Chapter 11
The Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

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Abstract The Middle East respiratory syndrome coronavirus (MERS-CoV) is one of the newly identified viral infections affecting humans. It was recently reported in the Arabian Peninsula (AP), particularly Saudi Arabia. The virus primarily causes respiratory failure in affected patients, but can also sometimes cause renal failure. The main reservoir of this virus is dromedary camels. Although a lot is known about the diseases pattern, pathogenesis, and immunity of MERS-CoV infection in humans, very little is known about these parameters in camels. The main goals of this book chapter are to discuss the current understandings about MERS-CoV infection in animals, particularly the dromedary camels. Special attention should be paid to the clinical pattern of MERS-CoV infection in animals, including clinical signs, pathological changes during virus infection, various methods of clinical and laboratory diagnosis, and the recent advances related to the control of MERS-CoV.

Keywords MERS-CoV · Clinical signs · Pathology · Diagnosis · Control · Vaccines

11.1 Prologue

There are 2458 laboratory-confirmed cases of MERS-CoV reported from 27 countries around the globe (till August 20, 2019). The disease was fatal in about 848 cases (34%) (WHO 2019). Unfortunately, until now, there is no effective medication or even vaccine against MERS-CoV. MERS-CoV represents a good example for the One Health concept (Hemida 2019). The virus infects humans and can cause severe illness in some patients, particularly those suffering from chronic diseases and other

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comorbidities such as cancer (Rahman and Sarkar 2019). The main known reservoir for the virus is the dromedary camels (Hemida et al. 2017a). MERS-CoV was isolated from dromedary camels in many countries across the Arabian Peninsula such as Saudi Arabia, UAE, Qatar, and Oman (Hemida et al. 2014a; Nowotny and Kolodziejek 2014; Yusof et al. 2015).

However, there is a scarcity of information about the actual natural infection of MERS-CoV in dromedary camels. The main objective of this article was to highlight the most up-to-date knowledge about MERS-CoV in various species of animals with special attention to dromedary camels.

11.2   Historical Background and Evolution of MERS-CoV

MERS-CoV was first identified in a patient from Saudi Arabia who presented with severe respiratory symptoms and later developed respiratory failure (Zaki et al. 2012). A retrospective study confirmed the detection of MERS-CoV in archived patient samples from Jordan (Hijawi et al. 2013). The virus was called *novel coronavirus* at that time (Zaki et al. 2012). Later on, based on the available epidemiological data, a study group decided to change the name of the virus to 'Middle East respiratory syndrome' (MERS-CoV) (de Groot et al. 2013). Initially, there was a debate about the origin of MERS-CoV. Some studies suggested bats as a potential reservoir for the MERS-CoV (Memish et al. 2013). This study sequenced a short fragment from a coronavirus genome collected from bats in Saudi Arabia and found high similarity to the circulating MERS-CoV strains at that time (Memish et al. 2013). Another recent study was conducted in Africa, in Ghana, where the camels tend to live in close contact with various species of bats. This study failed to detect a similar MERS-CoV in bats in these regions (de Groot et al. 2013). Scientists tried to speculate the potential reservoir of this new coronavirus in the Middle East, especially in the Arabian Peninsula. The first seroprevalence study for MERS-CoV in different livestock animals including dromedary camels was conducted in Oman (Reusken et al. 2013). This study revealed that only dromedary camels carried specific antibodies against MERS-CoV in their sera. Since then there have been quite a few reports of new cases coming from the Arabian Peninsula and other parts of Africa (Ali et al. 2017; Deem et al. 2015; Hemida et al. 2014b; Hemida et al. 2013). On the other hand, there are reports of detection of MERS-CoV nucleic acids in many cases in dromedary camels from both the AP and Africa (Hemida et al. 2017a; Hemida et al. 2014a; Yusof et al. 2015; El-Duah et al. 2019). MERS-CoV detected in archived serum samples, dating back to the 1990s, showed higher seroprevalence of MERS-CoV in the tested specimens (Reusken et al. 2013; Hemida et al. 2014b). This may suggest the possibility of the circulation of MERS-CoV in dromedary camels before its actual emergence in human as announced for the first time in 2012 (Zaki et al. 2012).

