bacteria

Many studies make no mention of additional testing for endemic human respiratory viruses or bacteria (Assiri et al., 2013a,b; Devi et al., 2014). When viruses are sought, they include some of the following: human herpesvirus (HHV), rhinovirus (HRV), enterovirus (EV), respiratory syncytial virus (RSV), parainfluenzavirus types 1, 2 and 3 (PIVs), influenzaviruses (IFVs), endemic HCoVs, adenoviruses (AdVs) and metapneumovirus (MPV) and co-detections with MERS-CoV do occur (Bermingham et al., 2012; Drosten et al., 2013; Health Protection Agency (HPA) UK Novel Coronavirus Investigation Team, 2013; Memish et al., 2013b). When included, other viruses are often absent in the samples positive for MERS-CoV (Al-Tawfiq et al., 2014; Bermingham et al., 2012; Kraaij-Dirkzwager et al., 2014; Omrani et al., 2013) but have been found in samples negative for MERS-CoV during such rare MERS investigations (Reuss et al., 2014). Tests on the first human case in the KSA used a LRT sample to isolate MERS-CoV in culture, conduct immunofluorescence for some viruses (negative for IFV, PIVs, RSV and AdVs) and RT-PCR for other viruses (negative for AdV, EVs, MPV and HHVs) (Zaki et al., 2012). RT-PCR also detected MERS-CoV. A case exported to Greece in April-2014 was tested (and found negative) for IFVs, *Legionella* and *Pneumococcus* (Tsiodras et al., 2014). Other bacterial testing has been conducted but the impact of bacterial co-presence is also unclear (Al-Tawfiq et al., 2014; Devi et al., 2014; Memish et al., 2013b; Tsiodras et al., 2014). Two MERS cases that travelled from the KSA to the Netherlands were negative for RSV, AdV, bocavirus (BoV), PIVs, HCoVs, HRV, IFVs, RSV and MPV (Kraaij-Dirkzwager et al., 2014). Testing for other respiratory pathogens is strongly recommended (World Health Organization, 2013b) but limited data address the occurrence of co-infections or alternative viral diagnoses among both cases and contacts suspected of MERS-CoV infection. Little is known of other causes of MERS-like pneumonia in the KSA or of the general burden of disease due to the known classical respiratory viruses including endemic other human coronaviruses.

4.3. Serological surveys to identify prior MERS-CoV infection

Despite widespread use in elucidating the role of DCs as a source for MERS-CoV, no strategic and widespread sero-surveys have been conducted in humans using samples collected post-2012. The development of robust serological assays hinges on the accessibility of a reliable panel of well-characterised animal or human sera including those positive for antibodies specific to MERS-CoV and to likely agents of cross-reaction (Meyer et al., 2014a). Obtaining these control materials has been problematic and has slowed the development and commercialization of assays for human testing (Meyer et al., 2014a). One company has produced an ELISA kit to detect IgG in camels using a recombinant S1 receptor-binding subunit of MERS-CoV S protein as the antigen and produces a diagnostic reagent for immunofluorescence (Drosten et al., 2014b; EUROIMMUN Medizinische Labordiagnostika AG, 2014). Sero-surveys are essential to determine a baseline of animal and

community exposures to MERS-CoV among countries in the Arabian Peninsula. Early sero-surveys of humans used conventional immunofluorescent assays (IFA) in which antibodies, if present in patient sera, attach to MERS-CoV infected cell cultures to identify the presence of IgG, IgM or neutralising antibodies (Corman et al., 2012b; Drosten et al., 2013; Zaki et al., 2012). No sign of MERS-CoV antibody was found among 2400 sera from patients visiting the Dr. Soliman Fakeeh Hospital in Jeddah, KSA from 2010 through 2012 (Zaki et al., 2012), and no sign of prior MERS-CoV infection was found among 130 healthy blood donors screened at King Abdulaziz University Hospital in Jeddah (collected between January and December 2012). Eight of 226 slaughterhouse workers were positive by IFA, but those results could not be confirmed by neutralization (NT) test. The study indicated that HCoV-HKU1 was a likely source of cross-reactive antigen by IFA (Aburizaiza et al., 2014). An absence of MERS-CoV antibodies among slaughterhouse workers may reflect the killing of older DCs which are less often MERS-CoV positive (see Table 1), the rarity of infected animals, a limited transmission risk associated with slaughtering DCs (Aburizaiza et al., 2014), a weak immune response by humans who do not get severe MERS, or an overall low risk of MERS-CoV transmission by contact. IFA also suffered from some cross-reactivity with convalescent SARS patient sera which could not be resolved by an NT test (Chan et al., 2013b). The need for well-validated assays was further emphasised when publicly released MERS-CoV antibody test results indicated that a handshake and two face-to-face meetings were sufficient for MERS-CoV transmission between two people in the USA (CDC Newsroom, 2014; Sampathkumar, 2014). These results were subsequently retracted because they did not withstand further confirmatory analysis with the less rapid, but highly specific, NT assay (CDC Newsroom, 2014).

A more biologically safe IFA was developed that did not require infectious virus but was instead based on transfected cells expressing recombinant portions of the MERS-CoV N and S genes (Corman et al., 2012b; Reuss et al., 2014). Recombinant lentiviruses expressing MERS-CoV S protein and luciferase are also safer and simpler diagnostic alternatives to working with infectious MERS-CoV (Perera et al., 2013; Zhao et al., 2013). A pseudo particle neutralization (ppNT) assay has seen widespread use in animal studies and is at least as sensitive as the microneutralization (MNT) test (Hemida et al., 2013, 2014a, 2015; Perera et al., 2013; Reusken et al., 2013b). In a study of sera collected at the King Fahd Hospital, Eastern region of the KSA (158 from children with LRT infections between May 2010 and May 2011 and 110 from 19 to 52 year old male blood donors) no evidence of MERS-CoV neutralising antibody could be found using the ppNT assay (Gierer et al., 2013). Similarly, in a study of four herdsmen in contact with an infected DC herd in Al-Ahsa, eight people who had intermittent contact with the herd, 30 veterinary surgeons and support staff who were not exposed to the herd, three unprotected abattoir workers in Al-Ahsa and 146 controls who were not exposed to camels in any professional role, none had serological evidence of past MERS-CoV infection using the ppNT assay (Hemida et al., 2015).

MERS-CoV does not appear to be easily transmitted from DCs to humans, or perhaps it does not trigger a detectable immune response if only mild disease or asymptomatic infection results. A study of such cases is an important missing link for interpretation of these negative human serology data. A Jordanian outbreak of acute LRT disease in a hospital in Al-Zarqa in 2012, which predated the first KSA case of MERS, was retrospectively found to have been associated with MERS-CoV infection, initially via RT-rtPCR, but subsequently, and on a larger scale, using positivity by ELISA and IFA or MNT test (Al-Abdallat et al., 2014; Hijawi et al., 2013; Payne et al., 2014). The ELISA used a recombinant N protein from the group 2 betacoronavirus (*Pipistrellus*) bat-CoV HKU5 to identify antibodies against the equivalent cross-reactive MERS-CoV protein and

Table 1
Animal epidemiology studies seeking evidence of MERS-CoV infections.

Animal type Number if known/DC age if known	Sample type/number	Year collected	Region of animal origin	Assays used	Positive for antibody/RNA	Reference
DC 88/Adult ^c 9/3–4 yrs 8/≤2 yrs	Sera/105	2012–13	Canary-Islands	S1 subunit (CoV) antibody capture protein-microarray ¹ MERS-CoV EMC/2012 neutralization	14% 18% of seropositive subset	Reusken et al. (2013c)
DC 50/Adult	Sera/50	2012–13	Oman (born in Africa)	S1 subunit (CoV) antibody capture microarray ^a Neutralization of MERS-CoV EMC/2012	100% 100%	Reusken et al. (2013c)
Llamas Alpacas Bactrian camels Cattle Goats Sheep Llamas Alpacas Bactrian camels Guanaco	Sera/2 Sera/6 Sera/2 Sera/40 Sera/40 Sera/40 Sera/5 Sera/18 Sera/2 Sera/2	2012–13	Netherlands	S1 subunit (CoV) antibody capture microarray ^a	0% 0% 0% 0% 0% 0% 0% 0% 0% 0%	Reusken et al. (2013c)
Goats	Sera/40	2012–13	Spain	S1 subunit (CoV) antibody capture microarray ^a	0%	Reusken et al. (2013c)
DC 110/Adult	Sera	2013	Egypt (4–5 months after import from Sudan)	MNT	94%	Perera et al. (2013)
Water buffalo 8 Cows 25 Sheep 5 Goat 13 Human/815	Sera	2013	Egypt	ppNT ^b MNT ppNT ^b	0	Perera et al. (2013)
Human 528 Birds 2040 Pig 260 DC 310 Sheep 100 Goats 45 Cattle 50 Chickens 240	Sera/815 Sera/528 Sera/2040 Sera/260 Sera/310 Sera/100 Sera/45 Cattle/50 Chickens/240	2012–2013 2011–2012 2010 2012 2012–2013 2012–2013 2010–2012 2010–2013 2012–2013	Egypt Hong Kong Controls Hong Kong Hong Kong KSA KSA	MNT ppNT ^b MNT ppNT ^b MNT ppNT ^b ppNT ^b ppNT ^b ppNT ^b	0% 0% 0% 0% 0% 0% 90% 0% 0% 0% 0%	Perera et al. (2013) Perera et al. (2013) Perera et al. (2013) Perera et al. (2013) Perera et al. (2013) Hemida et al. (2013) Hemida et al. (2013)

Table 1 (Continued)

Animal type Number if known/DC age if known	Sample type/number	Year collected	Region of animal origin	Assays used	Positive for antibody/RNA	Reference
DC 11 Juvenile	Sera	2013	Jordan	S1 subunit (CoV) Ab capture microarray ^a MNT PRNT	100% 100% 100%	Reusken et al. (2013b)
Sheep 126	Sera/126	2013	Jordan	S1 subunit (CoV) Ab capture microarray ^a MNT PRNT	5% 0%	Reusken et al. (2013b)
Goats 150	Sera/150	2013	Jordan	S1 subunit (CoV) Ab capture microarray ^a	0%	Reusken et al. (2013b)
Cows 91	Sera/91				0%	
DC 14	Nasal swab/14	2013	Qatar	RT-rtPCR (upE, N and ORF1a)	79% (43% by ≥2 assays)	Haagmans et al. (2014)
	Rectal/14				0%	
	Sera/14			RT-rtPCR (upE, N and ORF1a)	100% 100%	
DC 9/Adults	Sera/33	2005	UAE	S1 subunit (CoV) Ab capture microarray ¹ MNT S1-based ELISA (Sino biologicals) Neutralization	91% 100%	Alexandersen et al. (2014)
DC 2/Juvenile	Sera/14	2005	UAE	S1-based ELISA (Sino biologicals) Neutralization	0% 0%	Alexandersen et al. (2014)
Horse 3	Sera/17	2005	UAE	S1-based ELISA (Sino biologicals)	0%	Alexandersen et al. (2014)
Sheep 3	Sera/20				0%	
DC 6	Sera/6	2005	North America (born Australia, 1990s)	S1-based ELISA (Sino biologicals)	0%	Alexandersen et al. (2014)
DC 108/Adult	Sera/108	2013	KSA	ELISA (MERS-CoV Hu/Jordan-N3/2012)	95%	Alagaili et al. (2014)
	Nasal swabs/108				15%	
DC 98/Juvenile	Sera/98	2013	KSA	RT-rtPCR (upE, ORF1a) ELISA (MERS-CoV Hu/Jordan-N3/2012)	55%	Alagaili et al. (2014)
	Nasal swabs/98				35%	
Goats 36	Sera/36	2013	KSA	RT-rtPCR (upE, ORF1a) ELISA (MERS-CoV Hu/Jordan-N3/2012)	0%	Alagaili et al. (2014)
	Nasal swabs/36				0%	
Sheep 112	Sera/112	2013	KSA	RT-rtPCR (upE) ELISA (MERS-CoV Hu/Jordan-N3/2012)	0%	Alagaili et al. (2014)
	Nasal swabs/78				0%	
DC 264	Sera/264	1992–2010	KSA	RT-rtPCR (upE) ELISA (MERS-CoV Hu/Jordan-N3/2012)	87%	Alagaili et al. (2014)

Table 1 (Continued)

Animal type Number if known/DC age if known	Sample type/number	Year collected	Region of animal origin	Assays used	Positive for antibody/RNA	Reference
DC 151	Sera/151	2003	UAE	Recombinant MERS spike IFA (Corman et al., 2012b) MNT (>1280)	100% 57%	Meyer et al. (2014b)
DC 182	Sera/182	2013	UAE	Recombinant MERS spike IFA (Corman et al., 2012b) MNT (>1280)	96% 45%	Meyer et al. (2014b)
DC 100/2–8 yrs	Sera/100	2013	UAE	Recombinant MERS spike IFA (Corman et al., 2012b) MNT (>1280)	89% 42%	Meyer et al. (2014b)
DC 218/Adult	Sera/218	2013	UAE (from KSA, Sudan, Pakistan, Oman)	Recombinant MERS spike IFA (Corman et al., 2012b) MNT (>1280)	99% 84%	Meyer et al. (2014b)
Bactrian Camel 16 Controls DC ^d Adult/21	Sera/16 Nasal swabs/9 Rectal swabs/19 Blood/30	2013 2013–14	German Zoo KSA	Recombinant MERS spike IFA (Corman et al., 2012b) RT-rtPCR (UpE (Corman et al., 2012a), ORF1a (Corman et al., 2012b)) RT-rtPCR (UpE (Corman et al., 2012a), ORF1a (Corman et al., 2012b)) ppNT ^b	0% 22% 5% 100% 43% (3/7 PCR-positive)	Meyer et al. (2014b) Hemida et al. (2014a)
DC ^d 20/Juvenile	Nasal swabs/18 Rectal swabs/18 Blood/15	2013–14	KSA	Vero cell culture RT-rtPCR (UpE (Corman et al., 2012a), ORF1a (Corman et al., 2012b)) RT-rtPCR (UpE (Corman et al., 2012a), ORF1a (Corman et al., 2012b)) ppNT ^b	33% 0% 87%	Hemida et al. (2014a)
DC 76	Nasal and conjunctival swabs/76	2013	Oman	RT-rtPCR; ORF1a (Corman et al., 2012b) and ORF1b (Corman et al., 2012a)	7%	Nowotny and Kolodziejek (2014)
DC 358/Adults/ DC 204	Sera/358 Sera/204	2010–2011 2009, 2013	Nigeria Tunisia	S1 subunit (CoV) Ab capture microarray ^a	94%	Reusken et al. (2014b)
DC 188	Sera/188	2011–2013	Ethiopia	S1 subunit (CoV) Ab capture microarray ^a	30% of juveniles 54% of adults	Reusken et al. (2014b)
DC 774	Sera	1992–2013	Kenya	Recombinant spike (S1) ELISA (Memish et al., 2014f) Recombinant spike (S1) IFA (Corman et al., 2012b) MNT	93% of juveniles 97% of adults	Reusken et al. (2014b)
DC 110/Adult	Nasal swabs/110 Sera/52	2013	Egypt	Recombinant spike (S1) ELISA (Memish et al., 2014f) Recombinant spike (S1) IFA (Corman et al., 2012b) MNT RT-rtPCR (UpE (Corman et al., 2012a), ORF1a (Corman et al., 2012b)) Culture on RT pos ppNT ^b	30% 28% 15%	Corman et al. (2014b)
					4% 0% 92%	Chu et al. (2014)

Table 1 (Continued)

Animal type Number if known/DC age if known	Sample type/number	Year collected	Region of animal origin	Assays used	Positive for antibody/RNA	Reference
DC 5/Adult		2013	KSA	RT-rtPCR (UpE (Corman et al., 2012a)) Recombinant spike (S1) ELISA (Raj et al., 2013) Recombinant spike (S1) IFA (Corman et al., 2012b)	0% 100% (5/5) 0%	Memish et al. (2014f) ^e
DC 4/Juvenile		2013	KSA	RT-rtPCR (UpE (Corman et al., 2012a)) Recombinant spike (S1) ELISA (Raj et al., 2013) Recombinant spike (S1) IFA (Corman et al., 2012b)	22% (2/4) ^f 100% (4/4) 1/1 ^f	Memish et al. (2014f) ^e
DC ^d 4/Juvenile	Nasal swabs Sera	2013	KSA	RT-rtPCR (UpE (Corman et al., 2012a), ORF1a (Corman et al., 2012b), ORF1b (Corman et al., 2012b)) Vero cell culture IFA	25% (1/4) 25% 100%	Azhar et al. (2014a) ^e
DC 5/Adult	Nasal swabs Sera	2013	KSA	RT-rtPCR (UpE (Corman et al., 2012a), ORF1a (Corman et al., 2012b), ORF1b (Corman et al., 2012b)) Vero cell culture IFA	0% 0% 100%	Azhar et al. (2014a) ^e
DC 25/Adult	Sera	2014	Australia	ppNT ^b MNT ^h	0% 0%	Hemida et al. (2014b)
DC 131	Sera	1993	KSA	ppNT ^b MNT ^h	90% NS	Hemida et al. (2014b)

^a Assay from Reusken et al. (2013a); S1-spike subunit 1.

^b Pseudoparticle neutralization test (Perera et al., 2013).

^c Adult \geq 5 yrs.

^d Virus was cultured from samples.

^e Samples from same farmer and camel herd collected days apart.

^f Two positive camels were juveniles, only camel G could be confirmed externally and was further analysed by recombinant spike (S1) IFA.

^g Plaque reduction neutralization test (Reusken et al., 2013c).

^h Microneutralization test (Hemida et al., 2013); IFA – immunofluorescence assay; KSA – Kingdom of Saudi Arabia; LIPS – Luciferase immunoprecipitation assay (Alagaili et al., 2014) based on recombinant MERS-CoV nucleoprotein; MERS-CoV – Middle East respiratory syndrome coronavirus; MNT – microneutralization test; NS – not stated; RT-rtPCR – reverse transcriptase real-time polymerase chain reaction; UAE – United Arab Emirates.

was validated using 545 sera including some from people known to have had previous HCoV-OC43, HCoV-229E, SARS-CoV, HCoV-NL63, HRV, HMPV or influenza A(H1N1) infections (Al-Abdallat et al., 2014). A protein microarray expressing the S1 protein subunit has also been validated and widely used for DC testing (see Table 1) (Reusken et al., 2013a) but not for human screening to date. Using the most immunogenic portion or subunit of a specific viral antigen is considered the best approach for producing reliable serological assays (Meyer et al., 2014a). Detection of MERS-CoV infection using ELISA or S1 subunit protein microarray (Reusken et al., 2013a) has usually been followed by confirmatory IFA and/or a plaque-reduction neutralization (PRNT) test (Aburizaiza et al., 2014; Drosten et al., 2013; Reusken et al., 2013c) or MNT test (Meyer et al., 2014b; Perera et al., 2013; Reusken et al., 2013c). The confirmatory methods ensure the antibodies detected using more subjective screening methods that may also employ a spectrum of potentially cross-reactive antigens, are able to specifically neutralise the intended virus and are not more broadly reactive to other coronaviruses found in DCs (bovine CoV, BCoV) or humans (HCoV-OC43, HCoV-229E, HCoV-NL63, HCoV-HKU1, SARS-CoV).

5. MERS-CoV cell, tissue tropism and receptor

Early work identified that a range of cells underwent cytopathic changes when infected with MERS-CoV in culture. These included cells from (Eckerle et al., 2013; Zaki et al., 2012; Ziebeck et al., 2013):

- human tracheobronchial epithelium,
- human primary renal epithelium,
- African green monkey kidney (Vero and Vero E6; IFN-deficient),
- rhesus monkey kidney (LLC-MK2),
- human bronchial epithelium (Calu-3).

MERS-CoV grows more efficiently than SARS-CoV in primary bronchial cells and in primary kidney cells (Eckerle et al., 2013).

Other cells or cell lines that support MERS-CoV transcription include (Eckerle et al., 2014; Muller et al., 2012):

- porcine kidney cancer (PS),
- human kidney cancer (769-P),
- human alveolar adenocarcinoma (A549),
- bat kidney (Rousettus aegyptiacus, RoNi/7; Pipistrellus pipistrellus, PipNi/1 and PipNi/3; Carollia perspicillata, CarNi/1),
- bat lung (Rhinolophus landeri, RhiLu; Myotis daubentonii, MyDauiNi/2),
- goat kidney (ZN-R),
- goat lung (ZLu-R),
- alpaca kidney (LGK-1-R),
- dromedary umbilical cord (TT-R.B).

