One Health 3 (2017) 11–16

Contents lists available at ScienceDirect

One Health

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

MERS-coronavirus: From discovery to intervention

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ARTICLE INFO

Article history:
Received 16 November 2016
Received in revised form 15 December 2016
Accepted 21 December 2016
Available online 23 December 2016

Keywords:
MERS
Coronavirus
Intervention
Review
Dromedary camel

ABSTRACT

Middle East respiratory syndrome coronavirus (MERS-CoV) still causes outbreaks despite public awareness and implementation of health care measures, such as rapid viral diagnosis and patient quarantine. Here we describe the current epidemiological picture of MERS-CoV, focusing on humans and animals affected by this virus and propose specific intervention strategies that would be appropriate to control MERS-CoV. One-third of MERS-CoV patients develop severe lower respiratory tract infection and succumb to a fatal outcome; these patients would require effective therapeutic antiviral therapy. Because of the lack of such intervention strategies, supportive care is the best that can be offered at the moment. Limiting viral spread from symptomatic human cases to health care workers and family members, on the other hand, could be achieved through prophylactic administration of MERS-CoV neutralizing antibodies and vaccines. To ultimately prevent spread of the virus into the human population, however, vaccination of dromedary camels – currently the only confirmed animal host for MERS-CoV – may be the best option to achieve a sustained drop in human MERS cases in time. In the end, a One Health approach combining all these different efforts is needed to tackle this zoonotic outbreak.

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1. Introduction

Coronaviruses (CoV) are known to cause mild upper respiratory tract infections in humans. This paradigm was challenged when severe acute respiratory syndrome (SARS)-CoV emerged in 2002. SARS-CoV causes mainly lower respiratory tract infections, such as bronchitis and pneumonia. Approximately 10% of SARS-CoV patients developed severe complications and succumbed to this disease. This virus originated from bats and was transmitted to humans through civet cats, highlighting its zoonotic capacity. It spread worldwide and infected ~8000 individuals within a year, but was fortunately contained in 2003. There is currently no evidence of SARS-CoV circulating in the human population [1]. However, a SARS-like CoV that is able to directly infect human cells has been recently identified in horseshoe bats in China [2]; therefore, continuous surveillance for these viruses remains necessary.

A decade after the SARS-CoV epidemic, another novel CoV was isolated from a 60-year-old Saudi Arabian man who presented with acute pneumonia. He subsequently developed acute respiratory distress syndrome and renal failure with a fatal outcome [3]. This virus, later called the Middle East respiratory syndrome (MERS)-CoV, attracted public interest due to its resemblance to SARS-CoV. So far, at least 1800 individuals have been infected with an ~35% fatality rate. Different from SARS, some individuals infected by MERS-CoV remain asymptomatic or develop only mild clinical manifestations [4,5]. Efforts to develop effective preventive and therapeutic intervention strategies are

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http://dx.doi.org/10.1016/j.onehlt.2016.12.001

currently ongoing. Current interventions are mainly based on setting up surveillance studies and public health measures that include patient isolation and quarantine [1]. Although these actions resulted in successful containment of the SARS outbreak, MERS-CoV still remains a problem, mainly in the Arabian Peninsula. The widespread circulation of MERS-CoV in dromedary camels is most likely the driving force of these outbreaks as novel zoonotic introductions of MERS-CoV may occur frequently. Therefore, different intervention approaches may be necessary to treat MERS patients, control zoonotic and nosocomial transmission. Here we describe the affected groups in the ongoing MERS-CoV outbreak and how distinct intervention strategies for each of them may curb the spread of the virus.

2. The discovery of the virus and the subsequent rapid development of diagnostics

When MERS patients are admitted to hospitals their clinical symptoms mostly include fever, cough, expectoration and shortness of breath [1,6]. Ground glass opacities and consolidation in the lungs are commonly reported in chest radiographs or computed tomography scans [7]. However, these characteristics overlap with other lower respiratory tract infections and are not pathognomonic for MERS-CoV, indicating the need to develop laboratory-based diagnostic assays with high sensitivity and specificity.