11.3 Aetiology, Genome Organization, and Classification of MERS-CoV

MERS-CoV belongs to the genus Betacoronavirus in the family *Coronaviridae* and the order *Nidovirales*. Betacoronaviruses are further divided into four distinct clades labelled as 'A-D' (de Groot et al. 2013). MERS-CoV has been recently classified as one of the members of clade C coronavirus. MERS-CoV genome is a single strand of the positive-sense RNA molecule. The viral genome is close to 30 kilobases in length. The genome is organized as follow (5′UTR, non-structural genes- structural genes- 3′UTR-Poly A tail-3′). The virus genome is characterized by the production of sets of sub-genomic messenger RNAs. The viral genome is flanked by two untranslated regions. Two-third of the 5′ genome is occupied by the non-structural genes called Gene-1. It is composed of two overlapping open reading frames with ribosomal frameshifting site. While, one-third of the genome is occupied by various structural genes, including spike (S), envelope (E), matrix (M), and the nucleocapsid (N). There are some accessory genes scattered across the viral genome (ORF3, ORF4a, ORF4b, ORF5, and ORF8b). One recent study showed that the African lineage of MERS-CoV is distinct from those circulating in the Gulf area. This study showed that African lineages lack ORF4, which is considered to be one of the virulence factors for MERS-CoV. This may explain at least in part why there are no human cases reported in the African countries which have a significant population of dromedary camels (Chu et al. 2018).

11.4 MERS-CoV Receptors and Tropism

MERS-CoV is one of the pneumotropic viruses, which replicates well in the cells of the respiratory tract (Park et al. 2018). It is well known that most coronaviruses utilize their S protein in the process of attachment to the host cells. MERS-CoV uses the S1 protein to attach to the sialic acid in the host cell (Widagdo et al. 2019). Several studies confirmed that dipeptidyl peptidase 4 (DPP4) play an important role in the attachment and tropism identification of MERS-CoV (van Doremalen et al. 2014). This study confirmed the possibility of using the DPP4 receptors in various animal species such as cows, sheep, and goat to establish MERS-CoV infection. This suggests the potential roles of these animals as other possible hosts for MERS-CoV (van Doremalen et al. 2014). However, this study needs further confirmation. Another study showed the involvement of another protein called glucose-regulated protein (GRP-78), in the process of MERS-CoV attachment to the target cells (Chu et al. 2018). This cellular factor may act as a key player in the MERS-CoV tropism and tissue distribution (Chu et al. 2018).

11.5   MERS-CoV Infections in Animals

Some coronaviruses are zoonotic in origin, such as the severe acute respiratory syndrome coronavirus (SARS-CoV). Animals, particularly the Palm Civet cats were the main reservoir for the SARS-CoV (Shi and Hu 2008; Wang and Eaton 2007). Culling of this reservoir and banning its distribution in the public markets contributed substantially in the control and extinction of SARS-CoV (Wang and Eaton 2007). When MERS-CoV emerged, scientists started to look for potential reservoirs in the context of the virus emergence and replication cycle. Several studies were conducted to check various domestic animals that lived in close proximity to confirmed patients, for the presence of MERS-CoV antibodies or nucleic acids (Hemida et al. 2014a; Reusken et al. 2013; Hemida et al. 2014b). Interestingly enough, dromedary camels were the only positive animal for the MERS-CoV (Reusken et al. 2013). Since that time, several research groups started to focus on this line of research and to investigate the roles of MERS-CoV in the transmission cycle of MERS-CoV (Yusof et al. 2015; Xu et al. 2019).

11.6   MERS-CoV in Dromedary Camels

Several studies reported the seroprevalence and virus detection of MERS-CoV in dromedary camels from many countries from Asia and Africa (Yusof et al. 2015; Reusken et al. 2013; Hemida et al. 2014b; Kandeil et al. 2019). Earlier studies showed the high seroprevalence of MERS-CoV in most of the tested camel sera from those two regions in the world. However, animals from Australia were seronegative for the MERS-CoV (Hemida et al. 2014b; Crameri et al. 2015). Another recent study showed the seroconversion of dromedary camels from Canary Island but the absence of virus detection in specimens collected from these group of animals (Gutierrez et al. 2015). MERS-CoV was detected in the body fluids of the infected dromedary camels, especially the nasal and rectal swabs (Hemida et al. 2014a; Yusof et al. 2015; Hemida et al. 2017b).