DC umbilical cord (TT-R.B) and bat kidney (PipNi) hosted MERS-CoV replication detected by RT-PCR and those from goat also produced the greatest amount of viable virus, followed by monkey, human, alpaca and bat cells (Eckerle et al., 2014). MERS-CoV N protein was produced by a range of infected mammalian cells including (Chan et al., 2013a,c):

- human *ex vivo* bronchial and lung tissue,
- Calu-3,
- embryonic foetal lung fibroblasts (HFL),
- gastrointestinal (Caco-2),
- liver (Huh-7),
- kidney (HEK),
- histiocytoma (His-1),
- porcine kidney (PK-15),

- civet lung (CL-1),
- monkey kidney (LLC-MK2, Vero, Vero E6).

Some cell lines did not support MERS-CoV growth, including (Chan et al., 2013a; Eckerle et al., 2014; Muller et al., 2012):

- baby hamster kidney (BHK),
- African green monkey kidney (MA104),
- canine kidney (MDCK),
- feline kidney (CRFK),
- rabbit kidney (RK-13),
- mouse embryonic fibroblast (NIH-3T3),
- rat kidney (RK3E, RMC),
- chicken fibroblast (DF-1),
- insect (C6-36).

Kidney cells are a common source of passaged cell lines used in virology and they feature prominently in the list of MERS-CoV-permissive cell lines. It is noteworthy that diabetes is also one of the underlying diseases among two thirds of MERS-CoV positive people and that renal damage features among patients with severe disease (Assiri et al., 2013a). When a pseudovirus expressing the MERS-CoV S protein was used to examine the binding of S to the cellular receptor for SARS-CoV (angiotensin-converting enzyme 2; ACE2), MHV (carcinoembryonic antigen-related cell adhesion molecule; CEACAM1) or HCoV-229E (CD13) which were expressed in non-permissive 293T cells, it did not enter those cells, indicating these were not the receptor molecules for MERS-CoV (Payne et al., 2014). When ACE2 was expressed in otherwise non-permissive BHK cells, only SARS-CoV could infect them (Muller et al., 2012). Additionally, blockade of ACE2 by antibodies only prevented SARS-CoV infection of cells otherwise permissive to that virus while MERS-CoV could still infect them (Muller et al., 2012).

An extract from cells permissive for MERS-CoV infection (Huh-7 and Vero) yielded a ~110 kDa protein bound by a recombinant MERS-CoV S protein domain S1. It was identified by mass spectrometry as the exopeptidase dipeptidyl peptidase 4 (DPP4; also called CD26), a type-II transmembrane glycoprotein (Raj et al., 2013). The receptor's identity was confirmed by the use of soluble DPP4 to competitively inhibit MERS-CoV infection of Vero cells as well as by transient expression of DPP4 in COS-7 cells which rendered them permissive to MERS-CoV (Memish et al., 2014f; Raj et al., 2013). Anti-DPP4 antibodies also blocked human and DC MERS-CoV variants from infecting otherwise permissive human bronchial epithelial cells or Huh-7 cells respectively (Raj et al., 2013) while inhibitors of the DPP4 function did not, indicating that structure, rather than function, was important for MERS-CoV attachment (Raj et al., 2013).

DPP4 RNA-positive cells were detected by RT-PCR derived from goat lung, alpaca kidney, camel umbilical cord, bat kidney, human lung and monkey kidney (Eckerle et al., 2014). DPP4 protein was also found on epithelial cells of different species from organs including the kidney and renal ducts, small intestine, lung, umbilical cord, liver, prostate, activated leukocytes and in elevated levels in a soluble form in the blood of those with allergic asthma (Lun et al., 2007; Raj et al., 2013). Limited DPP4 expression was found in the lung of MERS-CoV non-permissive animals including mice and Syrian hamsters, but it was present. DPP4 expression was also seen in ferret, DC, sheep, goat and cow bronchial/bronchiolar tissues and renal tubular tissues (Coleman et al., 2014b; van Doremalen et al., 2014). When the structure of DPP4 from animals was modelled, a host-barrier restriction was predicted to exist among different mammals; DPP4 was present but some animals do not support MERS-CoV infection (van Doremalen et al., 2014). When a human DPP4 was "hamsterized" by exchanging five key MERS-CoV S-protein-interacting residues and introduced into non-permissive

BHK cells, they were not rendered permissive, whereas introduction of the human DPP4 into BHK cells successfully permitted their infection (van Doremalen et al., 2014). Because of the high affinity of MERS-CoV for human DPP4, similar to that for animal DPP4 molecules, it is possible that the MERS-CoV which was detected in humans in 2012 was already modestly adapted to humans and that both DCs and horses, and to a lesser extent goats, should all be considered as sources for intrusion of the virus into human populations because of their degree of binding affinity (Barlan et al., 2014; Raj et al., 2014a). To date, no sign of natural MERS-CoV infection has been found in goat, sheep, cow or alpaca, despite small seroprevalence studies. However, the DPP4 data suggest the possibility and these animals should be further examined as potential animal hosts (see Table 1 for detail on DCs).

Asthmatics have been identified among MERS patients. DPP4 secretion may decrease in proportion to the level of cellular inflammation and the molecule has a role in T-cell activation and hence immune regulation (Boonacker and Van Noorden, 2003), which may be important for asthmatic airway and MERS-CoV infection. While few studies have looked at DPP4 in the URT and none did so in relation to MERS-CoV, relevant enzymatic activity has been identified there and shown to decrease in the nasal mucosa of patients with rhinitis (inflammation), returning to normal after treatment and/or improvement (Grouzmann et al., 2002).

To date, the RBD of MERS-CoV have not undergone noteworthy genetic change, which could indicate that the RBD is not a crucial factor for species adaptation (Barlan et al., 2014), or that time spent in humans has been too short to see such change develop. There is wide genetic diversity among the DPP4 sequences from different animal species, but those with the strongest affinity for MERS-CoV recombinant S protein are more closely related and have the most conservation among 14 amino acids within the human DPP4 sequence predicted to come into contact with the S protein (Barlan et al., 2014).

6. Animal models and the pathogenesis of MERS

Autopsy material is not available from fatal MERS cases however such material reflects mostly late stage disease in patients who received numerous therapies and have spent time under mechanical ventilation (Chan et al., 2013c). Therefore a disease model system is essential to study pathogenesis without the confounding influence of a raft of supportive medical procedures and of pre-existing chronic disease.

6.1. Small animals

Neither mice nor Syrian hamsters have supported natural MERS-CoV replication (Coleman et al., 2014b; Drosten, 2013). To get around the divergence between murine and human DPP4, a mouse model was constructed by introducing a human DPP4 gene (Zhao et al., 2014). An adenoviral vector carrying the gene transduced cells in the mouse airway resulting in animals that were receptive to MERS-CoV infection for 17–22 days. Infection induced pneumonia in the animals and viral clearance followed 6–8 days after infection (Zhao et al., 2014). The model found limited cross-protection afforded by prior exposure to SARS-CoV. Using this model, a Venezuelan equine encephalitis-based replicon expressing MERS-CoV spike protein was shown to be an efficacious vaccine candidate, producing a protective immune response (Zhao et al., 2014). Subsequently a transgenic mouse model was created in which the human DPP4 was globally expressed resulting in a fully permissive model of severe MERS (Agrawal et al., 2015). A report of a mouse model for infection by a MERS-CoV close relative, batCoV HKU5, noted that vaccine designs for any emerging CoV should include

elements from that particular virus since different members of the same genus are insufficiently similar to elicit immunological cross-protection (Agnihothram et al., 2014). Use of transduced knockout mice revealed that TLR-dependent and IFN-signalling pathways were important for control of MERS-CoV replication (Zhao et al., 2014). Pre-infection delivery of poly I:C, IFN β or IFN γ speeded the clearance of MERS-CoV from infected mice and did so more effectively than if poly I:C was given post-infection (Zhao et al., 2014). IFN α 2b given with ribavirin improved clinical status and virological control in macaques, in association with reduced systemic and local levels of proinflammatory markers and viral load (Falzarano et al., 2013). T-cells were shown to be necessary for virus clearance in mice and immunodominant epitopes attracting CD8 T-cell responses are present in the MERS-CoV S protein (Zhao et al., 2014). The mouse model has shown that immune-deficient mice succumbed to more severe disease, as do humans with comorbidities that may affect their immune function.

6.2. Larger animals

To date the only non-transduced animal model for MERS-CoV infection is the rhesus macaque monkey; an expensive animal to house and work with. A multi-site infection protocol delivered 7×10^6 50% tissue culture infectious doses (TCID₅₀) of MERS-CoV to 6–12 year old macaques via a combination of intranasal, oral, ocular and intratracheal routes attempting to recreate the disease seen in humans (Yao et al., 2014). This resulted in a transient mild-to-moderate clinical disease, including a leucocytosis, with acute localised-to-widespread radiographic changes consistent with pneumonia (Munster et al., 2013). Three days after infection, viral RNA was detected in the conjunctiva, nasal mucosa and also at day six in the tonsils, trachea, bronchus, mediastinal lymph nodes and lung, but mostly at reduced levels to those from day three (deWit et al., 2013). By day six, virus was mostly undetectable in the URT, generally decreasing in quantity by day three and at its highest load one day after inoculation (deWit et al., 2013). This is much shorter than has been found when serial samples from a human case were analysed (Kraaij-Dirkzwager et al., 2014). Shedding from the respiratory tract continued until day six in two of three macaques (deWit et al., 2013). Levels of viral RNA remained high in BAL samples on day one and three (deWit et al., 2013). Virus or viral RNA was not found in the kidney, nor were pathological kidney changes or kidney failure noted among macaques (deWit et al., 2013; Yao et al., 2014). Microarray analysis of MERS-CoV infected macaque samples identified a rapid innate immune activation with increased expression of genes involved in proinflammatory processes and cell recruitment including interleukin 6 (IL-6), chemokine C-X-C ligand 1 (CXCL1) and matrix metalloproteinase 9 (MMP9) (deWit et al., 2013). By day six post inoculation, gene expression in macaque peripheral blood mononuclear cells and lung had returned to baseline (deWit et al., 2013). While the viral dosage and spread of delivery may be unrealistic, we have since learned that much milder disease is not uncommon among MERS-CoV-positive humans, especially those who are younger and do not have underlying comorbidities. The macaque model fulfilled the need to see a purified viral preparation produce disease akin to that seen among some human cases (Munster et al., 2013). The direct LRT component of virus delivery in this model likely reflects some proportion of the human route of virus acquisition and some of the notable human disease involving the LRT. However, it may also bias against study of any potential URT response to first contact between virus and host and therefore overlook routes of ingress and the length and nature of the prodrome. While MERS-CoV is believed to the cause of MERS in humans, such causality can only

be extrapolated from these studies in the macaque model (Munster et al., 2013).

7. The immunobiology of MERS-CoV in tissue and cell culture

MERS-CoV and SARS-CoV were compared using human airway epithelial (HAE) cultures. These comprised bronchial epithelial cells that had been differentiated at the air liquid interface to contain basal, secretory, columnar and ciliated cells that generated mucous (Kindler et al., 2013). This system aimed to recreate the main epithelial lining which is possibly the site of first contact between host and MERS-CoV (Kindler et al., 2013). Pre-treatment of cultures with interferon α (IFN α) and IFN γ 3 proved effective at reducing the replication of MERS-CoV, SARS-CoV and HCoV-229E (Kindler et al., 2013). When IFN α or IFN β was added to *ex vivo* bronchial or lung tissues one hour after MERS-CoV infection, virus titre and RNA levels decreased more noticeably for MERS-CoV than for SARS-CoV and MERS-CoV also elicited a comparatively poor proinflammatory and cytokine response, but to the same extent as when pre-treated (Chan et al., 2013c). MERS-CoV failed to induce IFN β or tumour necrosis factor alpha (TNF α) or to upregulate IL-1 β , MCP-1 or regulated on activation, normal T-cell expressed and secreted (RANTES) mRNA (Chan et al., 2013c). However, MERS-CoV was perhaps a better replicator in these tissues than SARS-CoV and its targeting of Type I and Type II alveolar cells within the lungs indicated its potential to hinder lung regeneration after infection, since Type II cells are important for repair (Chan et al., 2013c). By comparison, HCoV-229E, a generally more mild pathogen, did not replicate in lung tissue while influenza A(H5N1) virus, known to be associated with viral pneumonia, did (Chan et al., 2013c).

Further immunobiological analysis using cell lines supported that MERS-CoV grows better, but is more sensitive to IFN β , than SARS-CoV. MERS-CoV is also a very weak inducer of IFN, most likely through an immune dampening effect that both it and SARS-CoV mediate *via* retention of interferon regulatory factor 3 (IRF-3) in the cytoplasm of infected cells (Zielecki et al., 2013). MERS-CoV was again shown to be more sensitive to IFN β , although this was based on pre-treatment of cells, a situation that does not mimic clinical reality for human cases of MERS (Zielecki et al., 2013). Kindler et al. noted that the comparatively limited transcriptional response after infection of HAE cultures by any CoV, with no induction of IFN β and only mild induction of proinflammatory cytokines, may mean that MERS-CoV is already adapted to growing in these cells (Kindler et al., 2013). Professional cytokine-producing cells such as plasmacytoid and conventional dendritic cells and macrophages were lacking in the HAE system but were present in the *ex vivo* tissues above. These cells are needed to see the full extent of the immune response to CoVs (Kindler et al., 2013). Use of an embryonal lung fibroblast cell line (HFL), to allow growth of HCoV-229E, identified more IFN β and IP-10 induction than that by MERS-CoV. In polarised Calu-3 cells, MERS-CoV and SARS-CoV differed in their elicitation of cytokine gene transcription (Lau et al., 2013). In a three-way virus comparison of MERS-CoV, SARS-CoV and HCoV-229E, 15 cellular mRNAs were analysed in response to type I IFN and type III IFN and CoV infection (Kindler et al., 2013). Proinflammatory gene (IL-1 β , IL-6 and IL-8) mRNA (and IL-8 protein) levels were increased in MERS-CoV infections compared to SARS-CoV, while innate antiviral genes (TNF α , IFN β [and protein] and IP-10) were comparatively decreased from 30 h post-infection (Lau et al., 2013). Apart from a reduced innate antiviral response, timing of the response also differed, with MERS-CoV appearing to delay cytokine induction. MERS-CoV produced less IL-8 or IFN β at 48 h compared to SARS-CoV. SARS-CoV also grew less efficiently and destructively than MERS-CoV in Calu-3 cells, as it did in primary bronchial and

primary kidney cells and it took longer to reach peak viral production (Eckerle et al., 2013; Kindler et al., 2013).

The growth of MERS-CoV and SARS-CoV was also compared in cell cultures including primary undifferentiated human tracheo-bronchial epithelial cells (HTBE), Calu-3, Vero, human embryonic kidney (293) and in A549 cells. Both SARS-CoV and MERS-CoV grew with similar kinetics in Calu-3 cells (Zielecki et al., 2013), but Josset et al. noted MERS-CoV induced more significant cytopathic changes accompanying earlier and distinctive changes to the global transcriptome (Josset et al., 2013). Fourteen-fold more differentially expressed genes were noted from MERS-CoV; uniquely down-regulated genes related to antigen presentation and lymphocyte signalling and up-regulated related to cAMP-mediated signalling and ubiquitination (Josset et al., 2013). The increased relative growth of SARS-CoV in Calu-3 cells compared to using HAE cultures above might reflect the latter's variable expression of the SARS-CoV receptor (Josset et al., 2013). While Kindler et al. found little IFN β produced up to 24 h after infection in HAE cultures, Josset et al. identified upregulation of IFN α 5 and IFN β 1 from 18 h in response to MERS-CoV infection of the Calu-3 cell line, perhaps indicating some post-pattern recognition differences in IFN induction between cell types (Josset et al., 2013; Kindler et al., 2013). Both SARS-CoV and MERS-CoV upregulated viral recognition and innate immune pathway activation genes (IL17-related and interferon regulatory factor [IRF]) and down-regulated metabolic genes (Josset et al., 2013). Because of the rapidity of MERS-CoV host cell expression changes, post-infection treatment with a kinase inhibitor was not as effective as pre-treatment (Josset et al., 2013), reinforcing the challenges faced in treatment of MERS cases who present once disease processes are well engaged.

MERS-CoV has been shown to infect and replicate within primary human monocyte-derived macrophages and produce considerable virus, in contrast to SARS-CoV which could enter, but not propagate within dendritic cells (Zhou et al., 2014). Interestingly, MERS-CoV antigens were not found to co-localise with macrophages in infected *ex vivo* lung tissue (Chan et al., 2013c). In contrast to the work in the HAE system, dendritic cell infection resulted in up-regulation of IFN α (though not IFN β) and of the proinflammatory cytokines TNF α and IL-6 (Zhou et al., 2014). Also, infection triggered significantly higher levels of IP-10 and RANTES than the equivalent dose of SARS-CoV (Zhou et al., 2014). In clinical practice, two MERS patients in France were found to differ in their IFN α responses (Faure et al., 2014). Patient 1, who died, did not mount a robust type I IFN response while Patient 2, who survived, did mount a response; particularly notable in Patient 1 was the absence of IFN α and comparatively little RIG-I, MDA5, IRF3, IRF7, IL-12 and IFN γ (Faure et al., 2014). IFN α promotes the antiviral adaptive Th-1 immune response to clear virus. Patient 1 also had a comparatively increased level of IL-10 (with its immunosuppressive role (Sabat, 2010)), a spike in CXCL10 (a proinflammatory role) late in the course of disease and both patients had raised IL-17A (also proinflammatory).

These immune studies raise questions about whether age and immune status or genetic disorders which affect IFN signalling and production could be a risk factor for severe MERS or whether the actions of MERS-CoV itself are sufficient to interfere with signalling.

8. Animal origins and route of acquisition of the MERS-CoV

An animal as source and intermediate host(s) were considered very early on in the MERS outbreak with three of the first five cases having contact with DCs (Albarrak et al., 2012; ProMED, 2012; Reuss et al., 2014). While unclear at that time, approximately 12 MERS-CoV detections from a community outbreak in Hafr Al-Batin between June and August 2013 were possibly triggered by the index

case having had DC contact (Memish et al., 2014g). Today, animal MERS-CoV infections must be reported to the world organization for animal health (OIE) as an emerging disease (2014f). Animal contact with humans was defined in a summary of the first MERS cases as being direct and within 10 days prior to symptom onset (The WHO MERS-CoV Research Group, 2013). This definition made no specific allowance for acquisition through a droplet-based route, which is still assumed to be the most common route for acquisition of a virus that predominantly causes respiratory disease (Assiri et al., 2013a,b; Zumla and Memish, 2014). Providing some support for a droplet transmission route, one report identified viral RNA in an air sample collected in a barn housing an infected DC (Azhar et al., 2014b). The precise route by which humans acquire MERS-CoV remains unproven. From early on and continuing throughout the MERS epidemic in the KSA, older human males have featured prominently, especially among index cases, implying behavioural factors may play a role (Penttinen et al., 2013), and these factors may provide important clues as to why so few human cases report DC contact and are reported to have had contact with an infected human, leaving this group without any obvious route of acquisition (Fig. 7). Whether the above definition of animal contact is sufficient to capture exposure to this respiratory virus remains unclear. Cases are sometimes listed in WHO disease notices as being in proximity to camels or farms, but individuals may have denied coming into contact with the animals. No alternative path for acquiring infection is reported in many of these instances. Fewer than half of human cases have reported camel contact (Gossner et al., 2014) but what constitutes a definition of “contact” during these interviews is unclear. In a May 2014 WHO update and risk assessment, specific wording focused on consumption of camel products while

equivalent wording was not used to ascribe risk to a potential droplet route for acquisition of MERS-CoV from DC. For example:

“Until more is understood about MERS, people at high risk of severe disease (those with diabetes, renal failure, chronic lung disease, and immunocompromised persons), should take precautions when visiting farms and markets where camels are present. These precautions include: avoiding contact with camels; not drinking raw camel milk or camel urine; and not eating meat that has not been thoroughly cooked” (World Health Organization, 2014c).