Upon its discovery, MERS-CoV was found to replicate to high titers and induce cytopathic effects in various different cell lines, thus enabling its rapid full genome characterization [3,8]. Two large replicase open reading frames, ORF1a and ORF1b cover the 5′ region of the MERS-CoV genome, whereas the 3′ end of the genome encodes structural proteins, *i.e.* spike (S), membrane (M), nucleocapsid (N), envelope (E), and several accessory proteins (3, 4a, 4b, 5 and 8b). The nucleotide sequence of MERS-CoV was used as a template to design primers for genome-based assays, *i.e.* real-time reverse-transcription polymerase chain reaction (RT-PCR) and sequencing [9,10]. Primer pairs targeting a region upstream of E (upE), N, ORF1a, ORF1b and RdRp genes were then developed and shown to be highly sensitive and specific not only for the EMC isolate but also for other MERS-CoV isolates [9–11]. It is currently suggested to use upE RT-PCR as a screening assay and another target gene as a confirmatory assay [11].

Despite being highly specific and sensitive, RT-PCR-based assays still have limitations as MERS-CoV can only be detected when it is actively shed by the host. Serology-based assays were subsequently developed to distinguish those individuals that had been exposed to MERS-CoV in the past. Indirect immunofluorescence assays (IFA) and neutralization tests (plaque reduction neutralization test and microneutralization test) were set up using susceptible cell lines and whole virus particles [5,12]. These assays require biosafety level 3 facilities to work with the infectious MERS-CoV *in-vitro*, limiting their usage. In addition, whole virus IFA showed limited specificity to MERS-CoV due to cross-reactivity with other human CoV [5,12]. Alternative assays using a pseudoparticle virus and specific MERS-CoV antigens were then developed to solve this issue. Two CoV structural proteins known to be highly antigenic are the N and S proteins. Both proteins have been used to develop serology-based assays in various platforms, *i.e.* recombinant IFA, western blot, enzyme-linked immunosorbent assay (ELISA), luciferase-based antibody detection assay and protein microarray [5,13–16]. The N protein is relatively conserved among CoVs, whereas the S1 domain, located in S, is more divergent among CoVs, making it an ideal candidate for CoV specific diagnostic serological assays. However, it is important to note that none of the serological assays available to date has been fully validated for specificity and sensitivity, therefore due care must be taken in interpreting the results of large serosurveillance studies. Possible cross reactivity and/or low sensitivity of these assays can lead to failure in determining the prevalence of true MERS-CoV positive cases in a given population. In turn, this has an impact on the calculated fatality rate of the viral infection. Further studies using a set of well-characterized sera are required for the determination of cut-off values and assessing cross-reactivity between MERS-CoV and other human CoVs. It is crucial to properly determine the MERS-CoV prevalence at a population level to develop adequate control programs.

3. Treatment options for MERS patients

One third of the symptomatic patients develop severe pneumonia that ultimately leads to a fatal outcome. In general, these individuals are characterized by advanced age (>55 years old) and may have multiple underlying comorbidities, *i.e.* diabetes mellitus, asthma, chronic kidney failure, heart disease and immunosuppression [6,17]. Most importantly, they are also prone to develop life-threatening complications, such as sepsis, acute respiratory distress syndrome, and acute kidney failure [1,6]. Therefore, rapid and effective treatment options are required in order to limit the number of cases with a fatal outcome.

Several studies are dedicated to the development of effective treatments against MERS-CoV, either based on the use of broad-spectrum or MERS-CoV specific therapeutic agents. *In-vitro*, MERS-CoV is highly sensitive to type I interferon (IFN). While MERS-CoV replication in Vero cells could be efficiently reduced with low levels of recombinant IFNα, in the case of SARS-CoV much higher concentrations are needed to achieve a similar inhibition of viral replication [18]. Other broad spectrum antivirals, such as ribavirin, mycophenolic acid and cyclosporine-A, also have antiviral activity against MERS-CoV *in-vitro*. Combinations of ribavirin and IFNα may have a synergistic effect as shown by *in-vitro* and *in vivo* studies in rhesus macaques [19,20]. However, treatment should be initiated quite early after the infection; hence it has a limited effective therapeutic window of opportunity. Experimental SARS-CoV infection in mice showed that administration of type I IFN 6 h post inoculation (pi) is life-saving, while 24 h pi it is detrimental, supporting the limited effective therapeutic window of opportunity [21]. The importance of having sufficient type I IFN being produced early after the SARS-CoV infection has also been investigated in experimentally infected macaques. Advanced-age macaques do not mount sufficient type I IFN responses upon SARS-CoV inoculation but instead upregulate expression of interleukin-8 (IL-8), a neutrophil chemoattractant, leading to the development of acute lung injury (ALI). Most importantly, treatment with type I IFN at day 1 and 3 pi prevents this IL-8 mediated ALI [22]. The limited effective therapeutic window of opportunity of type I IFN might explain why a cocktail regimen of ribavirin and IFNα did not improve the overall survival rates seen in human MERS cases, despite these promising *in-vitro* and *in-vivo* results [18–20,23,24]. Other candidate drugs were identified by screening for MERS-CoV inhibitory activity using FDA-approved drugs that are used to treat other diseases. Two research groups reported that both chloroquine and chlorpromazine have potential therapeutic antiviral activity. Chlorpromazine affects the assembly of clathrin-coated pits at the plasma membrane, while chloroquine plays a role in endosomal acidification. By interrupting these biological processes, those drugs likely inhibit MERS-CoV endocytosis at the host cell membrane and fusion in endosomes intracellularly [25,26].