Meanwhile, high prevalence of MERS-CoV was detected in tissue specimens collected from the upper respiratory tract of dromedary camels (Khalafalla et al. 2015). This was in contrast to absence of the detection of any viral nucleic acids or particles in urine samples collected from camels in Qatar (Farag et al. 2019). It was found that the detection rate of MERS-CoV in naïve camels (less than 2 years old) is much higher than adult animals (Hemida et al. 2017b). This highlights the potential role of the young camels in the transmission of MERS-CoV. Another interesting phenomenon is the susceptibility of dromedary camels to the second round of MERS-CoV infection despite the presence of neutralizing antibodies in their sera (Hemida et al. 2017b). This also explains the high seroprevalence of adult camels to MERS-CoV. Another important study reported the detection of MERS-CoV in apparently normal dromedary camels (Mohran et al. 2016). This highlights the

confirmed roles of dromedary camels in the sustainability of MERS-CoV in the environment. Although the role of camels in the transmission/replication cycle of MERS-CoV was confirmed a while ago, until now, there is no clear evidence of MERS-CoV infection in dromedary camels under the natural conditions.

Some studies reported the results of the experimental infection of dromedary camels with MERS-CoV (Adney et al. 2014). These studies reported the course of the MERS-CoV infection in a limited number of animals and the curve of the virus shedding over the time of the experiments (Adney et al. 2014). According to these studies there were no characteristic clinical signs observed after the experimental challenges with MERS-CoV, except for very mild elevation of the body temperature and some mild nasal discharges (Adney et al. 2014). The nasal discharge ranged from very mild serous exudate to purulent and sometimes haemorrhagic (Adney et al. 2014). According to same study, virus shedding from the nasal secretions started in less than one-week post-inoculation (PI) and lasted for up to 35 days PI (Adney et al. 2014). However, no viral shedding was reported in faecal swabs or urine during infection (Adney et al. 2014). Interestingly, very low virus concentration was detected in the exhaled breath from the infected animals (Adney et al. 2014). Post-mortem inspection of these animals revealed that the viral antigens were detected in the upper respiratory tracts, especially nasal passages, trachea, and nasal turbinate bones by the immunohistochemistry technique (IHC) (Adney et al. 2014). However, no signal was detected on the alveolar epithelium (Adney et al. 2014). These studies were quite useful in the determination of the viral tropism and tissue distribution, which explains the pathogenesis of the viral infection in dromedary camels. Meanwhile, seroconversion of the inoculated animals was observed within 14 days PI (Adney et al. 2014).

11.7 MERS-CoV in Other Members of the Family *Camelidae*

The family *Camelidae* includes dromedary camels, alpaca, and llama. One recent study showed seroprevalence of alpaca kept in close proximity to dromedary camels in Qatar (Reusken et al. 2016). Another study was conducted to check the status of MERS-CoV in dromedary camels, alpaca, and llama. This study found all swabs were negative while some llama were seropositive indicating an active infection among this species of animals in Israel (David et al. 2018). Based on the susceptibility of alpaca to MERS-CoV infection, one study tested the efficacy of a new MERS-CoV-spike based subunit vaccine in both dromedary camels and alpaca. This study showed the induction of a high level of neutralizing antibodies in sera of the challenged animals, which reduced the titre of virus after challenging (Adney et al. 2019a). Recent studies showed that alpaca is susceptible to experimental infection with MERS-CoV (Crameri et al. 2016). The challenged animals shed the virus in

their secretions particularly the nasal tract and showed protection when challenged with the wild virus at the end of the experiment (Crameri et al. 2016).