Despite the early evidence of DC contact, the diversity of coronaviruses known to exist among bats made them a more widely discussed initial focus (Fig. 7) (Shi, 2013; Smith and Wang, 2013; Woo et al., 2009). Nonetheless, to date the only MERS-CoV-specific data pointing to bats originate with a 190nt amplified fragment of the RNA-dependent RNA polymerase gene of the MERS-CoV genome identified in the fecal pellet from an insectivorous *Emballonuridae* bat, *Taphozous perforatus*, commonly called the Egyptian tomb bat, from Bisha, KSA (Memish et al., 2013a). While very short, the sequence of the fragment defined it as a diagnostic discovery and at writing no better sequence match exists on the GenBank sequence database. Unfortunately further sequence from this sample could not be obtained because the cold chain was broken when samples shipped to the USA for study thawed and decayed. The role of bats as the natural host of MERS-CoV or the host of a MERS-CoV-like virus remains to be proven.

Dromedary camels, which make up 95% of all camels, have a central presence in the Arabian Peninsula and human-DC contact ranges from little to close (Gossner et al., 2014; MERS, 2014d).

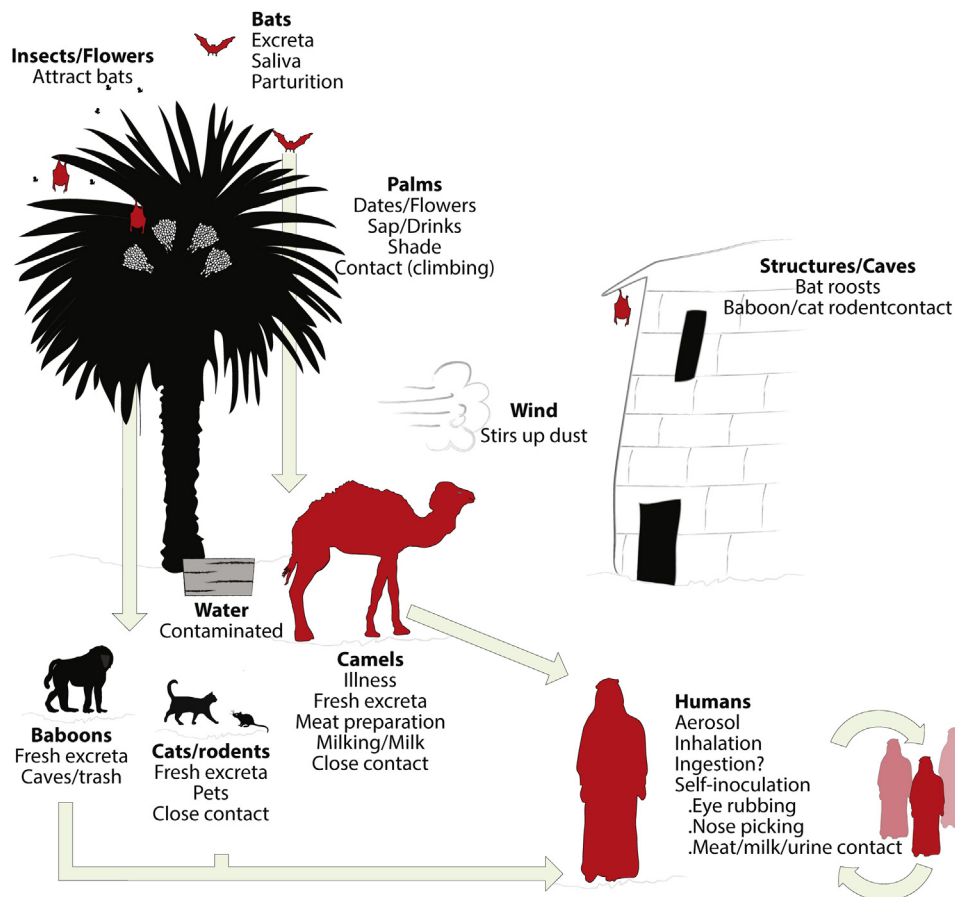


Fig. 7. A speculative model of how humans, camels and other animals may interact to acquire and spread MERS-CoV. Highlighted in red are MERS-CoV virus/RNA and/or antibody-positive hosts.

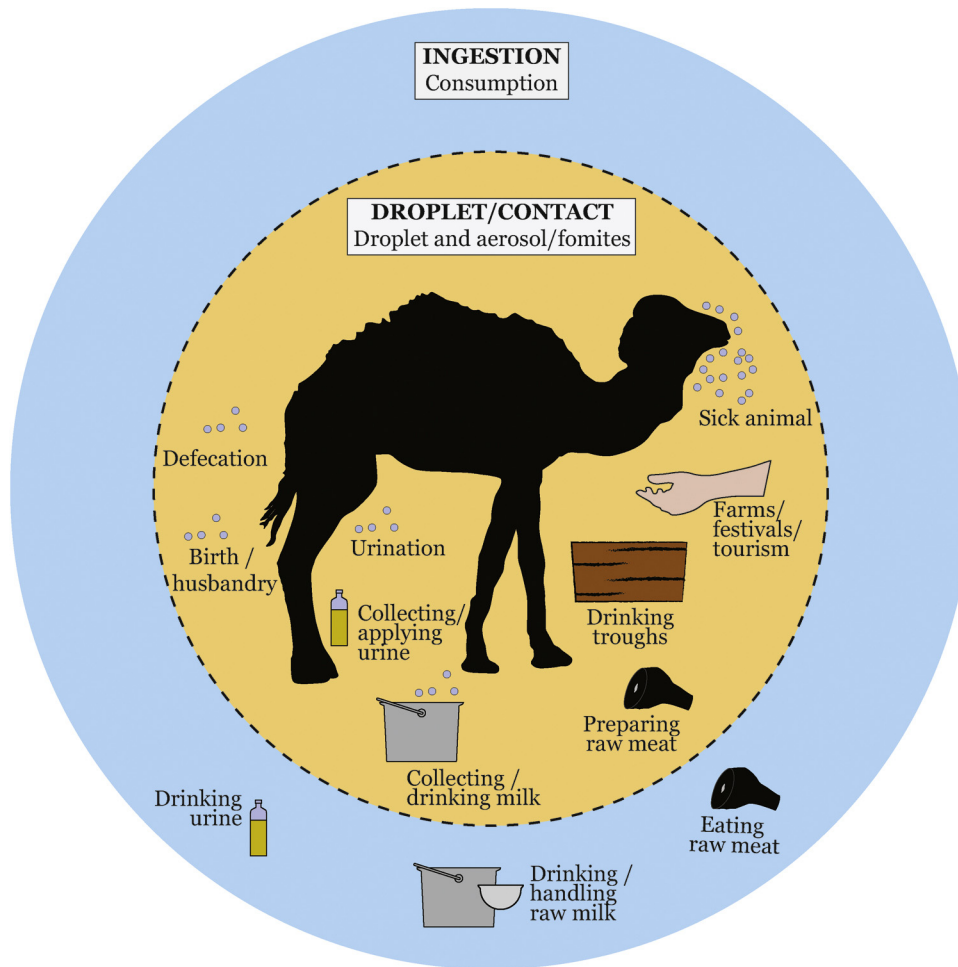


Fig. 8. Risks for acquiring MERS-CoV from a DC. This speculative illustration divides risks into those with a droplet component or those with a direct contact component (within the orange circle) and those with a route of ingestion involved (within the blue circle). No route of MERS-CoV acquisition has been proven to date. The dashed boundary between the two implies that both forms of acquisition may co-occur.

Camel densities are greatest in and around the horn of Africa, specifically Ethiopia, Somalia, Kenya, Sudan and South Sudan (Gossner et al., 2014). Near the KSA, Yemen has the highest density of camels, and within the country the Ha'il region is most densely populated with DCs (Gossner et al., 2014) despite there being no human MERS cases from this region. Contact may be commonplace and could occur in variety of ways (Fig. 8). There are several large well-attended festivals, races, sales and parades which feature DCs and DCs are also kept and bred close to populated areas in the KSA (2014d). DC milk and meat are widely collected and consumed and the DC is an animal of ritual significance after the Hajj pilgrimage. However, MERS-CoV infection frequency appears much lower than does the more widespread and frequent habit of eating, drinking and preparing camel products. Daily ingestion of fresh unpasteurised DC milk is common among the desert Bedouin and many others in the KSA. Apart from its nutritional value, other perceived health benefits and availability, camel milk has been reported as an adjunct to standard type 1 diabetes management (Agrawal et al., 2009; Mohamad et al., 2009). DC urine is also consumed or used for supposed health benefits including a belief that it will keep hair parasite-free and has reported application as an aspirin-like anti-platelet-aggregation substance (Alhaidar et al., 2011) and as a specific anti-cancer and immune modulation substance (Al-Yousef et al., 2012).

In August 2013, Reusken et al. reported the first sero-survey of livestock living in the region during 2012–2013 (Table 1) (Reusken

et al., 2013c). DCs were sampled from a mostly Canary Island-born (originally imported from the Horn of Africa in the 15th century) herd and from Omani DCs (mostly imported in high numbers and with high turnover, from Africa) retired from racing and tested using a protein microarray that expressed a portion of the receptor-binding S1 subunit of the S proteins from SARS-CoV, MERS-CoV and HCoV-OC43 (Reusken et al., 2013a,c). A range of other animals was also tested. All non-DC sera tested negative for MERS-CoV antibodies. A neutralising antibody assay found that 10% of strongly seropositive Canary Island DC sera could neutralise the virus while all of the Omani DC sera had high levels of specific MERS-CoV neutralising antibody (Reusken et al., 2013c). This indicated that DCs had in the past been infected by MERS-CoV, or a very similar virus, but its spread to camels beyond these borders remained unclear.

Since this study, a host of peer-reviewed reports have looked at DCs, and other animals, and the possibility that they may play host to MERS-CoV infection (Table 1). Seropositive DCs have been found throughout the Arabian Peninsula and in Africa (Corman et al., 2014b; Reusken et al., 2013c, 2014b). Other animals tested include sheep, cows, pigs, horses, birds, water buffalo, goats, Bactrian camels, llamas and guanaco (south American camelids). Many of these animals have only been tested in small numbers and rats, mice, cats, and baboons (wide-ranging in Saudi Arabia and known to come into contact with humans and travel to caves possibly frequented by bats) have yet to be tested at all (Alagaili et al., 2014;

Alexandersen et al., 2014; Meyer et al., 2014b; Perera et al., 2013; Reusken et al., 2013b,c). In a large study of 1053 sera from equids (horses, donkeys and mules) from the UAE and Spain, none harboured signs of neutralising antibody against MERS-CoV (Meyer et al., 2015).

Initially, serological tools were employed and cautious analysis reported positive DCs as being seropositive to MERS-CoV, or a closely related virus (Alexandersen et al., 2014; Perera et al., 2013; Reusken et al., 2013c). Subsequently, diagnostic detection of MERS-CoV RNA and recovery of infectious virus has occurred from DC samples (Alagaili et al., 2014; Azhar et al., 2014a; Chu et al., 2014; Haagmans et al., 2014; Hemida et al., 2014a; Nowotny and Kolodziejek, 2014; Raj et al., 2014a) and from some of these, the full or majority length genomes of MERS-CoV have been sequenced (Chu et al., 2014; Haagmans et al., 2014; Hemida et al., 2014a). These findings confirmed that DC versions of MERS-CoV were at least as similar to and just as genetically phylogenetically interrelated, as were variants detected from different humans over time and across distance (Fig. 4). Only one other animal, a *Taphozous perforatus* bat, has contained any traces of MERS-CoV RNA to date (Memish et al., 2013a).

Antibody screening assays sometimes detect cross-reactive antibodies in sera. These are identified as such by screening sera against likely viral culprits, for example BCoV or HCoV-OC43 (as an antigenic facsimile for BCoV). It is possible that other MERS-CoV-like viruses also reside within camels, but this would in no way detract from the current definitive characterization of identical MERS-CoV sequences found in camels.

Camel screening studies have shown that juvenile DCs are more often virus or viral RNA positive while older DCs are more likely to be seropositive and RNA or virus negative or if RNA positive in the process of sero-converting, indicative of recent infection (Hemida et al., 2013, 2014a). In adults, MERS-CoV RNA has been detected among animals with pre-existing antibody suggesting re-infection is also possible (Hemida et al., 2014a). Viral loads among positive camels can be very high (Alagaili et al., 2014; Hemida et al., 2013, 2014a; Nowotny and Kolodziejek, 2014) and camels have been found positive both when ill with URT respiratory signs (Adney et al., 2014; Azhar et al., 2014a; Hemida et al., 2014a; Memish et al., 2014f) or not obviously unwell. These findings indicate camels can host natural MERS-CoV infections. Furthermore, stored camel sera have revealed signs of MERS-CoV in camels which date back over two decades (Alagaili et al., 2014; Alexandersen et al., 2014). Older sera have not been tested and so precisely how long camels have been afflicted by MERS-CoV, whether the virus is enzootic among them, only introduced to them 20-years ago from bats, or they are the subject of regular but short-lived viral incursions perhaps occasionally from humans themselves, cannot yet be answered.

The next step was to prove a direction for infection; were DCs transmitting virus to humans or were humans infecting camels? At a Qatari site, a farm owner and his employee became ill in mid-October 2013 and tested positive for MERS-CoV RNA in a sputum and throat swab sample, respectively. RT-rtPCRs found MERS-CoV RNA in 11 of 14 positive camel nasal swabs at the farm; six (43%) met the international criteria of being positive by two or more assays (Haagmans et al., 2014). These results indicated a recent outbreak had occurred in this herd; the first indication of MERS-CoV RNA found within DCs with a temporal association to human infections. Three positive DC samples were confirmed by sequencing a 358nt portion of the S gene; these sequences were identical to each other, with close homology to other human and camel MERS-CoV sequences (Haagmans et al., 2014). The DCs and human contacts yielded ORF1a and ORF4b sequences differing by only a single nucleotide each, clustering closely with the Hafr-Al-Batin.1.2013 variant (Haagmans et al., 2014). Subsequently, another case study found evidence of a concurrent human and camel infection and the

direction of that infection was inferred to be from the ill DCs and to their human owners (Azhar et al., 2014a; Drosten et al., 2014a; Memish et al., 2014f). Partial genome sequences indicated that a human and a MERS-CoV RT-rtPCR positive camel had been infected by a variant of the same virus, harbouring the same distinct pattern of nucleotide polymorphisms (Memish et al., 2014f). All nine camels in the owner's herd, serially sampled, reacted in a recombinant S1 antigen ELISA, with the two animals that had been RT-rtPCR positive showing a small, verifiable rise in antibody titre (Memish et al., 2014f). A rise in titre theoretically begins 10–21 days after camel infection (Memish et al., 2014f). While samples were few, the authors of this and a subsequently published study of the same farmer and camel herd in which samples were collected a few days earlier, suggested that the rise in titre in camel sera which occurred alongside a declining RNA load, while the patient was actively ill and hospitalised, indicated that the camels were infected first followed by the owner (Azhar et al., 2014a; Memish et al., 2014f). Bovine coronavirus (BCoV) antibodies were also present and rising in one of the two RT-rtPCR positive animals but no animal's antibodies could neutralise BCoV infection (Memish et al., 2014f).

These studies show that DCs, but perhaps not one of the other animal species tested, are a reservoir for the MERS-CoV by which they seem to be often infected. Camels have been exposed to MERS-CoV, in some way, for at least two decades. It remains unknown whether isolating the reservoir would stop sporadic transmission of MERS-CoV to humans (Nishiura et al., 2014). When three adult DCs were infected intranasally, intracheally and conjunctivally with 10^7 TCID₅₀ of MERS-CoV in a study in the United States, an amount similar to that shed by an infected camel, mild URT disease and mostly URT-localised virus replication resulted (Adney et al., 2014). Infectious virus was also detected in the trachea and one of four lung lobes tested as well as in retropharyngeal, mediastinal, mesenteric, and tracheobronchial lymph nodes. URT shedding of infectious virus continued for seven days after inoculation while viral RNA could be detected for 35 days in nasal swabs. Small quantities of viral RNA, but not culturable virus, were detected in exhaled breath but no virus or viral RNA was detected in serum or whole blood and no viral RNA could be detected in faeces or urine for 42 days post inoculation (Adney et al., 2014). A strong neutralising antibody response was produced by the camels from 14 days post inoculation.

We also do not know whether camels are essential to maintaining chains of human infection which subsequently amplify into more apparent clusters and outbreaks by spreading from human-to-human. Parturition in DCs occurs in the winter months (early in the Gregorian calendar year) which may be a driver of the subsequent spike in human cases seen during 2013 and 2014 (Memish et al., 2014f). Juvenile camels appear to host active infection more often than adult camels and this may help explain why the slaughter of camels, which must be five years of age or older, does not contribute to significantly to exposure among slaughterhouse workers (Nowotny and Kolodziejek, 2014). Small numbers of tested DCs from Australia were not seropositive, but expanded virological investigations of Australian (a source of export) and African camels as well as bats may lead to findings of a more ancestral viral variant or more seropositive animals and geographic areas. Identifying the animal source for zoonotic spread is important to inform options for reducing human exposures (Corman et al., 2014b; Hemida et al., 2013).

The MERS-CoV genome does not appear to have changed significantly during its movement through humans in 2012–2014, nor during human and camel spillovers; human variants show very little divergence from camel MERS-CoV variants. This implies that the major source for human acquisition is the camel, rather than another animal, but more testing of other animal species is needed to support that conclusion. Over a month, a DC virus sequenced

on different occasions, did not change at all genetically, indicating a high level of genomic stability and supporting the possibility that DCs might be the natural, rather than intermediate, host for MERS-CoV we know today (Hemida et al., 2014a).

The precise role for camels and the route(s) of human acquisition of MERS-CoV from camels in sporadic infections remains to be determined. Nonetheless in the absence of any other likely animal source and in the interest of public health, risk reduction activities now recommend reducing contact with camels, especially when ill, and limiting contact with camel secretions and excretions including the handling of camel milk and meat and the butchering of camels. The potential for aerosol-generation by all these procedures, yet to be defined, described or studied, has not been acknowledged in plain language as a risk to date (see Section 9.1 for aerosols and risk). Thus, camels develop signs of URT disease, shed infectious virus in high quantities in URT secretions and the possibility exists that they may aerosolise that virus.

9. Transmission of MERS-CoV among humans

Transmission of MERS-CoV has been defined as sporadic, intra-familial and healthcare associated (Memish et al., 2014e). The virus was characterised because of its role in causing severe and therefore more obvious illness in humans; we were the early sentinels highlighting its incursion. Spread of MERS-CoV within families (Memish et al., 2013b; Omrani et al., 2013) and between people has been well documented (Drosten et al., 2014b; Health Protection Agency (HPA) UK Novel Coronavirus Investigation Team, 2013; MERS, 2014b; Puzelli et al., 2013) however the first known MERS outbreak was one of acute LRT disease in a healthcare setting (Hijawi et al., 2013). This occurred in Al-Zarqa, Jordan and was retrospectively linked to the MERS-CoV after some detailed laboratory and epidemiological analyses (Hijawi et al., 2013). A BAL and a serum sample from two cases were found to be MERS-CoV RT-rtPCR positive and a number of probable cases were subsequently added to this outbreak (Hijawi et al., 2013). Further investigations deployed an ELISA employing the genetically and antigenically similar btHKU5.2 recombinant nucleocapsid antigen (Chan et al., 2013c), a MERS-CoV Hu/Jordan-N3/2012 infected Vero cell IFA, and an MNT test (Al-Abdallat et al., 2014). These found additional cases from among the sera of 124 subjects (Al-Abdallat et al., 2014). While serology alone does not meet the current WHO definition of a case, optimised and thoroughly validated sero-assays employed alongside good clinical histories do still identify previous occurrences of infection. Just as with PCR testing on occasion, serology usually finds a viral footprint in the absence of any clear signs and symptoms of disease (Knibbs et al., 2014). And as is also the case with molecular testing, care is needed when moving a newly developed diagnostic serology algorithm from a research setting to one that informs public health decisions (CDC Newsroom, 2014).

For some time, testing of samples focussed on those collected from patients with severe illness and not on milder acute respiratory tract infections, nor on conducting prospective case-control studies. Contacts of confirmed MERS cases were observed for illness, but, in the absence of signs and symptoms, samples were generally not tested by a laboratory, biasing early data towards more ill and hospitalised patients; often described as just “the tip of the iceberg”. As testing paradigms changed with increased testing of contacts, more asymptomatic and mild infections were recognised.