Due to its limited effective therapeutic window of opportunity, broad spectrum antiviral agents might not be sufficient to treat severe MERS-CoV patients. Several promising CoV specific therapeutic agents have recently been reviewed extensively [27]. These include efforts to develop effective intervention measures aiming to disrupt the interaction of MERS-CoV with its host receptor, dipeptidyl peptidase 4 (DPP4). Although, the crystal structure of DPP4 and the S1 protein of MERS-CoV allowed visualization of the interacting amino acids in both proteins, no antagonists other than adenosine deaminase (ADA) and antibodies that recognize the receptor binding domain (RBD) have been identified so far [28–30]. Importantly, inhibitors of the enzymatic activity of the DPP4, generally used to treat type 2 diabetes patients, did not block infection of target cells with MERS-CoV [31]. Another potential target for treatment is the S2 protein of MERS-CoV, a subdomain

of S protein that mediates fusion of the virus in the endosome. Interaction between heptad region (HR) 1 and 2 within S2 protein is imperative for MERS-CoV entry into its target cell. This interaction can be disrupted using an artificial HR2 homologous peptide, making it a promising entry inhibitor for MERS-CoV [32,33]. There are also novel drugs aimed at inhibiting virus replication within host cells, targeting the non-structural proteins (nsp) of coronaviruses. Examples are K22, a novel inhibitor of nsp6 [34] and SSYA10-001 that acts on nsp13 [35]. The pocket binding ligand of these compounds is conserved among coronaviruses making them promising pan-coronavirus replication inhibitors.

4. Preventing human-to-human transmission of MERS-CoV

MERS patients require proper quarantine measures to hinder the spread of the MERS-CoV to other susceptible individuals. Although several studies performed in Saudi Arabia and South Korea indicated that human-to-human transmission is relatively limited, it has been found to be crucially important, especially in the hospital outbreaks [5,36,37]. Inefficient human-to-human transmission is partly due to the tropism of MERS-CoV. Autopsy results from one fatal human MERS case and *ex-vivo* experiments using human lung explants showed that this virus infects the lower respiratory tract epithelium [38,39]. These observations are consistent with the fact that in most cases MERS-CoV isolation from human samples was successful only when lower respiratory tract samples were used [3,40]. Although it is possible to detect MERS-CoV in the upper respiratory tract, viral RNA levels are generally very low compared to the lower respiratory tract [40–42]. This restricted tropism of MERS-CoV in the lower respiratory tract is supported by the distribution of its host receptor. DPP4 is expressed in the lower but not in the upper human respiratory tract epithelium [43].

As a result of the lower respiratory tract tropism, close contact between individuals is necessary to transmit MERS-CoV between humans. Healthcare workers and family members are therefore at particular risk to acquire secondary MERS-infections [5,36], as reflected by the MERS outbreaks which mostly occur in the healthcare centers [44,45]. Other groups that are at risk for MERS-CoV infection are slaughterhouse workers and camel shepherds. In cohort studies in Saudi Arabia and Qatar, these groups with camel contact were reported to have a higher percentage of MERS-CoV seropositivity as compared to the general population [15,46]. The fact that camel-farm-related outbreaks are generally less common compared to health-care-related outbreaks may also be helpful in containing the outbreak [47,48].

