Review

Middle East respiratory syndrome (MERS) coronavirus and dromedaries

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A B S T R A C T

Middle East Respiratory Syndrome (MERS) is a zoonotic viral disease that can be transmitted from dromedaries to human beings. More than 1500 cases of MERS have been reported in human beings to date. Although MERS has been associated with 30% case fatality in human beings, MERS coronavirus (MERS-CoV) infection in dromedaries is usually asymptomatic. In rare cases, dromedaries may develop mild respiratory signs. No MERS-CoV or antibodies against the virus have been detected in camelds other than dromedaries. MERS-CoV is mainly acquired in dromedaries when they are less than 1 year of age, and the proportion of seropositivity increases with age to a seroprevalence of 100% in adult dromedaries. Laboratory diagnosis of MERS-CoV infection in dromedaries can be achieved through virus isolation using Vero cells, RNA detection by real-time quantitative reverse transcriptase-PCR and antigen detection using respiratory specimens or serum. Rapid nucleocapsid antigen detection using a lateral flow platform allows efficient screening of dromedaries carrying MERS-CoV. In addition to MERS-CoV, which is a lineage C virus in the Betacoronavirus (betaCoV) genus, a lineage B betaCoV and a virus in the Alphacoronavirus (alphaCoV) genus have been detected in dromedaries. Dromedary CoV UAE-HKU23 is closely related to human CoV OC43, whereas the alphaCoV has not been detected in human beings to date.

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Introduction

Since the emergence of Middle East respiratory syndrome (MERS) in 2012, more than 1500 human cases have been reported, with a case fatality of ~30% (World Health Organization (WHO), 2016). The causative agent of MERS has been confirmed to be a novel coronavirus (CoV), named MERS-CoV (van Boheemen et al., 2012; Zaki et al., 2012). CoVs are positive-sense single-stranded RNA viruses with genome size of ~30 kb. They have the characteristic crown-shaped appearance on electron microscopic examination. CoVs are classified into four genera, Alphacoronavirus, Betacoronavirus (with four lineages), Gammacoronavirus and Deltacoronavirus (Woo et al., 2012). MERS-CoV belongs to lineage C of Betacoronavirus (Fig. 1) (van Boheemen et al., 2012).

Subsequent investigations have shown that dromedary or one humped camels (Camelus dromedarius) are so far the only reservoir of MERS-CoV (Alagaili et al., 2014; Chu et al., 2014; Wernery et al., 2015a, 2015b; Sabir et al., 2016). Adult dromedaries have almost 100% seropositivity against MERS-CoV while the virus is found mainly in dromedary calves (Alagaili et al., 2014; Wernery et al., 2015b). Camel-to-human transmission of MERS-CoV and subsequent human-to-human transmission result in MERS in human beings, many of whom develop severe lower respiratory tract infections, with renal failure in some cases (Arabi et al., 2014; Saad et al., 2014). In contrast to human infection, MERS-CoV causes no or mild disease in dromedaries. In this article, our current understanding of MERS-CoV infection in dromedaries is reviewed and the presence of other CoVs in dromedaries is also discussed.

MERS-CoV infection in dromedaries

More than 20 MERS-CoV isolates have been recovered from nasal swabs from young dromedaries from different farms in Dubai over the last 2 years at the Central Veterinary Research Laboratory. These animals died from diseases unrelated to MERS, such as selenium deficiency, cryptosporidiosis, salmonellosis, Escherichia coli septicaemia and Clostridium perfringens A enterotoxaemia. None had nasal discharge and it is believed that the virus isolation was incidental to the death of the dromedaries. Histopathological investigations did not show any lesions consistent with virus infection.

However, experimental infections of dromedaries with MERS-CoV in the USA and Spain predominantly induced respiratory tract disease with no or mild clinical respiratory signs (Adney et al., 2014; Haagmans et al., 2016). The experimentally infected animals demonstrated moderate rhinitis, with nasal discharge, tracheitis and bronchitis, but no involvement of the alveolar tissue. The
dromedaries were infected with high doses of MERS-CoV intranasally, i.e. 1 × 10^5 50% tissue culture infectious doses (TCID₅₀) in the study of Adney et al. (2014) and 5 × 10⁶ TCID₅₀ in the study of Haagmans et al. (2016). Experimental MERS-CoV infections were also performed in alpacas, with similar results (Adney et al., 2016). Experimentally infected alpacas transmitted the virus to two of three contact animals. Experimentally infected animals were protected against reinfection 70 days later and those infected by contact were only partially protected.