A rise in the proportion of asymptomatic cases (which enlarges the denominator for calculations of the proportion of fatal cases (Dudley and Mackay, 2013)) and a drop in the proportion of fatal cases (the numerator) was identified in September 2013 (Penttinen et al., 2013). Historically, such rises are consistent with changing

definitions and laboratory and clinical responses to, and understanding of, a newly discovered virus that was first noted among the severely ill. As adjustments to testing occurred, more cases of milder disease and those with subclinical infections were noted among MERS-CoV positive people. Over time there has been an apparent cyclical pattern to the average weekly age of people positive for MERS-CoV (Fig. 9A) however the age distribution changed most notably after the Jeddah-2014 outbreak during which a shift towards younger people became evident (Fig. 9B–G).

As a group, HCWs comprised 19% of MERS-CoV detections and it is apparent from closely following publicly released case data that the weekly proportion of infected HCWs increases dramatically alongside each spike in overall detections (Fig. 10). This is explainable because to date, each spike has been intimately associated with healthcare-facility related outbreaks (Penttinen et al., 2013). These spikes drive observed decreases in average age during each event because afflicted HCWs as well as families are usually younger than inpatients with MERS. Healthcare facilities have therefore been a regular target for suggested improvements aimed at bolstering weaknesses in infection prevention and control (IPC) procedures (Penttinen et al., 2013; World Health Organization, 2014f). In May 2013, the WHO published guidelines for IPC during care of probable or confirmed cases of MERS-CoV infection in a healthcare setting (World Health Organization, 2013a).

The Jeddah-2014 outbreak was the largest and most rapid accumulation of MERS-CoV detections to date (Fig. 11) with April having the greatest number of MERS-CoV detections of any month on record (Fig. 11B). The outbreak was mostly (>60% of cases) associated with human-to-human spread within hospital environments, and was thought to have resulted from a lack of, or breakdown in, IPC (Brown, 2014; Zumla and Hui, 2014). Following the rapid increase in case numbers, a rise in fatalities ensued (Fig. 11A).

Genome sequences indicate viral change, and if the virus is well characterised, such changes may flag alterations to transmissibility, replication, lethality and response to drugs. While genetic sequences examined in 2012 and 2013 showed that multiple variants circulated among people and camels in the KSA (Cotten et al., 2013b), by comparison to the massive genome sequencing approach taken towards tracking Ebola virus transmission and evolution in west Africa during 2014 (Chan et al., 2013b), only a handful of MERS-CoV genome sequences were reported during 2014, when the majority of all MERS-CoV detections to date occurred. The conclusions about variant diversity in 2014 are extrapolated from these relative few sequences. Those genomes from the Jeddah-2014 outbreak indicated no outstanding genetic or possibly replicative changes from earlier variants (Drosten et al., 2015; World Health Organization, 2014c). In parallel with the Jeddah-2014 outbreak, another HCW cluster occurred in the UAE. How the index case(s) for either outbreak acquired MERS-CoV remains unknown.

Genomic sequence can also be used to define the boundaries of a cluster or outbreak based on the similarity of the variants present among the infected humans and animals and at different healthcare facilities (Fig. 4) (Cotten et al., 2014). This approach was most clearly seen when defining the geographically constrained hospital MERS outbreak in Al-Ahsa, which occurred between 1st April and 23rd May 2013, as well as clusters in Buraidah and a community outbreak in Hafr Al-Batin, KSA. Cotton et al. employed molecular epidemiology and found that there had been no sign of transmission chains extending beyond two to three months, so extensive opportunities for the virus adaptation to humans through sustained serial transmission have been infrequent (Cotten et al., 2014). Sequencing of MERS-CoV genomes from the 2013 Al-Ahsa hospital outbreak indicated that multiple viral variants contributed to the cases but that most were similar enough to each other to be consistent with human-to-human transmission (Assiri et al., 2013a,b; Cotten et al., 2013b).

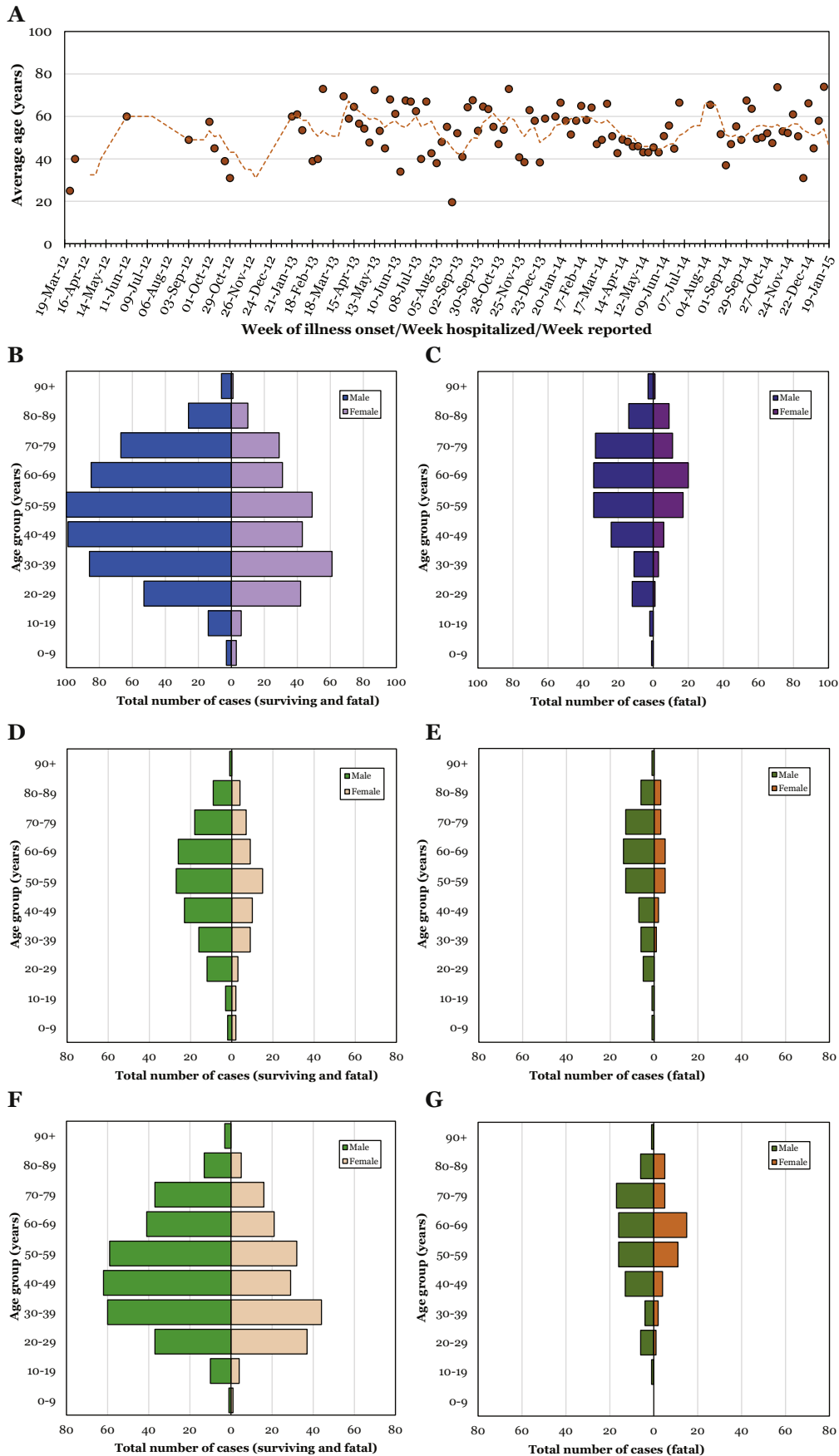


Fig. 9. MERS-CoV detections, age and sex. (A) Plot of the average age (orange circles) of laboratory cases detected in that week and a six day moving average (dashed line). (B) An age and sex pyramid for all MERS-CoV detections worldwide and (C) for those with fatal outcomes from infection. (D) The distribution of age and sex up to but not including the first identifiable case that began the Jeddah-2014 outbreak and (E) those with a fatal outcome. (F) The distribution

Contact tracing has been described in detail for MERS cases exported beyond the Middle East. Tracing usually identifies dozens of potential cases per confirmed case and while it is a time consuming and expensive process it is essential for understanding transmission and for containing a virus about which little is known. Eighty-three symptomatic or asymptomatic contacts of a case imported to Germany from the United Arab Emirates (UAE) harboured no sign of virus or antibody (Reuss et al., 2014) and similar examples of very limited to no onward transmission have been the hallmark of contact tracing results to date. In a study of 123 contacts of a case imported to France, only seven matched the definition for a possible case and were tested; one who had shared a 20 m² room while in a bed 1.5 m apart from the index case was positive and this was determined to be a nosocomial acquisition (Mailles et al., 2013). It is possible that further mild yet positive cases may have been identified had all contacts been tested regardless of symptoms. No contacts of the first two MERS cases imported into the USA in 2014 tested positive (Bialek et al., 2014) and none among 78 contacts of two cases imported to the Netherlands develop MERS-CoV antibodies (Kraaij-Dirkzwager et al., 2014). Apart from clearly defined case studies of nosocomial infection, nosocomial spread is also suspected among a number of MERS cases noted through analysis of public data (author's observations). These are cases admitted to hospital for support of a non-respiratory medical condition, who then develop respiratory symptoms and test positive for MERS-CoV within the incubation period of MERS-CoV infection. One example identified the likely role of a mild or asymptomatic case, present in a hospital during their admission for other reasons, as the likeliest index case for a family cluster (Omrani et al., 2013). Such studies validate the laboratory testing of mild cases when accompanied by a process that restricts exposure of others, especially older family members and friends with underlying disease, to a MERS-CoV infected person.

9.1. Virus survival in the environment

Infectious MERS-CoV added to camel, goat or cow milk and stored at 4 °C could be recovered at least 72 h later and, at 22 °C, for up to 48 h afterwards (van Doremalen et al., 2013). This is in keeping with HCoV-229E being capable of retaining viability in water for 2 days up to a predicted 588 days depending on the type of water and temperature (Gundy et al., 2009). MERS-CoV titre decreased somewhat when recovered from milk at 22 °C but pasteurization ablated MERS-CoV infectivity (van Doremalen et al., 2013). In a subsequent study, MERS-CoV RNA was identified in the milk, nasal secretion and faeces of camels from Qatar (Reusken et al., 2014a).

Plastic or steel surfaces were inoculated with 10⁶ TCID₅₀ of MERS-CoV at 20 °C, 47% relative humidity (RH) and virus recovery was attempted in cell culture (van Doremalen et al., 2013). At 30 °C and 80% RH, MERS-CoV remained viable for 8 h and for 24 h at 30 °C, 30% RH (van Doremalen et al., 2013). By comparison, influenza A virus A/Mexico/4108/2009 (H1N1) could not be recovered in Madin-Darby canine kidney (MDCK) cells beyond 4 h under any conditions (van Doremalen et al., 2013). Aerosol experiments found MERS-CoV viability decreased by 89% at 70% RH but only decreased 7% at 40% RH when an aerosol was generated at 20 °C while influenza A virus A/Mexico/4108/2009 (H1N1) decreased by 62% and 95% respectively (van Doremalen et al., 2013). MERS-CoV survival is inferior to that previously demonstrated by SARS-CoV (Chan et al., 2011) however for context, pathogenic bacteria can

remain viable for 45 min in a coughed aerosol and can spread 4 m, thus MERS-CoV's ability to remain viable over long time periods gives it the capacity to thoroughly contaminate a room occupied by an infected and symptomatic patient (Knibbs et al., 2014). Such findings expand our understanding of the risks associated with bioaerosols for transmission of respiratory viruses in many settings, including hospital waiting rooms, emergency departments, treatment rooms, open intensive care facilities and private patient rooms. The nature and quality of air exchange, circulation and filtration is an important variable in risk measurement and reduction. Droplet spread between humans is considered the mechanism of human-to-human transmission and the need for droplet precautions has been emphasised after the Al-Ahsa hospital outbreak (Al-Tawfiq and Memish, 2014; Assiri et al., 2013a,b; Zumla and Memish, 2014). By extrapolation, aerosol-generating events involving camels (urination, defecation, and the processes of preparation and consumption of camel products) should be factored into risk measurement and reduction efforts and the need to define whether a more stringent level of personal protective equipment should be worn by HCWs and animal handlers remains a priority.

9.2. MERS-CoV and pandemic potential

When the MERS-CoV detection tally sat below 120, analyses of the basic reproduction number (R_0) – the average number of infections caused by one infected individual in a fully susceptible population – returned values below 1, indicating that a pandemic was not likely (Bauch and Oraby, 2013; Breban et al., 2013; Cauchemez et al., 2014; Poletto et al., 2013). With more data and correction for observation bias, this conclusion was maintained (Chowell et al., 2014). These analyses reflected charting of small public data sources and indicated that while cases could climb suddenly, such as during the Al-Ahsa outbreak (Assiri et al., 2013b), the climb was not logarithmic. Methods used in these studies vary but some make allowance for more extensive case numbers than may have been publicly reported (Cauchemez et al., 2014; Poletto et al., 2013). If R_0 was greater than 1, sustained case climb would be expected. The impact of incomplete case contact tracing, limited community testing and clinically defined cases in the absence of laboratory confirmation might affect some R_0 calculations and make it difficult to identify patterns among case occurrence. The implication is that more positives among these population groups could have occurred and thus a greater transmission efficiency and higher R_0 value may be possible. When narrowing an analysis to index cases (the case with the earliest onset date of a cluster) and inferring secondary case numbers, one study predicted that the R_0 could be slightly above 1.0 (0.8–1.3 with an upper bound of 1.2–1.5 depending on method used) (Cauchemez et al., 2014). This study hypothesised that a high proportion of infections were not being detected (Cauchemez et al., 2014). Similar analyses are awaited following the Jeddah-2014 outbreak.

Most cases of MERS have resulted from human-to-human transmission, however that transmission was inefficient (Drosten et al., 2014b, 2015; Memish et al., 2013b; Omrani et al., 2013) and defined as sporadic rather than sustained (Fig. 12). Based on observational studies, human spread requires close contact to occur. Relevant data are scant but it appears that the majority of human cases of MERS-CoV do not transmit to more than one other human and to date, the localised epidemic of MERS-CoV has not been self-sustaining (Poletto et al., 2013). Index or sporadic cases have been

of age and sex during the Jeddah-2014 outbreak, arbitrarily defined as spanning from the week beginning 17th March 2014 and ending in the week beginning 7th July 2014; data are based on laboratory confirmed cases collated into the author's curated line list as at 19th January 2015. Note the changed x-axis scale between B/C and D/E/F/G. Sources of these public data include the World Health Organization, Ministries of Health and FluTrackers (Ministry of Health, Saudi Arabia, 2014). Earlier and subsequent versions of this chart are maintained on a personal website (Mackay, 1997) and blog (Mackay, 2013).

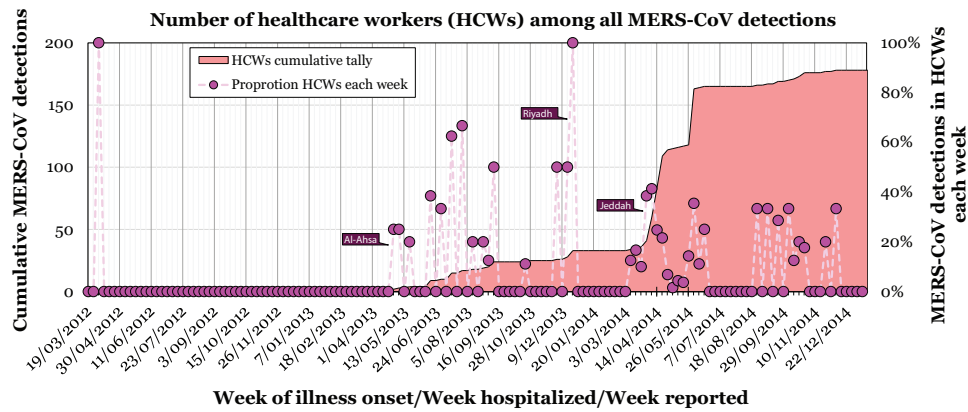


Fig. 10. Data on MERS-CoV detections among HCWs and based on laboratory confirmed cases collated into the author's curated line list as at 14th January 2015. Sources of these public data include the World Health Organization, Ministries of Health and FluTrackers. Earlier and subsequent versions of this chart are maintained on a personal website (Mackay, 1997) and blog (Mackay, 2013).

older and it has yet to be established whether infections thought to have been acquired from an animal source produce a more severe outcome than those spread between humans (The WHO MERS-CoV Research Group, 2013). Strategic sero-assays have yet to investigate the extent to which milder or asymptomatic cases contribute to the MERS-CoV transmission chain. In a household study, 14 of 280 (5%) contacts of 26 MERS-CoV positive index patients were RNA or antibody positive (Drosten et al., 2014b).

10. Therapeutic options for the MERS-CoV

There is no specific drug or vaccine currently available to treat infection by the MERS-CoV. For context, there is still no vaccine available against SARS, mostly attributable to the brevity of the SARS-CoV global emergence. It is impossible to predict whether a MERS-CoV vaccine for human use will fall victim to a lack of commercial interest or remain relevant in the time it will take to be developed, however a vaccine for use in camels is a more practical option given the identity shared between human and camel viral variants and the apparent rarity of MERS-CoV spillover to humans. If applied to young camels ahead of their first virus acquisition, such a vaccine may be capable of eradicating MERS-CoV from herds. But if the source of camel infections is bats, newly imported camels or other herds, any vaccine would need to be used in an ongoing manner and be able to reach feral camel herds as well. Care of hospitalised patients remains supportive, with vigilance for complications (The WHO MERS-CoV Research Group, 2013). No empirical use of antimicrobial agents has improved severe disease nor have high dose steroids succeeded in reversing the progression of respiratory disease (The WHO MERS-CoV Research Group, 2013). The development and registration of drugs for human use is not suitably rapid to use effectively against an emerging infectious agent (de Wilde et al., 2014; Dyall et al., 2014). However, research advances in this area have been made.

Testing of formulations previously approved by the Food and Drug Agency (FDA; USA) at the Small-molecule Inhibitor Leads Versus Emerging and neglected RNA viruses (SILVER) project described four existing compounds (chloroquine, chlorpromazine, loperamide and lopinavir) that inhibit MERS-CoV, SARS-CoV and HCoV-229E replication in cell culture (de Wilde et al., 2014; SILVER consortium, 2015). In another study, 33 drugs with the ability to impart >50% inhibition upon MERS-CoV growth in culture have been described as candidates for future clinical MERS intervention studies (Dyall et al., 2014). These were also identified following investigation of pre-existing, FDA-approved clinical drugs and

encompass 13 different classes of therapeutic (Dyall et al., 2014). A novel molecule, K22, showed promise both directly and as an example that it was possible to target and specifically disrupt very conserved viral replication processes such as double membrane vesicle-associated RNA replication, without causing cellular toxicity (Lundin et al., 2014).

Another approach has been to target the interface between the MERS-CoV RBD and the receptor by employing competitive substrates or inhibitors of the enzymatic function of DPP4 (Kawalec et al., 2014). DPP4 inhibitors, in their role as anti-diabetes drugs, already exist, are tolerated and are not associated with adverse events (Kawalec et al., 2014) although it is unclear whether they may already be in use among MERS patients with underlying kidney disease. Additionally, targeting the viral MERS-CoV 3C protease, important for replicase polyprotein maturation, may be a viable strategy in the future because it has so far remained conserved (Cotten et al., 2014).

The company Novavax has produced an experimental recombinant nanoparticle vaccine candidate based on the MERS-CoV S protein and it has generated important anti-MERS-CoV antibodies in a mouse model (Coleman et al., 2014a). Monoclonal antibodies (e.g. MERS-4, MERS-27 and 3B11) directed towards the S protein are capable of neutralising infection by the MERS-CoV and hold future promise for use as a therapeutic and prophylactic (Jiang et al., 2014; Tang et al., 2014). A replication-competent, propagation-deficient E gene-deleted mutant of MERS-CoV variant EMC/2012 (rMERS-CoV- Δ E) may also prove to be a useful vaccine candidate while a truncated RBD of MERS-CoV has been shown to elicit antibodies in mice (Almazán et al., 2013; Du et al., 2013a). A conserved peptide in the RdRp of all HCoVs was also identified, and that may provide the basis for an epitope-directed universal vaccine (Sharmin and Islam, 2014).