During the SARS-CoV outbreak in Hong Kong, active surveillance followed by proper quarantine measures was shown to be highly effective in limiting the spread of the virus to other susceptible individuals [4]. One important factor that helped to contain the outbreak relates to the fact that the virus rarely causes subclinical infection and virus excretion peaked approximately 10 days after the onset of symptoms, allowing successful containment when SARS patients are diagnosed early after disease onset [4,49,50]. Recent data from the MERS-CoV outbreak in South Korea also showed that viral shedding in severe cases peaked in the second week after the onset of disease [51]. Therefore, similar to SARS, rapid identification of MERS-CoV patients, followed by proper quarantine measures, should be sufficient to significantly limit the spread of the virus, especially in hospital settings where most MERS outbreaks occur. Indeed, this strategy has been reported to be useful in controlling the MERS-CoV outbreak in South Korea [37]. Most likely, such approaches had not been properly applied in the earlier outbreaks in Saudi Arabia. The fact that more recent MERS cases reported in 2016 from this region mostly seem to be restricted to primary cases with a history of camel contact but less to nosocomial outbreaks may indicate that more effective control measures have been implemented to contain the spread of the virus.

Studies in Hong Kong during the SARS-CoV outbreak also indicated that infection with SARS-CoV was unlikely to result in an asymptomatic infection [49,50], explaining why active surveillance and quarantine were so effective in stopping the outbreak [4]. In case of MERS, asymptomatic MERS-CoV-infected individuals that spread the virus may fuel the ongoing outbreak as these are currently not identified by diagnostic screening. It is difficult to precisely assess the percentage of asymptomatic MERS-CoV infections in the human population, partly due to the lack of active serological surveillance and fully validated commercial serological assays. However, asymptomatic individuals who had contact with MERS patients have been identified using a combination of RT-PCR, ELISA, IFA and PRNT assays; suggesting that in case of MERS, the virus can indeed cause mild to asymptomatic infections [5,36,52]. In addition, subclinical MERS-CoV infection with long term excretion of MERS-CoV RNA has been reported [52]. Unlike severe MERS cases these patients are mostly young to middle-aged without underlying comorbidities. This group is primarily dominated by health care workers in the hospitals where MERS-CoV patients were admitted and secondly by family members having close contact with the severe MERS-CoV patients [5,36]. In one contact tracing study, out of 280 individuals who had contact with MERS-CoV patients, 12 were concluded to have probable MERS-CoV infections. Only one out of these 12 contacts developed symptoms which later progressed to respiratory failure and fatal outcome, while the rest reported no symptoms [5]. Therefore, during an ongoing outbreak in a health care setting, it is imperative to protect the healthcare workers that are at high risk of acquiring the infection [36,45]. In this scenario, prophylactic regimens, for example using humanized monoclonal antibodies, would be theoretically suitable to limit the spread of the virus. Monoclonal antibodies mostly have limited therapeutic efficacy despite promising *in-vivo* results, yet they might still serve as a potent prophylactic measure, as exemplified by palivizumab, a monoclonal antibody against respiratory syncytial virus. This antibody is effective in reducing the frequency of hospitalizations in children at high risk of infection, but its efficacy for treatment purposes has not been demonstrated [53]. The receptor binding domain (RBD) located within the S1 protein could serve as a target for humanized monoclonal antibodies. These antibodies have been generated using different approaches, *i.e.* from non-immune human peripheral blood mononuclear cells [54,55], from yeast cells expressing human antibodies [56], from memory B cells obtained from infected humans [57], or from mice immunized with the RBD domain of the S protein of MERS-CoV [58]. Several of these antibodies have been reported to be effective *in-vitro* and *in-vivo* [55–59]. One monoclonal antibody against MERS-CoV, m336, has been shown to be effective as a prophylactic agent in rabbits [60]. Inhibiting virus attachment could also be achieved by targeting the host receptor, DPP4. Unfortunately, commercially available DPP4 inhibitors that are marketed as type II diabetes mellitus drugs were not able to serve this purpose, since they do not bind to the RBD [56]. Monoclonal antibodies targeting DPP4, on the other hand, also may inhibit MERS-CoV attachment *in vitro*. One of these antibodies, YS110, has been used in a phase I clinical trial for patients with DPP4-expressing cancers [61].

There are also examples of antiviral agents that can be used for prophylactic purposes. Post-exposure prophylaxis using oseltamivir was shown to be effective in reducing influenza transmission during the 2009 H1N1 influenza outbreak in Singapore military camps [62]. Intranasal pretreatment of aged mice with poly (I.C.), a TLR3 agonist, induces IFN expression and provides protection against lethal dose of SARS-CoV and influenza A virus [63].