11.8 MERS-CoV in Other Species of Animals

Earlier studies reported the absence of MERS-CoV antibodies in sera of Bactrian camels from Mongolia (Chan et al. 2015; Miguel et al. 2016). However, one recent study reported high virus shedding in secretions of the MERS-CoV experimentally infected Bactrians (Adney et al. 2019b). This explains the potential role of genetic factors in the susceptibility/resistance of the members of the family *Camelidae* to the MERS-CoV infection. This line of research requires further studies in future. Previous studies showed the absence of any detectable antibodies of MERS-CoV in sera of other livestock animals such as sheep, goat, cattle, horses, and many other animals (El-Duah et al. 2019; Zohaib et al. 2018). This is in contrast to a recent study conducted in Egypt, Tunisia, and Senegal, which showed the detection of specific antibodies in sera collected from of sheep, goat, and donkeys that lived in close contact with camels from these countries (Kandeil et al. 2019). Furthermore, viral nucleic acids were detected in specimens from sheep, goat, and cattle (Kandeil et al. 2019). This line of research requires large-scale studies to understand the dynamics of MERS-CoV among livestock animals in countries where camels live in close contact with these species of animals.

11.9 Animal Models for MERS-CoV

Since the emergence of MERS-CoV in 2012, there was a great demand in finding some potential animal models. Confirmation of the roles of dromedary camels in the transmission cycle of MERS-CoV increased this demand. This is because of the difficulty in access to dromedary camels in North America and Europe. There is also a scarcity of the animal biosafety, level-3 laboratories that are equipped to adopt large animals, especially camels. Initial trials considered the Syrian hamster as a good animal model for the SARS-CoV research (Schaecher et al. 2008); however, it is not a suitable animal model for MERS-CoV (de Wit et al. 2013). The MERS-CoV experimental infection in the golden Syrian hamster was not successful since the virus neither replicates in various body organs nor produces any detectable immune response or antibodies after infection (de Wit et al. 2013). The wild type mouse is non-susceptible to MERS-CoV infection, but recently a MERS-CoV transgenic mouse was developed by the expression of the human DPP4 receptors (hDPP4) (Agrawal et al. 2015). These transgenic mice developed severe clinical signs after the challenge with MERS-CoV. The infected transgenic mice suffered from ruffled fur, severe respiratory manifestations, and finally death, a few days after infection (Agrawal et al. 2015). Both rhesus macaques and marmosets are susceptible to

MERS-CoV infection. Both animals were shown to develop signs similar to humans infected with MERS-CoV, including several lesions on the respiratory tract with pulmonary oedema, haemorrhage, and pneumonia (Yu et al. 2017). Pigs were found to be refractory to MERS-CoV infection. Very low virus titre was detected in challenged animals after infection, suggesting that pigs are not a suitable animal model for MERS-CoV, in contrast to dromedary camels and alpaca.

11.10 Isolation of MERS-CoV

The success of isolating MERS-CoV from clinical specimens collected from human or animal mainly depends on the quality of the collected specimen and its preservation, as well as maintenance, until it is processed in the laboratory. In case of transportation of these specimens from the field to the laboratory, it should be transported on ice and kept cool till it reaches the destination laboratory. Transport medium like foetal bovine sera and antibiotic cocktail should be added to the transport medium containing minimal essential media. Some common cell lines are used in the isolation of MERS-CoV collected from patients or animals, especially Vero cell line (Chan et al. 2014; Park et al. 2016). Successful virus isolation is associated with the induction of cytopathic effects (CPE) on the inoculated cell culture. The observed CPE in case of MERS-CoV isolation is the rounding and detachment of cells from the confluent monolayer sheet within three days from the infection of the cell culture (Chan et al. 2014; Park et al. 2016).