IFN treatment has shown promise in culture-based experiments (MERS, 2014a) but in macaques, early application was required for effectiveness. Macaques were infected with 7×10^6 TCID₅₀ of MERS-CoV and, 8 h after infection, they were treated with substantial amounts of subcutaneous IFN- α 2b along with intravenous ribavirin, followed by ribavirin doses intramuscularly every 8 h and with IFN- α 2b every 16 h until 72 h after infection (Falzarano et al., 2013). Treatment prevented any signs of increased respiration, leucocytosis (neutrophilia) or reduction in oxygen saturation seen in the untreated animals plus treated macaques had very little to no radiographic evidence of pneumonia (Falzarano et al., 2013). The viral load in daily BALs did not differ between treated and untreated macaques but loads were reduced in the treated animal's post-mortem tissues and fewer tissues were MERS-CoV RT-PCR positive. Since MERS seems to be a prolonged disease that is well engaged

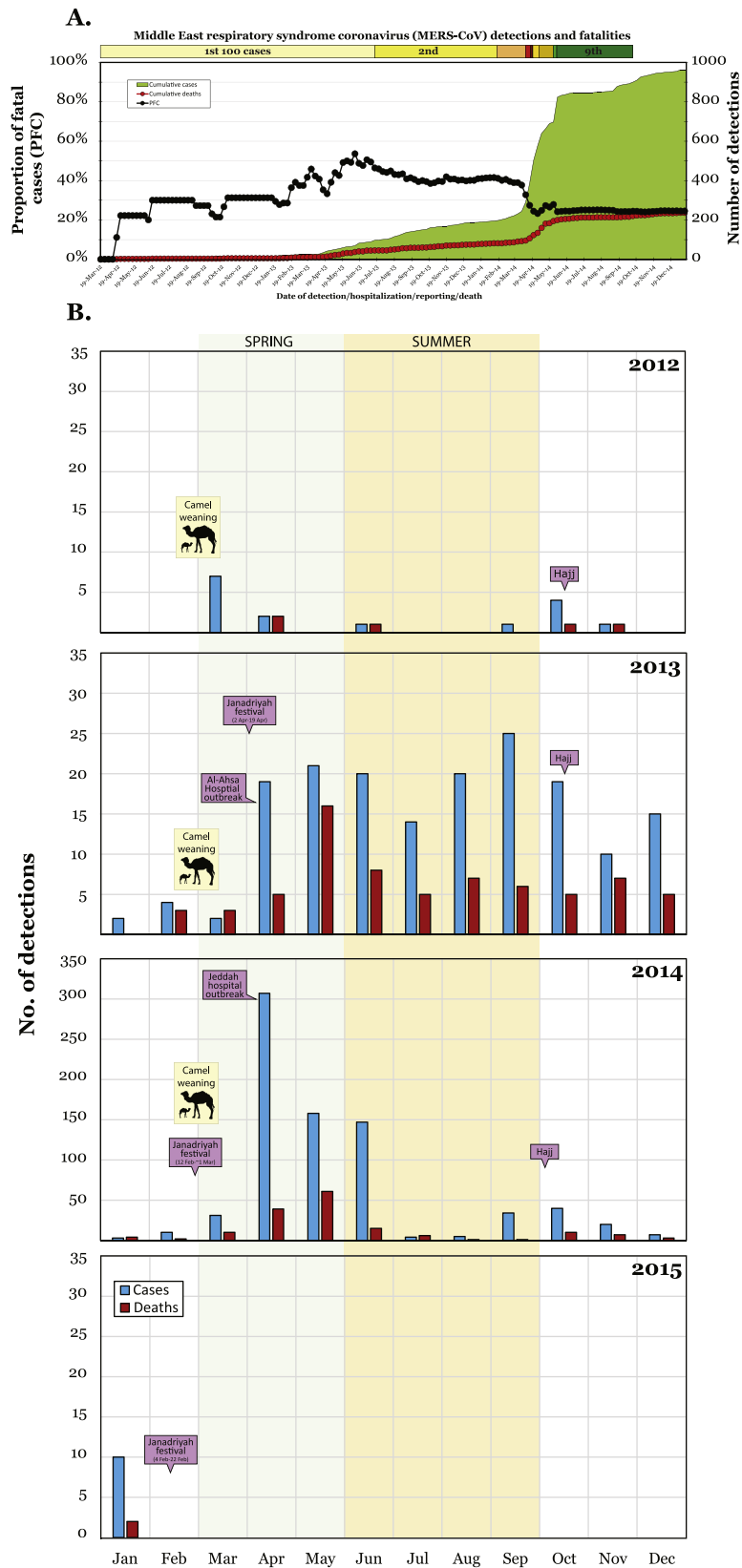


Fig. 11. The time taken for MERS-CoV detections to reach units of 100. The hospital outbreaks in Al-Ahsa and Jeddah triggered the biggest rises in cases. (A) Includes 963 cases worldwide, and 236 of 348 fatal cases as at 14th of January 2015. (B) Monthly detections of MERS-CoV (blue bars) and those of cases who died (red bars) with some dates of interest marked for 2012 to June 2nd 2014. An approximation of when recently born camels are active is indicated. Spring (green) and summer (orange) in the Arabian Peninsula are also shaded. Note the left-hand y-axis scale for 2014 which is 10-fold greater than for 2012/13/15. Sources of these public data include the World Health Organization, Ministries of Health and FluTrackers (Ministry of Health, Saudi Arabia, 2014). Earlier and subsequent versions of this chart are maintained on a personal website (Mackay, 1997) and blog (Mackay, 2013).

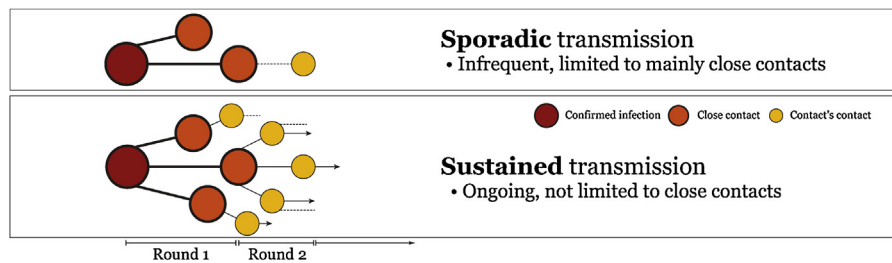


Fig. 12. A representation of sporadic *versus* sustained human-to-human transmission of a respiratory virus. MERS-CoV does not spread in a sustained manner.

upon presentation for medical help, it is unclear how this drug cocktail could be delivered early enough to moderate severe disease. When trialled on five critically ill patients with comorbidities and a median time between hospitalization and treatment of 19 days (range 10–22 days), no response to treatment was evident (Al-Tawfiq et al., 2014). When used as a primary treatment of an infected physician and as prophylaxis for his wife, it was unclear whether the cocktail had any effect (Khalid et al., 2014).

11. Communicating risk to the public

The WHO took the lead in collating data and providing an official voice for the emergence of MERS from 23rd September 2012 (Hartl, 2013; World Health Organization, 2014b). Through Global Alert and Response (GAR) Disease Outbreak News (DON) releases, the WHO kept the world updated with detailed, deidentified case information provided soon after they had been forwarded on to them from each country's Ministry of Health or equivalent, as per the transparency required by the International Health Regulations (IHR), 2005. However there were often delays in the posting of such information, most notably during the Jeddah-2014 outbreak when no new case details were confirmed by the WHO as originating from KSA for more than six weeks. Cases announced *via* the KSA Ministry of Health website have often had data inconsistencies including errors, format variations, have often lacked key information including dates, have presented deaths with insufficient information to permit linkage with the announced case and over one hundred cases remain devoid of all key data, excluding them from most epidemiological analyses. This was a particular problem during periods when rapid case accumulation became a concern for the global community. When available, WHO DONs fill in vital missing detail, with additional important demographic data like age, sex or essential dates permitting improved understanding of when illness onset occurred, when cases were hospitalised, whether they were asymptomatic and if the newly announced case was a contact of another case, an animal or an animal product. However DONs are only effective when the underlying data is forwarded to the WHO. Such comprehensive data permits other calculations, for instance the likely laboratory turnaround time, the possibility for nosocomial spread occurring and the location of cases as a determining factor in whether a cluster has become an outbreak or a local epidemic. WHO data can be viewed by the public but are also relied upon by WHO Member States' epidemiology analysts. Such experts report to many levels of Health management and government who seek to understand threats to their own population so as to make the most informed judgements. The decisions which follow may include the need to raise alert levels, prepare laboratory capacity, create educational materials, free specific response funds, ramp up messaging, manage and alter border controls or issue travel alerts. The activities of the WHO have been essential for global preparedness against MERS. The WHO rapidly and comprehensively adopted, updated and publicly promoted diagnostic tools and testing algorithms, case definitions and guidelines for investigations, research study protocols, IPC guidance, travel

advice, risk assessments and summary updates *via* a purpose-built coronavirus-focussed website. Their social media unit strove to answer questions posed by everyone and anyone through Twitter, which in turn allowed users to take control of further disseminating the information they found most interesting, informative, relevant or concerning to others in their social networks; a process that can also be informative to public health bodies who seek to provide their clients the detail they desire. The role of WHO as a nexus for collaboration laid the groundwork for the accumulation and advancement of knowledge about MERS and the MERS-CoV through the formation of, and reporting by, the IHR (2005) Emergency Committee concerning MERS-CoV. Their role is to advise the WHO Director-General on the need for action and, as yet, no Public Health Emergency of International Concern (PHEIC) has been declared. The IHR Emergency Committee has met eight times to date. Upon request, the WHO has also sent risk assessment teams to the region to gauge the MERS situation. Gregory Härtl, Coordinator of the Department of Communications for the WHO, noted that "the more answers public health experts can provide now, the greater the public's trust in these institutions will be if and when the virus should become easily transmissible between humans and cause more widespread morbidity and mortality" (Hartl, 2013). This is a comment that applies equally well to the emergence of any infectious disease. By comparison, officials within the KSA were widely and regularly criticised for moving slowly, shunning collaborations and failing to show proactive action in commencing local research into MERS and MERS-CoV (2014c; Editorial, 2014; Holmes, 2014; Kupferschmidt, 2014; Reuters, 2014; World Health Organization, 2014f).

Despite data in the scientific literature strongly indicating that camels harboured MERS-CoV or a very closely related virus, communication about camels posing a risk to humans as the zoonotic sources for infections did not become a mainstream public health message until late April of 2014; two years after the discovery of the virus. This shift correlated with a change in the KSA Minister of Health, requests for help in containing the Jeddah-2014 outbreak and a new WHO risk assessment, which included mention of camels (World Health Organization, 2014e). Shortly thereafter, the KSA Deputy Minister for Public Health and lead author of most peer-reviewed MERS-CoV research, Professor Ziad Memish, was also asked to step down. However, the message to date, underpinned as it is by a paucity of understanding about how and from where the virus transmits, may not yet capture or adequately communicate all the risks.

Much of what is considered social media is a volatile resource, yet a very valuable one. There is an understanding of the volatility of this non-peer reviewed medium but also a perception that social media is a place only for "selfies" and not science. Tweets can disappear to search after a short period, blog pages can come and go and be untraceably altered while online mainstream news stories, in some countries more than others, may suffer similar fates as they rapidly become old news. Despite that, internet-driven information has played a vital role in rapidly tracking and unearthing cases, clusters and outbreaks of disease in recent years. This has

been the case for MERS globally. At this stage, many who follow infectious disease clusters, outbreaks and pandemics through social media are well informed by exposure to a distillation of the most recent events, reports, commentary and news stories from all over the world. Professionals who use this communication medium are clearly appreciated by their funding stakeholders, the public. Social media and blogs communicate with a much broader audience than is usually reached by the traditional scientific literature and they are more accessible to the local and global communities who contribute to the funds that researchers so dearly seek. Sometimes these forms of mass communication are useful to those seeking information about a disease outbreak in their own country. Sometimes they are entirely misleading. The public audience may not spend the time and mostly do not have the background expertise to interpret the densely presented, often slow to appear, scientific and public health literature. The space between the scientific literature and mainstream media is occupied by those science communicators who may, sometimes with and sometimes without relevant scientific training, be as up-to-date on their topics as the best academic epidemiologist. The President John F. Kennedy quote, "One person can make a difference, and everyone should try" seems an apt one to describe these dedicated people who, often without any paid incentives, devote their personal time, effort and money to better understand and communicate about infectious disease. Frequently updated data repositories such as the small FluTrackers group constantly compile, actively curate and condense worldwide news sources into threads of information that often assemble into patterns that can predict emerging infectious disease events well before they reach the mainstream media, and their line list of MERS-CoV detections (Ministry of Health, Saudi Arabia, 2014) has been a cornerstone of case numbering, detail and clarity; Crawford Kilian's H5N1 blog (Kilian, 2014) outstrips its name by providing links to other reliable sources as well as excerpts and highly eloquent and thought-provoking commentary on a range of microbial infectious disease stories, of which MERS-CoV is just one; Michael Coston's Avian Flu Diary (Coston, 2014) also focusses and comments on emerging infectious diseases augmented by astute and experienced public health commentary, resources and a large inventory of past posts that are used to good effect when creating new and informative ones; the Programme for Monitoring Emerging Diseases (ProMED) is an internet-based reporting system maintained by a panel of authoritative professionals and rapporteurs who prepare daily digests of the latest infectious disease happenings which they freely circulate using widely read emails (Program for Monitoring Emerging Diseases, 2014); for a more workforce-oriented view of MERS and infectious disease, Shane Granger's Random Analytics provides an excellent perspective of the less frequently discussed impact of infectious diseases on humans (Granger, 2012); my own blog, Virology Down Under seeks to distill some of the more complex virology-related happenings and present data on emerging viral disease in a more graphical, accessible and open access way (Mackay, 1997, 2013); Maia Majumder who applies an engineering systems expertise to the epidemiology of the disease (Majumder, 2014). These are just a few resources and each is part of an interconnected web of other sources, contacts and personal experience that exist on the internet; they have all chased and tracked the emergence of MERS-CoV as doggedly as a professional science writer. Each resource is as distinct yet complimentary source of information that together are sometimes referred to as flublogia. While it is often overlooked and perhaps underestimated by some professional scientists and clinicians, flublogia compiles or uses publicly available, deidentified data and adds publicly relevant interpretation which is greatly appreciated, much more widely read and far, far more often cited than anything seen in the professional scientific literature. It is also a factual source of such information (Lau et al., 2014; Salathe et al., 2013).

12. Summary

The MERS-CoV appears to be an entrenched camel virus infecting the URT which may have its origins among bats. Human infection may result from rare zoonotic spillover to humans. Many potential animal, human and environmental sources await further testing but thanks to quick action, the sensitive and rapid molecular diagnostic tools required to achieve this goal have been in place and available since the virus was made known to the public. Serological tools for research application, after a much slower start, are now emerging as robust, validated and ready for wider use, with care. Commercial unavailability remains an issue for more widespread use and so collaboration with the relevant research groups must be the immediate option for future studies. The MERS-CoV can spread from human-to-human but seems to do so only sporadically and sustained chains of transmission have not been evident to date. There is also no evidence that MERS-CoV is a virus of pandemic concern, despite many opportunities for it to become so. But vigilance is key for a virus with a genetic makeup that has only been observed for three years. Of those known to be infected, over a third have died. Central to future vigilance is continued screening, sequencing, analysis and timely data sharing. While whole genome sequencing has been used extensively to study MERS-CoV, it remains a tool for experts and collaborations have again been key for less equipped or experienced researchers to decode the MERS-CoV as it moves through people, over time and across distance in the KSA and beyond its borders. A recently described routine genotyping target which is equivalently informative will also aid this vigilance. Nonetheless, figures modelling efficiency of transmission will need confirmation once better serological and RT-rtPCR-based studies of humans, covering more of the community, have been conducted. It has become very clear that the MERS-CoV may spread poorly from human-to-human, but that spread is at its most effective around a hospital environment. Indications are that this can be traced back to poor IPC practices and protocols (Brown, 2014; Editorial, 2014; Zumla and Hui, 2014). The virus has its greatest impact on those with underlying diseases and such cases, sometimes suffering multiple comorbidities, are likely to visit hospital for treatment, creating a perfect storm of exposure, transmission and mortality. Educational programmes will be important tools to combat the spread of MERS-CoV. While MERS and SARS have some similarities they also diverge significantly. Characteristics including the higher PFC among MERS cases (above 50% in 2013 and currently at 30–40%; well above the 9.5% of SARS), the association between fatal disease and older males with underlying comorbidities, the very broad tropism of MERS-CoV, its rapid *in vitro* growth, rapid induction of cytopathogenic change, robust yet distinct transcriptional responses, use of a different receptor, its induction of a more proinflammatory but reduced and delayed innate antiviral response and its sensitivity to external IFN α or IFN β all signal differences from SARS-CoV to some degree.

MERS has had little direct impact on populations outside the Arabian Peninsula with relatively few cases acquired outside the KSA to date. From those studies conducted thus far that screen people beyond the most severely ill, there appears to be a 2–3% prevalence of MERS-CoV in the KSA with a 5% chance of secondary transmission within the household. Despite two mass gatherings that have afforded the virus many millions of opportunities to spread, there have, remarkably, been no reported outbreaks of MERS or MERS-CoV during or immediately after these events. Nonetheless, hospitals across the KSA continue to describe MERS cases and so the other 22 countries who have experienced MERS and the rest of the world remain on alert for imported cases, especially during and after mass gathering events including Umrah, Ramadan and Hajj pilgrimages.

Much remains unknown about MERS-CoV and MERS. Further cooperative data-sharing and research is needed to address questions which include:

- What is the natural host for the MERS-CoV?
- Are camels the source of sporadic human infections and if so, why is MERS-CoV seroprevalence among humans working closely with camels, so low?
- What is the route of transmission to humans and between humans and what is the best personal protective equipment to be adopted by frontline HCWs?
- Which hospital IPC measures are insufficient or insufficiently employed to halt the transmission of MERS-CoV and what is the extent of transmission and clinical impact of, and nosocomial infection by, other co-occurring respiratory viruses in these settings in the KSA?
- Why are males more often afflicted than females? Are there differences in activity and exposure that could explain this? What is the prevalence of underlying disease between the sexes?
- Does MERS-CoV infection generally result in a subclinical outcome except in those with comorbidities?
- Do current serological tools have the sensitivity to detect the immune response to a mild, subclinical or asymptomatic MERS-CoV infection? If not, might MERS-CoV be a rare, seasonal and endemic infection of humans like other HCoV's?
- For how long does the average period of MERS-CoV shedding continue from infected humans and does this differ with age, sex and vocation?
- March and April seem to be when human outbreaks of MERS begin—what are the events occurring in and around this time of year that may increase human exposure to MERS-CoV?
- Why does MERS-CoV continue to affect such a wide area of the KSA but in such very low numbers—is there an as-yet-identified role for mass gatherings in virus acquisition and subsequent geographic dissemination?
- What proportion of acute respiratory tract disease cases continue to go untested because they do not manifest as severe disease and could these clarify gaps in the transmission chains of some cases?
- Have cases of MERS been going undiagnosed among people in African countries that are known to host seropositive or virus-positive camels?
- Are there divergent MERS-CoV variants and viral clades to be found in camels, humans and bats beyond the Arabian peninsula?

The localised MERS-CoV epidemic reminds us of the importance of communication at all levels and perhaps the need to rethink how stakeholders are informed of the progress of chasing down and studying new disease threats. In May 2014, two cases of MERS-CoV were imported into the USA. A third person was proposed to be the first local transmission event but was subsequently declared a false-positive. These cases served as an excellent example of “gumshoe epidemiology” working alongside excellent public communication, comprehensive laboratory support and a well-prepared clinical capacity. Social and mainstream media and press conferences comprehensively informed the public of the extensive findings generated by expert health care supported by laboratory analysis. And yet lessons were still learned about balancing real-time transparency and the need for suitable laboratory confirmation of results. This case highlighted the effectiveness of social media in communicating digestible information to expectant stakeholders compared to the use of a generally slower and more elite peer-reviewed scientific publication pipeline which is often better suited to detailed research description over the long haul, but with some exceptions, a process unsuited for informing a concerned global and always-online public of potential threats to its immediate health or providing context to those threats.