Besides these prophylactic measures, vaccines could also be applied in particular risk groups. Many different approaches have been taken to develop MERS vaccines (reviewed in [27]). An orthopoxvirus-based vaccine using modified vaccinia Ankara (MVA) expressing the spike protein has been developed and shown to induce neutralizing antibodies and specific cytotoxic T cell response against MERS-CoV [64]. The MVA itself has been validated as an effective vaccine platform in humans [65]. Unlike prophylactic regimens, protection induced by vaccine intervention is long lasting, although not immediate. Thus

vaccination would be more appropriate for camel shepherds and slaughterhouse workers who are at a continuous risk of being infected by MERS-CoV from the animals.

5. Intervention of the MERS-CoV outbreak at the source: vaccination of dromedary camels

The zoonotic capacity of MERS-CoV was suggested already at the beginning of its emergence, since it was observed that the virus is closely related to bat CoVs but not to other human CoVs [3,8]. Bats have been suggested to be one of the natural hosts of MERS-CoV but the evidence supporting transmission of this virus from bats to other species is currently lacking. Serology-based assays applied using sera from various animal species in search of the animal hosts for MERS-CoV, revealed the dromedary camel as the most likely zoonotic source for MERS-CoV [13,66,67]. The high percentage of seropositivity against MERS-CoV among camels in the Arabian Peninsula and Africa, as early as the 1980s, indicates that MERS-CoV did circulate in this animal long before being introduced to the human population [13,14,66–72]. Screening of dromedary camel nasal swabs subsequently led to identification and isolation of MERS-CoV from dromedaries, confirming its circulation in this animal species [14,47,73,74]. Camels develop mild upper respiratory tract infections upon experimental inoculation of MERS-CoV, consistent with localization of DPP4 at this location [43,75,76]. Furthermore, two studies of human MERS cases post-contact with infected camels reported high similarity in virus sequences obtained from both the camels and humans [47,77]. These studies along with a case control study identifying direct exposure to camels as a risk factor for MERS-CoV infection, and serology studies showing higher seropositivity among camel contacts compared to non-camel contacts, support camel-to-human transmission of MERS-CoV [15,17,46]. The risk of humans acquiring MERS-CoV infection from camels could be high due to the wide geographical distribution of MERS-CoV seropositive camels, also demonstrated by the fact that MERS-CoV seropositive livestock handlers were identified in Kenya [78], but it might be underestimated due to unrecorded subclinical infections in humans.

Efforts to screen other animal species for MERS-CoV are currently ongoing. Phylogenetic analysis using RBD within DPP4 of different species identified several livestock animals, *i.e.* sheep, goats, cattle, llamas and horses to cluster together with other susceptible animals, including dromedary camels. Transfection of non-susceptible cells with DPP4 of these different animal species converted these cells to become susceptible to MERS-CoV [79,80]. However, serological studies did not provide evidence for MERS-CoV circulating in these animals [13,66,81]. Experimental MERS-CoV infections showed that alpacas shed infectious virus up to twelve days post inoculation [82], and they could transmit infectious virus to other naïve alpacas [83]. These observations are consistent with findings from a study at a farm located in the Al Shahaniya region of Qatar which showed that 15 out of 20 alpacas were found positive for neutralizing antibodies against MERS-CoV. These alpacas were housed nearby to camels, also seropositive for MERS-CoV, and taken care of by the same shepherds [84]. Although pigs and llamas were found susceptible to MERS-CoV upon experimental infection [85], thus far no evidence was obtained from the field for seroconversion in these animal species. Limited shedding of infectious virus was reported in goat, sheep, and horse experimentally inoculated with MERS-CoV, suggesting that these animals play minimal to no role in spreading MERS-CoV in current outbreaks [86]. These studies suggest that among the livestock animals, dromedary camels are the main species responsible for spreading the virus.