11.11 Laboratory Techniques for the Diagnosis of MERS-CoV

Since the clinical diagnosis of MERS-CoV in dromedary camels is not accurate or even suggestive due to the absence of any pathognomonic clinical signs or post-mortem lesions, laboratory diagnosis is the only way to confirm the presence or absence of MERS-CoV infection in a certain animal. There are two strategies for the diagnosis of MERS-CoV infection in dromedary camels (Fig. 11.1). The first one is the detection of specific viral antibodies in sera of dromedary camels. Several in house and commercial diagnostic antibody detention and titration kits, particularly the enzyme-linked immunosorbent assay (ELISA) are commercially available for MERS-CoV antibodies (Hashem et al. 2019a; Lee et al. 2018; Okba et al. 2019). These tests showed good aptitude in the detection of MERS-CoV in sera of dromedary camels. However, their specificity and sensitivity still need further evaluation. This may be due to the possibility of cross-reactivity that reported between MERS-CoV and other closely related coronaviruses affecting dromedary camels particularly the Bovine coronavirus (Hemida et al. 2014b).
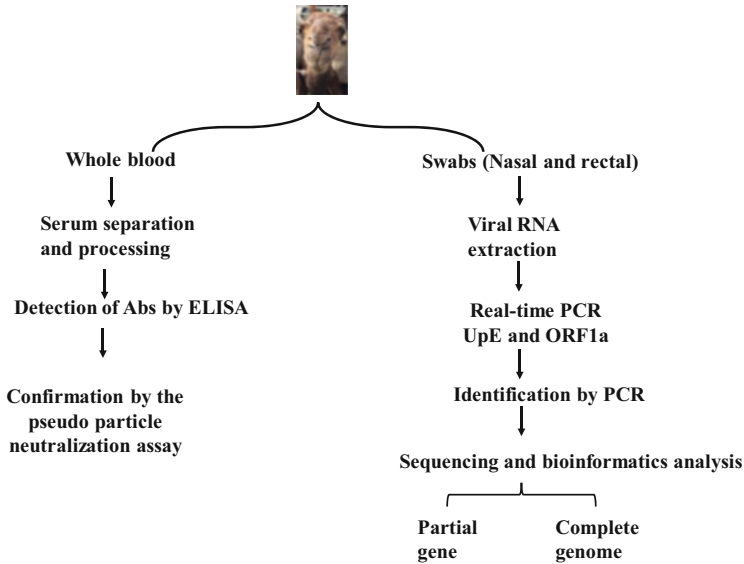
Fig. 11.1 A standard protocol for the laboratory diagnosis of MERS-CoV in animals detection and titration of MERS-CoV antibodies in sera of dromedary camels by commercial or in house ELISA. Confirmation of the ELIA results by the pseudoparticle naturalization assay. Detection and titration of MERS-CoV nucleic acids in swabs (nasal and rectal) collected from animals. Extraction of the viral nucleic acids by standard commercial kits. Detection of the viral nucleic acids by real-time PCR using two targets (up-E and ORF1a). Confirmation of the real-time PCR results by amplification of some viral target genes or the full-length genome by the next-generation sequencing. Revealing the identity of the amplicons can be done by blasting obtained sequences against the available MERS-CoV in the GenBank

Meanwhile, there are some other reliable laboratory accurate techniques used for the detection and quantification of the MERS-CoV specific antibodies in dromedary camel sera. These techniques include, for example, the pseudoparticle neutralization assay (Hemida et al. 2014b). The other strategy is the detection of MERS-CoV infectious virus particles or the viral nucleic acids in the body secretions of the tested animals. The ideal samples for the detecting of the virus or its nucleic acids are the nasal and rectal swabs (Hemida et al. 2014a). There are some quick latex agglutination based tests recently introduced for the quick identification of positive MERS-CoV camels (Kasem et al. 2018). However, these methods are not accurate and prone to false positive/negative results. It is mainly depended on the concentration of the viral antigens in the tested samples. The most accurate technique, considered the gold standard, is the detection of the viral nucleic acids in body secretions by the reverse transcriptase-polymerase chain reactions (RT-PCR) (Hemida et al. 2013). There are many modifications for the RT-PCR technique in the diagnosis of MERS-CoV infection in both humans and animals. One of the robust and user-friendly techniques is the isothermal PCR technique (Shirato et al. 2014). As per the WHO's instruction, confirmation of active MERS-CoV case depends on the testing of

suspected samples by RT-PCR using two different gene targets particularly (up-E and ORF1a) (Corman et al. 2012). The definite diagnosis is the sequencing of the obtained PCR products then blasting the results against the available sequences in the Genebank. Currently, the application of the next-generation sequencing of the partial or the full-length genome is of great value in the diagnosis, classification, and mentoring of changes on the MERS-CoV genomes on the genomic levels (Yusof et al. 2015; Hemida et al. 2013). The application of the IHC techniques to study the antigen localization of the MERS-CoV in various tissues is a good diagnostic approach (Adney et al. 2014).