However, while MERS-CoV is not a rapidly spreading global contagion, it may evolve further to become that or another may emerge in the future and vigilance and rapid communication will be key to a timely response and early containment. Every time a new virus emerges, we learn more, requiring us to re-examine what we previously “knew”. One constant in all this however is the human; it is we who fan the flames of a disease cluster until it becomes an outbreak, epidemic or pandemic. Viruses do not emerge and gain a foothold without our aid, or our lapses in vigilance. MERS highlighted that some paradigms can be stumbling blocks to seeking and communicating knowledge and containing and understanding the spread and nature of a virus. It also served as yet another reminder to us that global human and animal health, politics, agriculture and economies are all intimately interwoven and it takes just a single tiny virus to tip a very fine balance.

References

- 2014a. Alferon® N Effective Against MERS (Middle East Respiratory Syndrome) Virus In-Vitro. <http://www.hemispherx.net/content/investor/default.asp?goto=781>
- Abdel-Moneim, A.S., 2014. Middle East respiratory syndrome coronavirus (MERS-CoV): evidence and speculations. *Arch. Virol.* 159, 1575–1584.
- Aburizaiza, A.S., Mattes, F.M., Azhar, E.I., Hassan, A.M., Memish, Z.A., Muth, D., Meyer, B., Lattwein, E., Muller, M.A., Drosten, C., 2014. Investigation of anti-middle East respiratory syndrome antibodies in blood donors and slaughterhouse workers in Jeddah and Makkah, Saudi Arabia, fall 2012. *J. Infect. Dis.* 209, 243–246.
- Adney, D.R., van, D.N., Brown, V.R., Bushmaker, T., Scott, D., de, W.E., Bowen, R.A., Munster, V.J., 2014. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. *Emerg. Infect. Dis.* 20, 1999–2005.
- Agnihothram, S., Yount Jr., B.L., Donaldson, E.F., Huynh, J., Menachery, V.D., Gralinski, L.E., Graham, R.L., Becker, M.M., Tomar, S., Scobey, T.D., Osswald, H.L., Whitmore, A., Gopal, R., Ghosh, A.K., Mesecar, A., Zambon, M., Heise, M., Denison, M.R., Baric, R.S., 2014. A mouse model for Betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. *MBio.* 5, e00047–14.
- Agrawal, A.S., Garron, T., Tao, X., Peng, B.H., Wakamiya, M., Chan, T.S., Couch, R.B., Tseng, C.T., 2015. Generation of transgenic mouse model of middle east respiratory syndrome-coronavirus infection and disease. *J. Virol.*
- Agrawal, R.P., Dogra, R., Mohta, N., Tiwari, R., Singhal, S., Sultania, S., 2009. Beneficial effect of camel milk in diabetic nephropathy. *Acta Biomed.* 80, 131–134.
- Al-Abdallat, M.M., Payne, D.C., Alqasrawi, S., Rha, B., Tohme, R.A., Abedi, G.R., Al, N.M., Iblan, I., Jarour, N., Farag, N.H., Haddadin, A., Al-Sanouri, T., Tamin, A., Harcourt, J.L., Kuhar, D.T., Swerdlow, D.L., Erdman, D.D., Pallansch, M.A., Haynes, L.M., Gerber, S.I., 2014. Hospital-associated outbreak of Middle East respiratory syndrome coronavirus: a serologic, epidemiologic, and clinical description. *Clin. Infect. Dis.*
- Al-Gethamy, M., Corman, V.M., Hussain, R., Al-Tawfiq, J.A., Drosten, C., Memish, Z.A., 2014. A case of long-term excretion and subclinical infection with Middle East respiratory syndrome coronavirus in a healthcare worker. *Clin. Infect. Dis.*
- Al-Tawfiq, J.A., Memish, Z.A., 2014. Middle East respiratory syndrome coronavirus: transmission and phylogenetic evolution. *Trends Microbiol.* 22, 573–579.
- Al-Tawfiq, J.A., Momattin, H., Dib, J., Memish, Z.A., 2014. Ribavirin and interferon therapy in patients infected with the Middle East respiratory syndrome coronavirus: an observational study. *Int. J. Infect. Dis.* 20, 42–46.
- Al-Yousef, N., Gaafar, A., Al-Otaibi, B., Al-Jammaz, I., Al-Hussein, K., Aboussekhra, A., 2012. Camel urine components display anti-cancer properties in vitro. *J. Ethnopharmacol.* 143, 819–825.
- Alagaili, A.N., Briesse, T., Mishra, N., Kapoor, V., Sameroff, S.C., Burbelo, P.D., de, W.E., Munster, V.J., Hensley, L.E., Zalmout, I.S., Kapoor, A., Epstein, J.H., Karesh, W.B., Daszak, P., Mohammed, O.B., Lipkin, W.I., 2014. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. *MBio.* 5, e00884–e914.
- Albarrak, A.M., Stephens, G.M., Hewson, R., Memish, Z.A., 2012. Recovery from severe novel coronavirus infection. *Saudi Med. J.* 33, 1265–1269.
- Alexandersen, S., Kobinger, G.P., Soule, G., Wernery, U., 2014. Middle East respiratory syndrome coronavirus antibody reactors among camels in Dubai, United Arab Emirates, in 2005. *Transbound. Emerg. Dis.* 61, 105–108.
- Alhaidar, A., bdel Gader, A.G., Mousa, S.A., 2011. The antiplatelet activity of camel urine. *J. Altern. Complement. Med.* 17, 803–808.
- Almazán, F., DeDiego, M.L., Sola, I., Zuniga, S., Nieto-Torres, J.L., Marquez-Jurado, S., Andres, G., Enjuanes, L., 2013. Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. *MBio.* 4, e00650–e00713.
- Arabi, Y.M., Arifi, A.A., Balkhy, H.H., Najm, H., Aldawood, A.S., Ghabashi, A., Hawa, H., Althman, A., Khaldi, A., Al Raiy, B., 2014. Clinical course and outcomes of critically ill patients with Middle East respiratory syndrome coronavirus infection. *Ann. Intern. Med.* 160, 389–397.
- Assiri, A., Al-Tawfiq, J.A., Al-Rabeah, A.A., Al-Rabiah, F.A., Al-Hajjar, S., Al-Barrak, A., Flemban, H., Al-nassir, W.N., Balkhy, H.H., Al-Hakeem, R.F., Makhdoom, H.Q., Zumla, A.I., Memish, Z.A., 2013a. Epidemiological, demographic, and clinical

- characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect. Dis.* 13, 752–761.
- Assiri, A., McGeer, A., Perl, T.M., Price, C.S., Al Rabeeah, A.A., Cummings, D.A., Alabdullatif, Z.N., Assad, M., Almulhim, A., Makhdoom, H., Madani, H., Alhakeem, R., Al-Tawfiq, J.A., Cotten, M., Watson, S.J., Kellam, P., Zumla, A.I., Memish, Z.A., 2013b. Hospital outbreak of Middle East respiratory syndrome coronavirus. *N. Engl. J. Med.* 369, 407–416.
- Azhar, E.I., El-Kafrawy, S.A., Farraj, S.A., Hassan, A.M., Al-Saeed, M.S., Hashem, A.M., Madani, T.A., 2014a. Evidence for camel-to-human transmission of MERS coronavirus. *N. Engl. J. Med.*
- Azhar, E.I., Hashem, A.M., El-Kafrawy, S.A., Sohrab, S.S., Aburizaiza, A.S., Farraj, S.A., Hassan, A.M., Al-Saeed, M.S., Jamjoom, G.A., Madani, T.A., 2014b. Detection of the Middle East respiratory syndrome coronavirus genome in an air sample originating from a camel barn owned by an infected patient. *MBio.* 5, e01450–e1514.
- Barasheed, O., Rashid, H., Alfelali, M., Tashani, M., Azeem, M., Bokhary, H., Kalantan, N., Samkari, J., Heron, L., Kok, J., Taylor, J., El, B.H., Memish, Z.A., Haworth, E., Holmes, E.C., Dwyer, D.E., Asghar, A., Booy, R., 2014. Viral respiratory infections among Hajj pilgrims in 2013. *Virology.* 519, 364–371.
- Barlan, A., Zhao, J., Sarkar, M.K., Kun, L., McCray Jr., P.B., Perlman, S., Gallagher, T., 2014. Receptor variation and susceptibility to Middle East respiratory syndrome coronavirus infection. *J. Virol.*
- Bauch, C.T., Oraby, T., 2013. Assessing the pandemic potential of MERS-CoV. *Lancet* 382, 662–664.
- Bermingham, A., Chand, M.A., Brown, C.S., Aarons, E., Tong, C., Langrish, C., Hoschler, K., Brown, K., Galiano, M., Myers, R., Pebody, R.G., Green, H.K., Boddington, N.L., Gopal, R., Price, N., Newsholme, W., Drosten, C., Fouchier, R.A., Zambon, M., 2012. Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012. *Euro Surveill.* 17, 20290.
- Bialek, S.R., Allen, D., varado-Ramy, F., Arthur, R., Balajee, A., Bell, D., Best, S., Blackmore, C., Breakwell, L., Cannons, A., Brown, C., Cetron, M., Chea, N., Chommanard, C., Cohen, N., Conover, C., Crespo, A., Creviston, J., Curns, A.T., Dahl, R., Dearth, S., DeMaria, A., Echols, F., Erdman, D.D., Feikin, D., Frias, M., Gerber, S.I., Gulati, R., Hale, C., Haynes, L.M., Heberlein-Larson, L., Holton, K., Ijaz, K., Kapoor, M., Kohl, K., Kuhar, D.T., Kumar, A.M., Kundich, M., Lippold, S., Liu, L., Lovchik, J.C., Madoff, L., Martell, S., Matthews, S., Moore, J., Murray, L.R., Onofrey, S., Pallansch, M.A., Pesik, N., Pham, H., Pillai, S., Pontones, P., Pringle, K., Pritchard, S., Rasmussen, S., Richards, S., Sandoval, M., Schneider, E., Schuchat, A., Sheedy, K., Sherin, K., Swerdlow, D.L., Tappero, J.W., Vernon, M.O., Watkins, S., Watson, J., 2014. First confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV) infection in the United States, updated information on the epidemiology of MERS-CoV infection, and guidance for the public, clinicians, and public health authorities – May 2014. *MMWR Morb. Mortal. Wkly. Rep.* 63, 431–436.
- Boonacker, E., Van Noorden, C.J., 2003. The multifunctional or moonlighting protein CD26/DPPIV. *Eur. J. Cell Biol.* 82, 53–73.
- Breban, R., Riou, J., Fontanet, A., 2013. Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. *Lancet* 382, 694–699.
- Brown, C., 2014. Call for infection control to stem MERS. *CMAJ.*
- 2014b. CDC Telet briefing – Updates on Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Investigation in the United States, <http://www.cdc.gov/media/releases/2014/t0517-mers.html>
- Cauchemez, S., Fraser, C., Van, K., Donnelly, C.A., Riley, S., Rambaut, A., Enouf, V., van der, W.S., Ferguson, N.M., 2014. Middle East respiratory syndrome coronavirus: quantification of the extent of the epidemic, surveillance biases, and transmissibility. *Lancet Infect. Dis.* 14, 50–56.
- CDC, 2014. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Version 2.7-6-2014, <http://www.cdc.gov/coronavirus/mers/guidelines-clinical-specimens.html>
- CDC Newsroom, 2014. CDC Concludes Indiana MERS Patient did not Spread Virus to Illinois Business Associate. 28-5-2014, <http://www.cdc.gov/media/releases/2014/p0528-mers.html>
- Chan, J.F., Chan, K.H., Choi, G.K., To, K.K., Tse, H., Cai, J.P., Yeung, M.L., Cheng, V.C., Chen, H., Che, X.Y., Lau, S.K., Woo, P.C., Yuen, K.Y., 2013a. Differential cell line susceptibility to the emerging novel human betacoronavirus 2c EMC/2012: implications for disease pathogenesis and clinical manifestation. *J. Infect. Dis.* 207, 1743–1752.
- Chan, K.H., Chan, J.F., Tse, H., Chen, H., Lau, C.C., Cai, J.P., Tsang, A.K., Xiao, X., To, K.K., Lau, S.K., Woo, P.C., Zheng, B.J., Wang, M., Yuen, K.Y., 2013b. Cross-reactive antibodies in convalescent SARS patients' sera against the emerging novel human coronavirus EMC (2012) by both immunofluorescent and neutralizing antibody tests. *J. Infect.* 67, 130–140.
- Chan, K.H., Peiris, J.S., Lam, S.Y., Poon, L.L., Yuen, K.Y., Seto, W.H., 2011. The effects of temperature and relative humidity on the viability of the SARS coronavirus. *Adv. Virol.* 2011, 734690.
- Chan, R.W., Chan, M.C., Agnihothram, S., Chan, L.L., Kuok, D.I., Fong, J.H., Guan, Y., Poon, L.L., Baric, R.S., Nicholls, J.M., Peiris, J.S., 2013c. Tropism of and innate immune responses to the novel human betacoronavirus lineage C virus in human ex vivo respiratory organ cultures. *J. Virol.* 87, 6604–6614.
- Chen, Y., Rajashankar, K.R., Yang, Y., Agnihothram, S.S., Liu, C., Lin, Y.-L., Baric, R.S., Li, F., 2013. Crystal structure of the receptor-binding domain from newly emerged Middle East respiratory syndrome coronavirus. *J. Virol.* 87, 10777–10783.
- Chowell, G., Blumberg, S., Simonsen, L., Miller, M.A., Viboud, C., 2014. Synthesizing data and models for the spread of MERS-CoV, 2013: key role of index cases and hospital transmission. *Epidemics* 9, 40–51.
- Chu, D.K.W., Poon, L.L.M., Gomaa, M.R., Shehata, M.M., Perera, R.A.P.M., Zeid, D.A., El Rifay, A.S., Siu, L.Y., Guan, Y., Webby, R.J., Ali, M.A., Peiris, M., Kayali, G., 2014. MERS coronaviruses in dromedary camels, *Egypt. Emerg. Infect. Dis.*, 20.
- Chu, K.H., Tsang, W.K., Tang, C.S., Lam, M.F., Lai, F.M., To, K.F., Fung, K.S., Tang, H.L., Yan, W.W., Chan, H.W., Lai, T.S., Tong, K.L., Lai, K.N., 2005. Acute renal impairment in coronavirus-associated severe acute respiratory syndrome. *Kidney Int.* 67, 698–705.
- Coleman, C.M., Liu, Y.V., Mu, H., Taylor, J.K., Massare, M., Flyer, D.C., Smith, G.E., Frieman, M.B., 2014a. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. *Vaccine.*
- Coleman, C.M., Matthews, K.L., Goicochea, L., Frieman, M.B., 2014b. Wild-type and innate immune-deficient mice are not susceptible to the Middle East respiratory syndrome coronavirus. *J. Gen. Virol.* 95, 408–412.
- Corman, V.M., Eckerle, I., Bleicker, T., Zaki, A., Landt, O., Eschbach-Bludau, M., van, B.S., Gopal, R., Ballhouse, M., Bestebroer, T.M., Muth, D., Muller, M.A., Drexler, J.F., Zambon, M., Osterhaus, A.D., Fouchier, R.M., Drosten, C., 2012a. Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. *Euro Surveill.*, 17.
- Corman, V.M., Ithete, N.L., Richards, L.R., Schoeman, M.C., Preiser, W., Drosten, C., Drexler, J.F., 2014a. Rooting the phylogenetic tree of Middle East respiratory syndrome coronavirus by characterization of a conspecific virus from an African bat. *J. Virol.* 88, 11297–11303.
- Corman, V.M., Jores, J., Meyer, B., Younan, M., Liljander, A., Said, M.Y., Gluecks, I., Lattwein, E., Bosch, B.J., Drexler, J.F., Bornstein, S., Drosten, C., Muller, M.A., 2014b. Antibodies against MERS Coronavirus in Dromedary Camels, Kenya, 1992–2013. *Emerg. Infect. Dis.*, 20.
- Corman, V.M., Muller, M.A., Costabel, U., Timm, J., Binger, T., Meyer, B., Kreher, P., Lattwein, E., Eschbach-Bludau, M., Nitsche, A., Bleicker, T., Landt, O., Schweiger, B., Drexler, J.F., Osterhaus, A.D., Haagmans, B.L., Dittmer, U., Bonin, F., Wolff, T., Drosten, C., 2012b. Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. *Euro Surveill.*, 17.
- Coston, M., 2014. Avian Flu Diary: Michael Coston's Blog on Emerging Infectious Diseases. <http://afidiary.blogspot.com.au/>
- Cotten, M., Lam, T.T., Watson, S.J., Palser, A.L., Petrova, V., Grant, P., Pybus, O.G., Rambaut, A., Guan, Y., Pillay, D., Kellam, P., Nastouli, E., 2013a. Full-genome deep sequencing and phylogenetic analysis of novel human betacoronavirus. *Emerg. Infect. Dis.* 19, 736–42B.
- Cotten, M., Watson, S.J., Kellam, P., Al-Rabeeah, A.A., Makhdoom, H.Q., Assiri, A., Al-Tawfiq, J.A., Alhakeem, R.F., Madani, H., AlRabiah, F.A., Al, H.S., Al-nassir, W.N., Albarrak, A., Flemban, H., Balkhy, H.H., Alsubaie, S., Palser, A.L., Gall, A., Bashford-Rogers, R., Rambaut, A., Zumla, A.I., Memish, Z.A., 2013b. Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study. *Lancet* 382, 1993–2002.
- Cotten, M., Watson, S.J., Zumla, A.I., Makhdoom, H.Q., Palser, A.L., Ong, S.H., Al Rabeeah, A.A., Alhakeem, R.F., Assiri, A., Al-Tawfiq, J.A., Albarrak, A., Barry, M., Shibl, A., AlRabiah, F.A., Hajjar, S., Balkhy, H.H., Flemban, H., Rambaut, A., Kellam, P., Memish, Z.A., 2014. Spread, circulation, and evolution of the Middle East respiratory syndrome coronavirus. *MBio.*, 5.
- 2014c. Deadly MERS Virus Circulates Among Arabian Camels, <http://www.npr.org/blogs/health/2014/02/25/282136478/deadly-mers-virus-circulates-among-arabian-camels>
- de Groot, R.J., Baker, S.C., Baric, R.S., Brown, C.S., Drosten, C., Enjuanes, L., Fouchier, R.A., Galiano, M., Gorbalenya, A.E., Memish, Z.A., Perlman, S., Poon, L.L., Snijder, E.J., Stephens, G.M., Woo, P.C., Zaki, A.M., Zambon, M., Ziebuhr, J., 2013. Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *J. Virol.* 87, 7790–7792.
- de Wilde, A.H., Jochmans, D., Posthuma, C.C., Zevenhoven-Dobbe, J.C., van, N.S., Bestebroer, T.M., van den Hoogen, B.G., Neyts, J., Snijder, E.J., 2014. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob. Agents Chemother.*
- Devi, J.P., Noraini, W., Norhayati, R., Kheong, C.C., Badrul, A.S., Zainah, S., Fadzilah, K., Hirman, I., Hakim, S.L., Hisham, A.N., 2014. Laboratory-confirmed case of Middle East respiratory syndrome coronavirus (MERS-CoV) infection in Malaysia: preparedness and response, April 2014. *Eurosurveillance* 19, 20797.
- de Wit, E., Rasmussen, A.L., Falzarano, D., Bushmaker, T., Feldmann, F., Brining, D.L., Fischer, E.R., Martellaro, C., Okumura, A., Chang, J., Scott, D., Bencke, A.G., Katze, M.G., Feldmann, H., Munster, V.J., 2013. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. *Proc. Natl. Acad. Sci. U. S. A.* 110, 16598–16603.
- Drosten, C., 2013. Is MERS another SARS? *Lancet Infect. Dis.* 13, 727–728.
- Drosten, C., Kellam, P., Memish, Z.A., 2014a. Evidence for camel-to-human transmission of MERS coronavirus. *N. Engl. J. Med.* 371, 1359–1360.
- Drosten, C., Meyer, B., Muller, M.A., Corman, V.M., Al-Masri, M., Hossain, R., Madani, H., Sieberg, A., Bosch, B.J., Lattwein, E., Alhakeem, R.F., Assiri, A.M., Hajomar, W., Albarrak, A.M., Al-Tawfiq, J.A., Zumla, A.I., Memish, Z.A., 2014b. Transmission of MERS-coronavirus in household contacts. *N. Engl. J. Med.* 371, 828–835.
- Drosten, C., Muth, D., Corman, V.M., Hussain, R., al, M.M., Hajomar, W., Landt, O., Assiri, A., Eckerle, I., Al, S.A., Al-Tawfiq, J.A., Albarrak, A., Zumla, A., Rambaut, A., Memish, Z.A., 2015. An observational, laboratory-based study of outbreaks of MERS-coronavirus in Jeddah and Riyadh, Kingdom of Saudi Arabia, 2014. *Clin. Infect. Dis.* 60, 369–377.