Given the fact that livestock animals, especially dromedary camels, can potentially transmit MERS-CoV to humans, preventive measures need to be applied to reduce the risk of zoonotic transmission. During the SARS outbreak, civet cats, which posed a risk of transmitting the virus to humans, were culled [87], thus potentially contributing in limiting the outbreak. In case of MERS, vaccine based strategies have to be applied to reduce the transmission of the virus to humans. The MVA-based vaccine against MERS-CoV might be a suitable intervention strategy for this purpose. This vaccine has been shown to induce neutralizing antibodies and significantly reduce MERS-CoV shedding in camels [76]. This vaccination strategy may especially be applied to young dromedary calves at 5–6 months post-parturition, when they lose their maternal antibodies [88]. Further studies should also address whether this vaccine is able to provide protection on the long term. The fact that this vaccine may provide additional protection against camelpox virus, another pox virus that could cause severe disease in camels, should be taken into account when evaluating the cost-effectiveness of this vaccine [76].

6. One Health approach for intervention

Despite current implementation of rapid diagnostic and public health measures, new MERS-CoV cases are still continuously being reported. This suggests that multiple interventions targeting different affected groups would be necessary to stop these outbreaks, as summarized in Fig. 1. As described in this review, different intervention

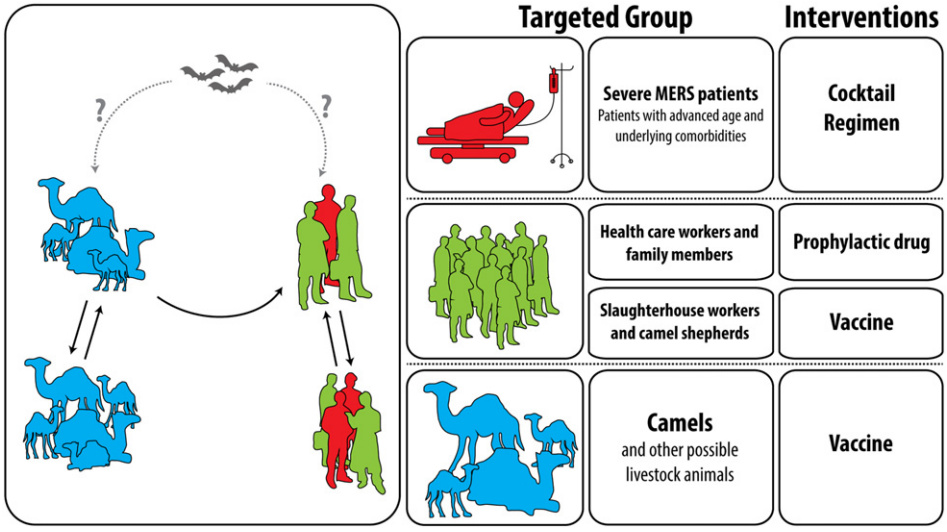

Fig. 1. Animals and humans potentially involved in the MERS outbreak and the intervention strategies that would apply to these different groups. Bats have been suggested to be one of the natural hosts of MERS-CoV, however the evidence supporting transmission of this virus from bats to other species is currently lacking. On the other hand, MERS-CoV has been isolated from both camels and humans. A combined One Health approach may be needed in order to stop ongoing outbreaks of MERS-CoV.

approaches may be needed to limit the MERS-CoV outbreaks by treating MERS-CoV patients and by controlling both zoonotic and nosocomial transmission. Prophylactic drugs would be appropriate to use when immediate protection is required to limit the spread of the virus, for example to protect health care workers and family members of MERS-CoV patients. Camel contacts that have higher chances of infection, on the other hand, may benefit from the long term protection induced by vaccines. Such vaccination could also be applied to the animal host of MERS-CoV which has close contact with humans, mainly dromedary camels, to limit zoonotic transmission. Effective treatment strategies for severe human MERS-CoV patients are the most problematic to implement. These MERS-CoV patients may require a cocktail treatment regimen consisting of potent antivirals, immunomodulatory agents and other supportive treatments to control disease progression and improve the prognosis of this severe lung infection. Although Type I IFN remains a promising treatment, its beneficial effect in severe and fatal MERS-CoV patients might be limited due to the timing of the drug administration as it has a narrow effective therapeutic window of opportunity [21]. Further efforts to better understand MERS-CoV pathogenesis are needed to identify viral and host factors that play a significant role in the progression of MERS in humans and offer potential novel treatment and intervention options.

Acknowledgements

This study was supported by the TOP Project grant (91213066) funded by ZonMw and partly by the Zoonotic Anticipation and Preparedness Initiative (ZAPI project; IMI Grant Agreement no. 115760), with the assistance and financial support of IMI and the European Commission.

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