**Laboratory diagnosis of MERS-CoV infection in dromedaries**

Detection of MERS-CoV in dromedaries is performed to understand the epidemiology and evolutionary dynamics of the virus and to reduce the risk of human transmission. MERS-CoV was first isolated from a human patient suffering from fatal lower respiratory tract infection and acute renal failure in Saudi Arabia in 2012 (van Boecken et al., 2012; Zaki et al., 2012). Since then, the virus has been isolated from both human beings and dromedaries. Although Vero cells are usually the cell line used in clinical laboratories, the virus can be propagated in a variety of human and non-human cell lines, which may have implications for its tissue tropism and the high fatality associated with MERS (Chan et al., 2013; Eckerle et al., 2014; Zhou et al., 2015).

MERS-CoV has been isolated from nasal swabs of young dromedaries (Wernery, 2014; Wernery et al., 2015a; Bin et al., 2016), but has not been recovered from adult dromedaries. Over the last 3 years, several hundred samples have been tested at the Central Veterinary Research Laboratory, Dubai, for the presence of MERS-CoV at post-mortem examination from serologically positive MERS-CoV adult dromedaries greater than 4 years of age. Most of the dromedaries came from farms where MERS-CoV was isolated from the nasal cavity of young animals. However, no MERS-CoV has been recovered from any sample from adult dromedaries, including nasal swabs, tonsils, lungs, intestinal lymph nodes, mammary lymph nodes and milk. Although isolating the virus will be useful for further research, such as antiviral susceptibility testing, diagnosis of MERS-CoV infection in dromedaries is mainly achieved through nucleic acid detection. Among all the nucleic acid detection technologies, the most widely used for MERS-CoV diagnosis is real-time quantitative reverse transcriptase (RT)-PCR (RT-qPCR), using a number of possible targets, including the RNA-dependent RNA polymerase, the region upstream to the envelope gene, or the nucleocapsid gene (Corman et al., 2012a, 2012b). RT-qPCR has high sensitivity and specificity for diagnosis of MERS-CoV infection.

Rapid diagnosis of MERS–CoV infections in dromedaries can also be achieved by direct antigen detection assays. In 2015, we published a monoclonal antibody-based ELISA for the detection of the nucleocapsid antigen of MERS-CoV in respiratory samples, with high sensitivity and specificity (Chen et al., 2015). Recently, we have used a lateral flow immunoassay platform for rapid detection of nucleocapsid antigen in respiratory samples of dromedaries; this has a sensitivity of ~80% and a specificity of 100% when compared to RT-qPCR (Chen et al., 2016). This technology has enabled the on-site screening of a large number of dromedaries in a short period of time. It also avoids the use of expensive equipment and does not require expertise in molecular diagnostics.

**Seroepidemiology**

The first evidence of dromedaries being the reservoir of MERS-CoV came from serological studies. High levels of MERS–CoV antibodies have been observed in dromedaries in the Middle East and Africa (Reusken et al., 2013; Corman et al., 2014a; Hemida et al., 2014; Meyer et al., 2014; Muller et al., 2014; Mackay and Arden, 2015). Serological follow-up of dromedary dams and their calves has shown a typical pattern of juvenile infection. Maternal antibodies against MERS-CoV in dromedary calves generally disappear between 4 and 8 months of age, permitting infection to occur during the seronegative period (Alagaili et al., 2014; Bin et al., 2016); young
infected dromedaries then develop antibodies that persist for a long time. However, in a few cases, MERS-CoV has been isolated at the age of 20 days or even at younger, indicating that maternal antibodies are not necessarily protective. So far, the specific source of infection for young dromedaries is not known, although it is likely to be from other dromedaries. Extensive investigations in other animal species, including rodents, ticks, horses and small ruminants, have not demonstrated other reservoirs of infection to date.