- Drosten, C., Seilmaier, M., Corman, V.M., Hartmann, W., Scheible, G., Sack, S., Guggemos, W., Kallies, R., Muth, D., Junglen, S., Muller, M.A., Haas, W., Guberina, H., Rohnisch, T., Schmid-Wendtner, M., Aldabbagh, S., Dittmer, U., Gold, H., Graf, P., Bonin, F., Rambaut, A., Wendtner, C.M., 2013. **Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection.** *Lancet Infect. Dis.* 13, 745–751.
- Du, L., Kou, Z., Ma, C., Tao, X., Wang, L., Zhao, G., Chen, Y., Yu, F., Tseng, C.T., Zhou, Y., Jiang, S., 2013a. **A truncated receptor-binding domain of MERS-CoV spike protein potentially inhibits MERS-CoV infection and induces strong neutralizing antibody responses: implication for developing therapeutics and vaccines.** *PLOS ONE* 8, e81587.
- Du, L., Zhao, G., Kou, Z., Ma, C., Sun, S., Poon, V.K., Lu, L., Wang, L., Debnath, A.K., Zheng, B.J., Zhou, Y., Jiang, S., 2013b. **Identification of a receptor-binding domain in the S protein of the novel human coronavirus Middle East respiratory syndrome coronavirus as an essential target for vaccine development.** *J. Virol.* 87, 9939–9942.
- Dudley, J.P., Mackay, I.M., 2013. **Age-specific and sex-specific morbidity and mortality from avian influenza A(H7N9).** *J. Clin. Virol.* 58, 568–570.
- Dyall, J., Coleman, C.M., Hart, B.J., Venkataraman, T., Holbrook, M.R., Kindrachuk, J., Johnson, R.F., Olinger Jr., G.G., Jahrling, P.B., Laidlaw, M., Johansen, L.M., Lear, C.M., Glass, P.J., Hensley, L.E., Frieman, M.B., 2014. **Repurposing of clinically developed drugs for treatment of Middle East Respiratory Coronavirus Infection.** *Antimicrob. Agents Chemother.*
- Eckerle, I., Corman, V.M., Muller, M.A., Lenk, M., Ulrich, R.G., Drosten, C., 2014. **Replicative capacity of MERS coronavirus in livestock cell lines.** *Emerg. Infect. Dis.* 20, 276–279.
- Eckerle, I., Muller, M.A., Kallies, S., Gotthardt, D.N., Drosten, C., 2013. **In-vitro renal epithelial cell infection reveals a viral kidney tropism as a potential mechanism for acute renal failure during Middle East Respiratory Syndrome (MERS) Coronavirus infection.** *Virology* 461, 359.
- Editorial, 2014. **MERS-CoV: address the knowledge gaps to move forward.** *Lancet* 383, 1782.
- EUROIMMUN Medizinische Labordiagnostika AG, 2014. **Test Characteristics: Anti-MERS-CoV ELISA Camel (IgG).** http://www.vet.euroimmun.de/fileadmin/vet.euroimmun/pdf/EL2604V_D_UK_A.pdf
- Falzarano, D., de W.E., Rasmussen, A.L., Feldmann, F., Okumura, A., Scott, D.P., Brining, D., Bushmaker, T., Martellaro, C., Baseler, L., Beneke, A.G., Katze, M.G., Munster, V.J., Feldmann, H., 2013. **Treatment with interferon-alpha2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques.** *Nat. Med.* 19, 1313–1317.
- Faure, E., Poissy, J., Goffard, A., Fournier, C., Kipnis, E., Titecat, M., Bortolotti, P., Martinez, L., Dubucquoi, S., Dessein, R., Gosset, P., Mathieu, D., Guery, B., 2014. **Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside?** *PLOS ONE* 9, e88716.
- Frey, K.G., Redden, C.L., Bishop-Lilly, K.A., Johnson, R., Hensley, L.E., Raviprakash, K., Luke, T., Kochel, T., Mokashi, V.P., Defang, G.N., 2014. **Full-genome sequence of human betacoronavirus 2c_jordan-n3/2012 after serial passage in Mammalian cells.** *Genome Announc.* 2.
- Gautret, P., Charrel, R., Belhouach, K., Drali, T., Benkouiten, S., Nougaiere, A., Zandotti, C., Memish, Z.A., al Masri, M.M., Gaillard, C., Brouqui, P., Parola, P., 2013. **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.* 19, E315–E317.
- Gierer, S., Hofmann-Winkler, H., Albuali, W.H., Bertram, S., Al-Rubaish, A.M., Yousef, A.A., Al-Nafaie, A.N., Al-Ali, A.K., Obeid, O.E., Alkharsah, K.R., Pohlmann, S., 2013. **Lack of MERS coronavirus neutralizing antibodies in humans, eastern province, Saudi Arabia.** *Emerg. Infect. Dis.* 19, 2034–2036.
- Gossner, C., Danielson, N., Gervelmeyer, A., Berthe, F., Faye, B., Kaasik, A.K., Adl-hoch, C., Zeller, H., Penttinen, P., Coulombier, D., 2014. **Human–Dromedary camel interactions and the risk of acquiring zoonotic Middle East respiratory syndrome coronavirus infection.** *Zoonoses Public Health.*
- Granger, S., 2012. **Random Analytics.** <http://gmgranger.wordpress.com/>
- Grouzmann, E., Monod, M., Landis, B., Wilk, S., Brakch, N., Nicoucar, K., Giger, R., Malis, D., Szalay-Quinodoz, I., Cavadas, C., Morel, D.R., Lacroix, J.S., 2002. **Loss of dipeptidylpeptidase IV activity in chronic rhinosinusitis contributes to the neurogenic inflammation induced by substance P in the nasal mucosa.** *FASEB J.* 16, 1132–1134.
- Gundy, P.M., Gerba, C.P., Pepper, I.L., 2009. **Survival of coronaviruses in water and wastewater.** *Food Environ. Virol.* 1, 10–14.
- Haagmans, B.L., Al Dhahiry, S.H., Reusken, C.B., Raj, V.S., Galiano, M., Myers, R., Godeke, G.J., Jonges, M., Farag, E., Diab, A., Ghobashy, H., Alhajri, F., Al-Thani, M., Al-Marri, S.A., Al Romaihi, H.E., Al, K.A., Bermingham, A., Osterhaus, A.D., Alhajri, M.M., Koopmans, M.P., 2014. **Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation.** *Lancet Infect. Dis.* 14, 140–145.
- Hartl, G., 2013. **Novel coronavirus: the challenge of communicating about a virus which one knows little about.** *East. Mediterr. Health J.* 1 (19 Suppl.), S26–S30.
- Health Protection Agency (HPA) UK Novel Coronavirus Investigation Team, 2013. **Evidence of person-to-person transmission within a family cluster of novel coronavirus infections, United Kingdom, February 2013.** *Eurosurveillance* 18, 20427.
- Hemida, M.G., Al-Naeem, A., Perera, R.A.P.M., Chin, A.W.H., Poon, L.L.M., Peiris, M., 2015. **Lack of Middle East respiratory syndrome coronavirus transmission from infected camels.** *Emerg. Infect. Dis.* 21.
- Hemida, M.G., Chu, D.K.W., Poon, L.L.M., Perera, R.A.P.M., Alhammadi, M.A., Ng, H.-Y., Siu, L.Y., Guan, Y., Alnaeem, A., Peiris, M., 2014a. **MERS coronavirus in dromedary camel herd, Saudi Arabia.** *Emerg. Infect. Dis.* 20.
- Hemida, M.G., Perera, R.A., Al Jassim, R.A., Kayali, G., Siu, L.Y., Wang, P., Chu, K.W., Perlman, S., Ali, M.A., Alnaeem, A., Guan, Y., Poon, L.L., Saif, L., Peiris, M., 2014b. **Seroepidemiology of Middle East respiratory syndrome (MERS) coronavirus in Saudi Arabia (1993) and Australia (2014) and characterisation of assay specificity.** *Euro Surveill.* 19.
- Hemida, M.G., Perera, R.A., Wang, P., Alhammadi, M.A., Siu, L.Y., Li, M., Poon, L.L., Saif, L., Alnaeem, A., Peiris, M., 2013. **Middle East respiratory syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010 to 2013.** *Euro Surveill.* 18, 20659.
- Hijawi, B., Abdallat, M., Sayaydeh, A., Alqasrawi, S., Haddadin, A., Jaarour, N., Alsheikh, S., Alsanouri, T., 2013. **Novel coronavirus infections in Jordan, April 2012: epidemiological findings from a retrospective investigation.** *East. Mediterr. Health J.* 1 (19 Suppl.), S12–S18.
- Holmes, D., 2014. **MERS-CoV enigma deepens as reported cases surge.** *Lancet* 383, 1793.
- Jiang, L., Wang, N., Zuo, T., Shi, X., Poon, K.M., Wu, Y., Gao, F., Li, D., Wang, R., Guo, J., Fu, L., Yuen, K.Y., Zheng, B.J., Wang, X., Zhang, L., 2014. **Potent neutralization of MERS-CoV by human neutralizing monoclonal antibodies to the viral spike glycoprotein.** *Sci. Transl. Med.* 6, 234ra59.
- Josset, L., Menachery, V.D., Gralinski, L.E., Agnihotram, S., Sova, P., Carter, V.S., Yount, B.L., Graham, R.L., Baric, R.S., Katze, M.G., 2013. **Cell host response to infection with novel human coronavirus EMC predicts potential antiviral and important differences with SARS coronavirus.** *MBio.* 4, e00165–e213.
- Kawalec, P., Mikrut, A., Lopuch, S., 2014. **The safety of dipeptidyl peptidase-4 (DPP-4) inhibitors or sodium-glucose cotransporter 2 (SGLT-2) inhibitors added to metformin background therapy in patients with type 2 diabetes mellitus: a systematic review and meta-analysis.** *Diabetes Metab. Res. Rev.*
- Khalid, M., Al, R.F., Khan, B., Al, M.A., Butt, T.S., Al, M.E., 2014. **Ribavirin and interferon (IFN)-alpha-2b as primary and preventive treatment for Middle East respiratory syndrome coronavirus (MERS-CoV): a preliminary report of two cases.** *Antivir. Ther.*
- Khuri-Bulos, N., Payne, D.C., Lu, X., Erdman, D., Wang, L., Faouri, S., Shehabi, A., Johnson, M., Becker, M.M., Denison, M.R., Williams, J.V., Halasa, N.B., 2013. **Middle East respiratory syndrome coronavirus not detected in children hospitalized with acute respiratory illness in Amman, Jordan, March 2010 to September 2012.** *Clin. Microbiol. Infect.*
- Kilian, C., 2014. **H5N1: Crawford Kilian's Infectious Disease Blog.** 8-6-2014. <http://crofsblogs.typepad.com/h5n1/>
- Kilianski, A., Mielech, A.M., Deng, X., Baker, S.C., 2013. **Assessing activity and inhibition of Middle East respiratory syndrome coronavirus papain-like and 3C-like proteases using luciferase-based biosensors.** *J. Virol.* 87, 11955–11962.
- Kindler, E., Jonsdottir, H.R., Muth, D., Hamming, O.J., Hartmann, R., Rodriguez, R., Geffers, R., Fouchier, R.A., Drosten, C., Muller, M.A., Dijkman, R., Thiel, V., 2013. **Efficient replication of the novel human betacoronavirus EMC on primary human epithelium highlights its zoonotic potential.** *MBio.* 4, e00611–e00612.
- Knibbs, L.D., Johnson, G.R., Kidd, T.J., Cheney, J., Grimwood, K., Kattenbelt, J.A., O'Rourke, P.K., Ramsay, K.A., Sly, P.D., Wainwright, C.E., Wood, M.E., Morawska, L., Bell, S.C., 2014. **Viability of *Pseudomonas aeruginosa* in cough aerosols generated by persons with cystic fibrosis.** *Thorax.*
- Kraaij-Dirkzwager, M., Timen, A., Dirksen, K., Gelnick, L., Leyten, E., Groeneveld, P., 2014. **Middle East respiratory syndrome coronavirus (MERS-CoV) infections in two returning travellers in the Netherlands, May 2014.** *Euro Surveill.* 19, 20817.
- Kupferschmidt, K., 2014. **The camel connection.** *Science* 343 (1422–3), 1425.
- 2014d. **Link Between MERS Virus and Camels Worries Breeders – 10-8-2014d.** <http://www.arabnews.com/news/460814>
- Lau, E.H.Y., Zheng, J., Tsang, T.K., Liao, Q., Lewis, B., Brownstein, J.S., Sanders, S., Wong, J.Y., Mekaru, S.R., Rivers, C., Wu, P., Jiang, H., Li, Y., Yu, J., Yu, J., Zhang, Q., Chang, Z., Liu, F., Peng, Z., Leung, G.M., Feng, L., Cowling, B.J., Yu, H., 2014. **Accuracy of epidemiological inferences based on publicly available information: retrospective comparative analysis of line lists of human cases infected with influenza A(H7N9) in China.** *BMC Med.* 12.
- Lau, S.K., Lau, C.C., Chan, K.H., Li, C.P., Chen, H., Jin, D.Y., Chan, J.F., Woo, P.C., Yuen, K.Y., 2013. **Delayed induction of proinflammatory cytokines and suppression of innate antiviral response by the novel Middle East respiratory syndrome coronavirus: implications for pathogenesis and treatment.** *J. Gen. Virol.* 94, 2679–2690.
- Lin, M.H., Chuang, S.J., Chen, C.C., Cheng, S.C., Cheng, K.W., Lin, C.H., Sun, C.Y., Chou, C.Y., 2014. **Structural and functional characterization of MERS coronavirus papain-like protease.** *J. Biomed. Sci.* 21, 54.
- Lu, G., Hu, Y., Wang, Q., Qi, J., Gao, F., Li, Y., Zhang, Y., Zhang, W., Yuan, Y., Bao, J., Zhang, B., Shi, Y., Yan, J., Gao, G.F., 2013. **Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26.** *Nature* 500, 227–231.
- Lun, S.W., Wong, C.K., Ko, F.W., Hui, D.S., Lam, C.W., 2007. **Increased expression of plasma and CD4+ T lymphocyte costimulatory molecule CD26 in adult patients with allergic asthma.** *J. Clin. Immunol.* 27, 430–437.
- Lundin, A., Dijkman, R., Bergstrom, T., Kann, N., Adamiak, B., Hannoun, C., Kindler, E., Jonsdottir, H.R., Muth, D., Kint, J., Forlenza, M., Muller, M.A., Drosten, C., Thiel, V., Trybala, E., 2014. **Targeting Membrane-Bound Viral RNA Synthesis Reveals Potential Inhibition of Diverse Coronaviruses Including the Middle East Respiratory Syndrome Virus.** *PLoS Pathog.* 10, e1004166.
- 2014e. **Middle East Respiratory Syndrome Coronavirus (MERS-CoV) – Update 15-May-2014e.** <http://www.who.int/csr/don/2014.05.15.mers/en/>