11.12 Zoonotic Potential of MERS-CoV

MERS-CoV is one of the most important newly emerged viral zoonosis (Azhar et al. 2017). The first observation about the potential zoonotic roles of MERS-CoV was reported in 2014 (Azhar et al. 2014). This study sequenced MERS-CoV from both patients who died from severe pneumonia and from some of the patients' camels that suffered from mild respiratory signs (Azhar et al. 2014). Sequences from both the patient and camels were identical, suggesting the zoonotic transmission of MERS-CoV from camels to human (Azhar et al. 2014). Since that time, there is an ongoing evaluation of the zoonotic potential of MERS-CoV, particularly in the at-risk group of people who come in close contact with dromedary camels on a daily basis. Although some research failed to detect any specific antibodies for MERS-CoV in sera of people in close contact with positive camel herds (Hemida et al. 2015), some other studies reported the seroprevalence of MERS-CoV in people who came in close contact with dromedary camels in Qatar (Reusken et al. 2015). One large-scale serosurveillance study was conducted across Saudi Arabia to investigate the nationwide prevalence of MERS-CoV in people with a history of close contact experience with dromedary camels. This study showed that 0.15% of tested individuals had antibodies against MERS-CoV (Muller et al. 2015). Another recent study conducted in UAE to assess the risk seroprevalence of MERS-CoV among workers in two slaughterhouses and local camel markets found that around 1.7% of the tested individuals have specific antibodies against MERS-CoV (Adney et al. 2019b). Taking into consideration all these facts, dromedary camels pose some risk to human in the context of MERS-CoV transmission cycle. Further studies are needed to unveil the exact mechanisms of MERS-CoV transmission from camels to humans.

11.13 Control Measures for MERS-CoV

Unfortunately, until now, there is no effective medication or vaccine to treat or prevent MERS-CoV infection in human or animals. The control measures for most viral infections are mainly based on the variability of effective vaccines and the

adoption of high standard hygienic measures in both the health care settings and in the farm animals. Implementation of novel strategies based on the One Health concept will help in the reduction of virus shedding from dromedary camels. This will have great benefits on the spillover of MERS-CoV from dromedary camels to human (Hemida and Alnaeem 2019). One of these approaches is the development of novel vaccines against MERS-CoV in dromedary camels. There is some progress in the development and testing of potential vaccines for MERS-CoV in dromedary camels. Some trials have used various approaches to prepare and test these vaccine candidates in laboratory animals as well as in camels. These approaches include some common vaccine strategies such as DNA vaccines, vector-based vaccines, the subunit vaccines, and the viral-like particles based vaccines (Yong et al. 2019). One recent subunit vaccine showed a high degree of protection and reduction in the time of viral shedding in MERS-CoV challenges alpaca and dromedary camels, respectively (Adney et al. 2019a). Several vaccine candidates used the full-length spike protein or its receptor binding domains (RBD) as a base for the preparation of recombinant vaccines against EMRS-CoV (Song et al. 2013; Volz et al. 2015). Several studies used the adenovirus-based recombinant MERS-CoV-S glycoprotein and proved to induce a high level of potent neutralizing antibodies against MERS-CoV (Hashem et al. 2019b; Jia et al. 2019; Kim et al. 2019). Currently, there are two potential MERS-CoV vaccines which have entered the clinical trials phase—the GLS-5300 and the MERS001 (Xu et al. 2019). The other arm of the control measures against MERS-CoV is the implementation of high degree of biosafety precautions among the at-risk people. Those include people who are in daily close contact with dromedary camels, particularly the camel owners, herders, veterinarians, and slaughterhouse personnel (Hemida et al. 2017a).

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