**Prevention and control**

Protective experimental immunisations in dromedaries have already started using a modified vaccinia virus Ankara (MVA) vaccine expressing the MERS-CoV spike protein (Haagmans et al., 2016). Preliminary data showed a significant reduction in excretion of infectious virus and viral RNA in small numbers of vaccinated and challenged dromedaries compared to controls. Protection is correlated with the presence of serum neutralising antibodies against MERS-CoV. MVA-specific antibodies that cross-neutralise camelpox virus are another very important advantage of this vaccine, since outbreaks of camelpox still occur in dromedaries (Higgins et al., 1992). Another approach would be to add a MERS-CoV component to the already existing attenuated camelpox vaccine Ducapox. Since Ducapox has been used in the Middle East for many years, the acceptance of such a vaccine can be anticipated (Wernery et al., 2014). However, it is important for the success of a vaccine to adhere strictly to the exact time of vaccination, since the window of disappearance of maternal antibodies and appearance of antibodies as a result of infection is narrow.

**MERS as a zoonotic disease**

MERS is a zoonotic viral disease that can be transmitted from dromedaries to human beings. Several groups have performed seroepidemiological studies in different camel species in various countries and continents. So far, no antibodies to MERS-CoV have been detected in South American camelds or in Bactrian camels (Chan et al., 2015; Mackay and Arden, 2015). It seems that infection of dromedaries with MERS-CoV is limited to Africa and the Middle East (Perera et al., 2013; Reusken et al., 2013; Corman et al., 2014a; Hemida et al., 2014; Meyer et al., 2014; Muller et al., 2014). Over 90% of all dromedaries investigated from this region possess antibodies to the virus, some of them at least for more than 30 years (Muller et al., 2014). MERS-CoV infection in dromedaries is not a new entity and only very few young animals (<1%) may develop nasal discharge without other clinical signs. This picture changes when dromedaries are experimentally infected through the intranasal route (Haagmans et al., 2016).

In human beings, close contact with these young dromedaries may result in MERS-CoV infection, sometimes with fatal consequences. Although the mode of human-to-human transmission of MERS-CoV is not fully understood, the virus has caused major nosocomial outbreaks at hospitals in Saudi Arabia (Assiri et al., 2013; Azhar et al., 2014) and South Korea (Oboho et al., 2015). In South Korea, environmental contamination has been demonstrated, with MERS-CoV detected in patients’ rooms, medical devices and air ventilating units, leading to closure of some hospitals (Cowling et al., 2015). It is worthwhile mentioning that no human MERS-CoV cases have been seen on camel dairy farms in the United Arab Emirates, even though the virus has been isolated regularly from dromedary calves.

Transmission from dromedaries to human beings is presumably uncommon, because the seroprevalence in human beings is low, even in people in frequent contact with dromedaries (Hemida et al., 2015). There may be several reasons for the low frequency of transmission from camels to human beings. Only young dromedaries with no or low maternal antibodies to MERS-CoV are susceptible to infection and the virus is shed only for 8 days (Wernery et al., 2015b). These young dromedaries are reared with their mothers for a year and have no or very little contact with human beings. Additionally, less than 1% of infected calves exhibit nasal discharge and therefore the quantity of virus excreted may be low. Another reason for the low transmission rate is likely to be the lack expression of the MERS-CoV receptor-dipeptidyl peptidase 4 (DPP4) in the human upper respiratory tract (Widagdo et al., 2016). Care must be taken when camel calves have to be treated. In these cases, caretakers and veterinarians should wear protective gear to avoid any transmission from camel calves to human beings.

In contrast to the monophyletic origin of SARS-CoV, MERS-CoV strains from human beings are polyphyletic as a result of multiple camel-to-human transmission events (Arabi et al., 2014; Ling et al., 2015). This implies that MERS-CoV has been present in dromedaries for many decades. It also provides an explanation why MERS has now persisted for more than 3 years, unlike SARS which disappeared rapidly after civets were removed by closure of animal markets in Southern China. MERS-CoV is likely to continue to be a threat to human beings unless vaccines are available.

**Coronaviruses other than MERS-CoV in camels**

Coronaviruses are widespread and infect a broad range of species. An alphaCoV has been isolated from New World camels (NWCs, also known as South American camels) with respiratory disease (Jin et al., 2007). A betaCoV (bovine CoV-like CoV with no genome sequenced) was isolated from NWCs and dromedaries with diarrhoea (Crossley et al., 2010, 2012). Transmission of the betaCoV occurs directly through close contact and aerosols, and the incubation period in crias is short (24–48 h). Disease in NWCs occurs in association with infection with rotavirus, E. coli, and Cryptosporidium and Salmonella spp. (Wernery et al., 2014). CoVs have been linked to several outbreaks of diarrhoea affecting NWCs of all ages on farms in north west USA and South America. Serological surveys and virus identification have also been carried out. CoVs were also detected in dromedary faecal samples and antibodies have been detected in dromedary sera in East Africa and on the Arabian Peninsula (Reusken et al., 2013; Meyer et al., 2014; Muller et al., 2014). However, there are no reports on CoV in Bactrian camels (Chan et al., 2015).