- Mackay, I.M., 2014. MERS-CoV Daily, Monthly and Cumulative Numbers Over Time. 1-12-0015. <http://virologydownunder.blogspot.com.au/2014/08/mers-cov-daily-monthly-and-cumulative.html>
- Mackay, I.M., 1997. Virology Down Under. 21-6-2014a. <http://www.uq.edu.au/vdu/>
- Mackay, I.M., 2013. Virology Down Under Blog. 2-6-2014b. <http://virologydownunder.blogspot.com.au/>
- Mackay, I.M., Arden, K.E., Speicher, D.J., O'Neill, N.T., McErlean, P.K., Greer, R.M., Nissen, M.D., Sloots, T.P., 2012. Co-circulation of four human coronaviruses (HCoVs) in Queensland children with acute respiratory tract illnesses in 2004. *Viruses* 4, 637–653.
- Mailles, A., Blanckaert, K., Chaud, P., van der, W.S., Lina, B., Caro, V., Campese, C., Guery, B., Prouvost, H., Lemaire, X., Paty, M.C., Haeghebaert, S., Antoine, D., Ettahar, N., Noel, H., Behillil, S., Hendrick, S., Manuguerra, J.C., Enouf, V., La, R.G., Semaille, C., Coignard, B., Levy-Bruhl, D., Weber, F., Saura, C., Che, D., 2013. First cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infections in France, investigations and implications for the prevention of human-to-human transmission, France, May 2013. *Euro Surveill.*, 18.
- Majumder, M., 2014. Mens et Manus: Maia Majumder's Blog. 8-6-2014. <http://maimunamajumder.wordpress.com/>
- Maltezzou, H.C., Tsioudras, S., 2014. Middle East respiratory syndrome coronavirus: Implications for health care facilities. *Am. J. Infect. Control* 42, 1261–1265.
- Memish, Z.A., Al-Tawfiq, J.A., Assiri, A., AlRabiah, F.A., Hajjar, S.A., Albarrak, A., Flemban, H., Alhakeem, R.F., Makhdoom, H.Q., Alsubaie, S., Al-Rabeeah, A.A., 2014a. Middle East Respiratory Syndrome Coronavirus Disease in Children. *Pediatr. Infect. Dis. J.*
- Memish, Z.A., Al-Tawfiq, J.A., Makhdoom, H.Q., Al-Rabeeah, A.A., Assiri, A., Alhakeem, R.F., AlRabiah, F.A., Al, H.S., Albarrak, A., Flemban, H., Balkhy, H., Barry, M., Alhassan, S., Alsubaie, S., Zumla, A., 2014b. Screening for Middle East respiratory syndrome coronavirus infection in hospital patients and their healthcare worker and family contacts: a prospective descriptive study. *Clin. Microbiol. Infect.*
- Memish, Z.A., Al-Tawfiq, J.A., Makhdoom, H.Q., Assiri, A., Alhakeem, R.F., Albarrak, A., Alsubaie, S., Al-Rabeeah, A.A., Hajomar, W.H., Hussain, R., Kheyami, A.M., Almutairi, A., Azhar, E.I., Drosten, C., Watson, S.J., Kellam, P., Cotten, M.W., Zumla, A., 2014c. Respiratory Tract Samples, Viral Load and Genome Fraction Yield in patients with Middle East Respiratory Syndrome. *J. Infect. Dis.*
- Memish, Z.A., Assiri, A., Almasri, M., Alhakeem, R.F., Turkestani, A., Al Rabeeah, A.A., Al-Tawfiq, J.A., Alzahrani, A., Azhar, E., Makhdoom, H.Q., Hajomar, W.H., Al-Shangiti, A.M., Yezli, S., 2014d. Prevalence of MERS-CoV Nasal Carriage and Compliance With the Saudi Health Recommendations Among Pilgrims Attending the 2013 Hajj. *J. Infect. Dis.*
- Memish, Z.A., Assiri, A.M., Al-Tawfiq, J.A., 2014e. Middle East respiratory syndrome coronavirus (MERS-CoV) viral shedding in the respiratory tract: an observational analysis with infection control implications. *Int. J. Infect. Dis.* 29, 307–308.
- Memish, Z.A., Cotten, M., Meyer, B., Watson, S.J., Alshahfi, A.J., Al Rabeeh, A.A., Corman, V.M., Sieberg, A., Makhdoom, H.Q., Assiri, A., Al Masri, M., Aldabbagh, S., Bosch, R.J., Beer, M., Müller, M.A., Kellam, P., Drosten, C., 2014f. Human Infection with MERS Coronavirus after Exposure to Infected Camels, Saudi Arabia, 2013. *Emerg. Infect. Dis.*, 20.
- Memish, Z.A., Cotten, M., Watson, S.J., Kellam, P., Zumla, A., Alhakeem, R.F., Assiri, A., Rabeeah, A.A., Al-Tawfiq, J.A., 2014g. Community Case Clusters of Middle East Respiratory Syndrome Coronavirus in Hafr Al-Batin, Kingdom of Saudi Arabia: A Descriptive Genomic study. *Int. J. Infect. Dis.*
- Memish, Z.A., Mishra, N., Olival, K.J., Fagbo, S.F., Kapoor, V., Epstein, J.H., Alhakeem, R., Durosintoun, A., Al, A.M., Islam, A., Kapoor, A., Briese, T., Daszak, P., Al Rabeeah, A.A., Lipkin, W.I., 2013a. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. *Emerg. Infect. Dis.* 19, 1819–1823.
- Memish, Z.A., Zumla, A.I., Al-Hakeem, R.F., Al-Rabeeah, A.A., Stephens, G.M., 2013b. Family cluster of Middle East respiratory syndrome coronavirus infections. *N. Engl. J. Med.* 368, 2487–2494.
- Meyer, B., Drosten, C., Müller, M.A., 2014a. Serological assays for emerging coronaviruses: challenges and pitfalls. *Virus Res.*
- Meyer, B., Garcia-Bocanegra, I., Wernery, U., Wernery, R., Sieberg, A., Müller, M.A., Drexler, J.F., Drosten, C., Eckerle, I., 2015. Serologic Assessment of Possibility for MERS-CoV Infection in Equids. *Emerg. Infect. Dis.* 21, 181–182.
- Meyer, B., Müller, M.A., Corman, V.M., Reusken, C.B., Ritz, D., Godeke, G.J., Lattwein, E., Kallies, S., Siemens, A., van, B.J., Drexler, J.F., Muth, D., Bosch, B.J., Wernery, U., Koopmans, M.P., Wernery, R., Drosten, C., 2014b. Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. *Emerg. Infect. Dis.* 20, 552–559.
- Ministry of Health, Saudi Arabia, 2014. 2012–2014 Case List of MoH/WHO Novel Coronavirus MERS nCoV Announced Cases. 7-6-2014. <http://www.flutracker.com/forum/showthread.php?t=205075>
- Mohamad, R.H., Zekry, Z.K., Al-Mehdar, H.A., Salama, O., El-Shaieb, S.E., El-Basmy, A.A., Al-said, M.G., Sharawy, S.M., 2009. Camel milk as an adjuvant therapy for the treatment of type 1 diabetes: verification of a traditional ethnomedical practice. *J. Med. Food* 12, 461–465.
- Mou, H., Raj, V.S., van Kuppeveld, F.J., Rottier, P.J., Haagmans, B.L., Bosch, B.J., 2013. The receptor binding domain of the new Middle East respiratory syndrome coronavirus maps to a 231-residue region in the spike protein that efficiently elicits neutralizing antibodies. *J. Virol.* 87, 9379–9383.
- Muller, M.A., Raj, V.S., Muth, D., Meyer, B., Kallies, S., Smits, S.L., Wollny, R., Bestebroer, T.M., Specht, S., Suliman, T., Zimmermann, K., Binger, T., Eckerle, I., Tschapka, M., Zaki, A.M., Osterhaus, A.D., Fouchier, R.A., Haagmans, B.L., Drosten, C., 2012. Human coronavirus EMC does not require the SARS-coronavirus receptor and maintains broad replicative capability in mammalian cell lines. *MBio.*, 3.
- Munster, V.J., de, W.E., Feldmann, H., 2013. Pneumonia from human coronavirus in a macaque model. *N. Engl. J. Med.* 368, 1560–1562.
- Nishiura, H., Ejima, K., Mizumoto, K., 2014. Missing information in animal surveillance of MERS-CoV. *Lancet Infect. Dis.* 14, 100.
- Nowotny, N., Kolodziejek, J., 2014. Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, Oman, 2013. *Euro Surveill.*, 19.
- Omrani, A.S., Matin, M.A., Haddad, Q., Al-Nakhli, D., Memish, Z.A., Albarrak, A.M., 2013. A family cluster of Middle East Respiratory Syndrome Coronavirus infections related to a likely unrecognized asymptomatic or mild case. *Int. J. Infect. Dis.* 17, e668–e672.
- 2014f. Public Health and Animal Health Experts Review Knowledge on MERS-CoV. 8-8-2014f. <http://www.oie.int/en/for-the-media/press-releases/detail/article/public-health-and-animal-health-experts-review-knowledge-on-mers-cov/>
- Payne, D.C., Iblan, I., Alqasrawi, S., Al, N.M., Rha, B., Tohme, R.A., Abedi, G.R., Farag, N.H., Haddadin, A., Al, S.T., Jarour, N., Swerdlow, D.L., Jamieson, D.J., Pallansch, M.A., Haynes, L.M., Gerber, S.I., Al Abdallat, M.M., 2014. Stillbirth During Infection With Middle East Respiratory Syndrome Coronavirus. *J. Infect. Dis.*
- Penntinen, P.M., Kaasik-Aaslav, K., Friaux, A., Donachie, A., Sudre, B., Mato-Gauci, A.J., Memish, Z.A., Coulombier, D., 2013. Taking stock of the first 133 MERS coronavirus cases globally – Is the epidemic changing? *Euro Surveill.*, 18.
- Perera, R.A., Wang, P., Gomaa, M.R., El-Shesheny, R., Kandeil, A., Bagato, O., Siu, L.Y., Shehata, M.M., Kayed, A.S., Moatasim, Y., Li, M., Poon, L.L., Guan, Y., Webby, R.J., Ali, M.A., Peiris, J.S., Kayali, G., 2013. Seroprevalence of MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt, June 2013. *Euro Surveill.* 18 (ii).
- Poletto, C., Pelat, C., Levy-Bruhl, D., Yazdanpanah, Y., Boelle, P.-Y., Colizza, V., 2013. Assessment of the MERS-CoV Epidemic Situation in the Middle East Region. Program for Monitoring Emerging Diseases, 2014. *ProMED Mail.* 8-6-2014. <http://www.promedmail.org/>
- ProMED, 2012. Novel Coronavirus – Saudi Arabia: Human Isolate. 11-4-2012. <http://www.promedmail.org/direct.php?id=20120920.1302733>
- ProMED, 2014. Novel Coronavirus – Saudi Arabia: Human Isolate. <http://www.promedmail.org/direct.php?id=20120920.1302733>
- Puzelli, S., Azzi, A., Santini, M.G., Di, M.A., Facchini, M., Castrucci, M.R., Meola, M., Arvia, R., Corcioli, F., Pierucci, F., Baretti, S., Bartoloni, A., Bartolozzi, D., de, M.M., Galli, L., Poma, M.G., Rezza, G., Balocchini, E., Donatelli, I., 2013. Investigation of an imported case of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) infection in Florence, Italy, May to June 2013. *Euro Surveill.*, 18.
- Raj, V.S., Farag, E.A.B.A., Reusken, C.B.E.M., Lamers, M.M., Pas, S.D., Voermans, J., Smits, S.L., Osterhaus, A.D.M.E., Al-Mawlawi, N., Al-Romaihi, H.E., AlHajri, M.M., El-Sayed, A.M., Mohran, K.A., Ghobashy, H., Alhajri, F., Al-Thani, M., Al-Marri, S.A., El-Maghraby, M.M., Koopmans, M.P.G., Haagmans, B.L., 2014a. Isolation of MERS Coronavirus from a Dromedary Camel, Qatar, 2014. *Emerg. Infect. Dis.*, 20.
- Raj, V.S., Mou, H., Smits, S.L., Dekkers, D.H., Müller, M.A., Dijkman, R., Muth, D., Demmers, J.A., Zaki, A., Fouchier, R.A., Thiel, V., Drosten, C., Rottier, P.J., Osterhaus, A.D., Bosch, B.J., Haagmans, B.L., 2013. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 495, 251–254.
- Raj, V.S., Osterhaus, A.D., Fouchier, R.A., Haagmans, B.L., 2014b. MERS: emergence of a novel human coronavirus. *Curr. Opin. Virol.* 5C, 58–62.
- Reusken, C., Mou, H., Godeke, G.J., van der, H.L., Meyer, B., Müller, M.A., Haagmans, B., De, S.R., Schuurman, N., Dittmer, U., Rottier, P., Osterhaus, A., Drosten, C., Bosch, B.J., Koopmans, M., 2013a. Specific serology for emerging human coronaviruses by protein microarray. *Euro Surveill.* 18, 20441.
- Reusken, C.B., Ababneh, M., Raj, V.S., Meyer, B., Eljarah, A., Abutarbush, S., Godeke, G.J., Bestebroer, T.M., Zutt, I., Müller, M.A., Bosch, B.J., Rottier, P.J., Osterhaus, A.D., Drosten, C., Haagmans, B.L., Koopmans, M.P., 2013b. Middle East Respiratory Syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, June to September 2013. *Euro Surveill.* 18, 20662.
- Reusken, C.B., Farag, E.A., Jonges, M., Godeke, G.J., El-Sayed, A.M., Pas, S.D., Raj, V.S., Mohran, K.A., Moussa, H.A., Ghobashy, H., Alhajri, F., Ibrahim, A.K., Bosch, B.J., Pasha, S.K., Al-Romaihi, H.E., Al-Thani, M., Al-Marri, S.A., AlHajri, M.M., Haagmans, B.L., Koopmans, M.P., 2014a. Middle East respiratory syndrome coronavirus (MERS-CoV) RNA and neutralising antibodies in milk collected according to local customs from dromedary camels, Qatar, April 2014. *Euro Surveill.*, 19.
- Reusken, C.B., Haagmans, B.L., Müller, M.A., Gutierrez, C., Godeke, G.J., Meyer, B., Muth, D., Raj, V.S., Smits-De, V.L., Corman, V.M., Drexler, J.F., Smits, S.L., El Tahir, Y.E., De, S.R., van, B.J., Nowotny, N., van, M.K., Hidalgo-Hermoso, E., Bosch, B.J., Rottier, P., Osterhaus, A., Gortazar-Schmidt, C., Drosten, C., Koopmans, M.P., 2013c. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. *Lancet Infect. Dis.* 13, 859–866.
- Reusken, C.B.E.M., Messadi, L., Feyisa, A., Ularumu, H., Godeke, G.J., Danmarwa, A., Dawo, F., Jemli, M., Melaku, S., Shamaki, D., Woma, Y., Wungak, Y., Gebremedhin, E.Z., Zutt, I., Bosch, B.J., Haagmans, B.L., Koopmans, M.P.G., 2014b. Geographic distribution of MERS Coronavirus among dromedary camels, Africa. *Emerg. Infect. Dis.*, 20.
- Reuss, A., Litterst, A., Drosten, C., Seilmaier, M., Bohmer, M., Graf, P., Gold, H., Wendtner, C.M., Zanzuzdana, A., Schaade, L., Haas, W., Buchholz, U., 2014. Contact investigation for imported case of Middle East respiratory syndrome, Germany. *Emerg. Infect. Dis.* 20, 620–625.

- Reuters, 2014. Saudi Arabia Replaces Health Minister Amid MERS Virus Fears. <http://www.reuters.com/article/2014/04/21/us-saudi-minister-mers-idUSBREA3K0QJ20140421>
- 2014g. Soaring MERS Cases Cause Pandemic Jitters, but Causes are Unclear. <http://news.sciencemag.org/health/2014/04/soaring-mers-cases-cause-pandemic-jitters-causes-are-unclear>
- Sabat, R., 2010. IL-10 family of cytokines. *Cytokine Growth Factor Rev.* 21, 315–324.
- Salathe, M., Freifeld, C.C., Mekaru, S.R., Tomasulo, A.F., Brownstein, J.S., 2013. Influenza A (H7N9) and the importance of digital epidemiology. *N. Engl. J. Med.* 369, 401–404.
- Sampathkumar, P., 2014. Middle East respiratory syndrome: what clinicians need to know. *Mayo Clin. Proc.* 89, 1153–1158.
- Sciencemag, 2014. As Outbreak Continues, Confusion Reigns Over Virus Patents. <http://news.sciencemag.org/people-events/2013/05/outbreak-continues-confusion-reigns-over-virus-patents>
- Sharmin, R., Islam, A.B., 2014. A highly conserved WDYPKCDRA epitope in the RNA directed RNA polymerase of human coronaviruses can be used as epitope-based universal vaccine design. *BMC Bioinformatics* 15, 161.
- Shi, Z., 2013. Emerging infectious diseases associated with bat viruses. *Sci. China Life Sci.* 56, 678–682.
- SILVER consortium, 2015. SILVER MERS-Coronavirus Outbreak Pipeline. 22-1–2015. <http://www.silver-europe.com/spip.php?article229>
- Smith, I., Wang, L.F., 2013. Bats and their virome: an important source of emerging viruses capable of infecting humans. *Curr. Opin. Virol.* 3, 84–91.
- Smits, S.L., Raj, V.S., Pas, S.D., Reusken, C.B.E.M., Mohran, K., Farag, E.A.B.A., Al-Romaihi, H.E., AlHajri, M.M., Haagmans, B.L., Koopmans, M.P., 2015. Reliable typing of MERS-CoV variants with a small genome fragment. *J. Clin. Virol.*
- Spanakis, N., Tsiodras, S., Haagmans, B.L., Raj, V.S., Pontikis, K., Koutsoukou, A., Koulouris, N.G., Osterhaus, A.D., Koopmans, M.P., Tsakris, A., 2014. Virological and serological analysis of a recent Middle East respiratory syndrome coronavirus infection case on a triple combination antiviral regimen. *Int. J. Antimicrob. Agents* 44, 528–532.
- Tang, X.C., Agnihothram, S.S., Jiao, Y., Stanhope, J., Graham, R.L., Peterson, E.C., Avnir, Y., Tallarico, A.S., Sheehan, J., Zhu, Q., Baric, R.S., Marasco, W.A., 2014. Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. *Proc. Natl. Acad. Sci. U. S. A.*
- The WHO MERS-CoV Research Group, 2013. State of Knowledge and Data Gaps of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Humans. *PLoS Curr. Ed.*, 1.
- Tsiodras, S., Baka, A., Mentis, A., Iliopoulos, D., Dedoukou, X., Papamavrou, G., Karadima, S., Emmanouil, M., Kossyvakis, A., Spanakis, N., Pavli, A., Maltezos, H., Karageorgou, A., Spala, G., Pitiriga, V., Kosmas, E., Tsiagklis, S., Gkatzias, S., Koulouris, N., Koutsoukou, A., Bakakos, P., Markozanhs, E., Dionellis, G., Pontikis, K., Rovina, N., Kyriakopoulou, M., Efstathiou, P., Papadimitriou, T., Kremastinou, J., Tsakris, A., Saroglou, G., 2014. A case of imported Middle East Respiratory Syndrome coronavirus infection and public health response, Greece, April 2014. *Euro Surveill.* 19.
- van Boheemen, S., de Graaf, M., Lauber, C., Bestebroer, T.M., Raj, V.S., Zaki, A.M., Osterhaus, A.D., Haagmans, B.L., Gorbelenya, A.E., Snijder, E.J., Fouchier, R.A., 2012. Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *MBio.*, 3.
- van Doremalen, N., Bushmaker, T., Munster, V.J., 2013. Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions. *Euro Surveill.*, 18.
- van Doremalen, N., Miazgowiec, K.L., Milne-Price, S., Bushmaker, T., Robertson, S., Scott, D., Kinne, J., McLellan, J.S., Zhu, J., Munster, V.J., 2014. Host Species Restriction of Middle East Respiratory Syndrome Coronavirus through its Receptor Dipeptidyl Peptidase 4. *J. Virol.*
- Wang, N., Shi, X., Jiang, L., Zhang, S., Wang, D., Tong, P., Guo, D., Fu, L., Cui, Y., Liu, X., Arledge, K.C., Chen, Y.H., Zhang, L., Wang, X., 2013. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Res.* 23, 986–993.
- Woo, P.C., Lau, S.K., Huang, Y., Yuen, K.Y., 2009. Coronavirus diversity, phylogeny and interspecies jumping. *Exp. Biol. Med. (Maywood)* 234, 1117–1127.
- World Health Organization, 2013a. Infection Prevention and Control During Health Care for Probable or Confirmed Cases of Novel Coronavirus (nCoV) infection. 5-6-2013a. http://www.who.int/csr/disease/coronavirus_infections/IPCNCoVguidance_06May13.pdf
- World Health Organization, 2013b. Laboratory Testing for Middle East Respiratory Syndrome Coronavirus. 16-9-2013b. http://who.int/csr/disease/coronavirus_infections/MERS_Lab_recos_16_Sept_2013.pdf?ua=1
- World Health Organization, 2014a. Case Definition for Case Finding Severe Respiratory Disease Associated with Novel Coronavirus: Interim Case Definition as of 25 September 2012. 12-5-2014. http://www.who.int/csr/disease/coronavirus_infections/case_definition_25_09_2012/en/
- World Health Organization, 2014b. Global Alert and Response (GAR): Coronavirus Infections. http://www.who.int/csr/don/archive/disease/coronavirus_infections/en/
- World Health Organization, 2014c. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Summary and Literature Update-as of 9 May 2014. 5-9-2014c. http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_Update_09_May_2014.pdf?ua=1
- World Health Organization, 2014d. Revised Interim Case Definition for Reporting to WHO – Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Interim Case Definition as of July 2013. 12-5-2014. http://www.who.int/csr/disease/coronavirus_infections/case_definition/en/
- World Health Organization, 2014e. WHO Risk Assessment Middle East Respiratory Syndrome Coronavirus (MERS-CoV). 24-4-2014e. http://www.who.int/csr/disease/coronavirus_infections/MERS_CoV_RA_20140424.pdf?ua=1
- World Health Organization, 2014f. WHO Statement on the Fifth Meeting of the IHR Emergency Committee Concerning MERS-CoV: WHO Statement. <http://www.who.int/mediacentre/news/statements/2014/mers-20140514/en/>
- Yao, Y., Bao, L., Deng, W., Xu, L., Li, F., Lv, Q., Yu, P., Chen, T., Xu, Y., Zhu, H., Yuan, J., Gu, S., Wei, Q., Chen, H., Yuen, K.Y., Qin, C., 2014. An animal model of MERS produced by infection of rhesus macaques with MERS coronavirus. *J. Infect. Dis.* 209, 236–242.
- Zaki, A.M., van, B.S., Bestebroer, T.M., Osterhaus, A.D., Fouchier, R.A., 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N. Engl. J. Med.* 367, 1814–1820.
- Zhao, G., Du, L., Ma, C., Li, Y., Li, L., Poon, V.K., Wang, L., Yu, F., Zheng, B.J., Jiang, S., Zhou, Y., 2013. A safe and convenient pseudovirus-based inhibition assay to detect neutralizing antibodies and screen for viral entry inhibitors against the novel human coronavirus MERS-CoV. *Virol. J.* 10, 266.
- Zhao, J., Li, K., Wohlford-Lenane, C., Agnihothram, S.S., Fett, C., Zhao, J., Gale Jr., M.J., Baric, R.S., Enjuanes, L., Gallagher, T., McCray Jr., P.B., Perlman, S., 2014. Rapid generation of a mouse model for Middle East respiratory syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 111, 4970–4975.
- Zhou, J., Chu, H., Li, C., Wong, B.H., Cheng, Z.S., Poon, V.K., Sun, T., Lau, C.C., Wong, K.K., Chan, J.Y., Chan, J.F., To, K.K., Chan, K.H., Zheng, B.J., Yuen, K.Y., 2014. Active replication of middle East respiratory syndrome coronavirus and aberrant induction of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis. *J. Infect. Dis.* 209, 1331–1342.
- Zielecki, F., Weber, M., Eickmann, M., Spiegelberg, L., Zaki, A.M., Matrosovich, M., Becker, S., Weber, F., 2013. Human cell tropism and innate immune system interactions of human respiratory coronavirus EMC compared to those of severe acute respiratory syndrome coronavirus. *J. Virol.* 87, 5300–5304.
- Zumla, A., Hui, D.S., 2014. Infection control and MERS-CoV in health workers. *Lancet* 383, 1869–1871.
- Zumla, A.I., Memish, Z.A., 2014. Middle East respiratory syndrome coronavirus: epidemic potential or a storm in a teacup? *Eur. Respir. J.* 43, 1243–1248.