In 2014, we discovered a novel CoV named dromedary camel CoV UAE-HKU23 (DdCoV UAE-HKU23); the complete genome of this virus was sequenced directly from dromedary faecal samples and its phylogenetic relationship with other CoVs was determined (Table 1) (Wernery et al., 2014). We speculate that the bovine CoV-like CoV, with limited sequence information, described previously (Crossley

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<td>DdCoV UAE-HKU23</td>
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et al., 2010, 2012), is probably DcCoV UAE-HKU23. Phylogenetic analysis shows that CoV UAE-HKU23 is closely related to other CoVs of the Betacoronavirus 1 species in betaCoV lineage A, of which bovine CoV and human CoV OC43 are members (Woo et al., 2014d). Recently, we successfully isolated DcCoV UAE-HKU23 using the human rectal tumour HRT-18G cell line and confirmed that it is different from the betaCoV in alpacas using bioinformatics approaches (Woo et al., 2016b). Although both MERS-CoV and DcCoV UAE-HKU23 are beta CoVs, there is minimal cross antigenicity between them, as shown by various serological tests (Woo et al., 2014d). Last year, another alphaCoV that has been found in alpacas was also discovered in nasal samples of dromedaries (Table 1). The cameld alphaCoV, together with MERS-CoV and DcCoV UAE-HKU23, were observed to be co-circulating in camels in Saudi Arabia (Sabir et al., 2016).

Discussion

In the last 15 years, we have witnessed unprecedented changes in the CoV field. Two novel human CoVs (human CoV NL63 and human CoV HKU1) have been discovered, along with more than 40 novel animal CoVs (Lau et al., 2005, 2010, 2012, 2015; Woo et al., 2005, 2009, 2012, 2014a; van der Hoek et al., 2006; Chu et al., 2008; Mihindukulasuriya et al., 2008; Berghamming et al., 2012; Corman et al., 2014b). These include three novel lineages (B, C and D) of betaCoVs and a novel CoV genus (Deltacoronavirus) in the Coronavirus family. In addition, two large human CoV epidemics have affected numerous countries globally. The number of publications on CoVs in PubMed search has doubled in the last 10 years and a model of CoVs and its unique mechanism of replication, it is expected that more and more ‘new’ CoVs will be generated. Some of these CoVs may have the capability to jump from one species to another and some of these interspecies transmission events may result in further major epidemics in human beings and animals.

In addition to CoVs, the MERS epidemic has also boosted interest in the discovery of other novel viruses in dromedaries. Since 2013, novel viruses of at least five different families (Astroviridae, Circoviridae, Hepeviridae, Picobirnaviridae and Picornaviridae) have been discovered in dromedaries (Woo et al., 2014b, 2014c, 2015, 2016a). Furthermore, West Nile virus has also been isolated from dromedaries (Joseph et al., 2016). Notably, shortly after the discovery of a novel genotype of hepatitis E virus in dromedaries in Dubai, the same virus was found in a human liver transplant recipient from the Middle East with chronic hepatitis E virus infection. The patient had the habit of regular consumption of camel meat and milk, suggesting that the virus may be transmitted from dromedaries to human beings (Lee et al., 2016). Both the transmission of MERS-CoV and hepatitis E virus from camels to human beings have shown that some viruses from dromedaries can be transmitted to human beings.

Conclusions

MERS-CoV is a zoonotic virus that can be transmitted from dromedaries to human beings, in which it causes severe respiratory disease with a high case fatality. Dromedaries develop no or only mild respiratory signs. In addition to MERS-CoV, a lineage B betaCoV and a virus in the Alphacoronavirus (alphaCoV) genus have been detected in dromedaries. More ‘new’ CoVs are likely to be generated due to the high recombination rate in this group of viruses. More intensive surveillance of CoVs in camels should be performed to improve understanding of these viruses in this unique group of animals, which have been closely associated with human beings for thousands of years.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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