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MERS-CoV INCIDENCE IN THE KINGDOM OF SAUDI ARABIA AND WORLDWIDE: GENERAL REVIEW ARTICLE

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Abstract

In recent years, several outbreaks of the Middle East Respiratory Syndrome Corona Virus (MERS-CoV) were reported to the WHO by Saudi Arabia (2014, 2015, and 2017) and the Republic of Korea (2015). These large outbreaks indicate that MERS-CoV, if not adequately controlled, can cause severe outbreaks and negative socio-economic consequences. The disease was identified and reported in 27 countries around the world, particularly in the Middle East, Africa, Asia, Europe, and North America. The total number of laboratory-confirmed MERS-CoV cases reported to the WHO between 2012 and 21 July 2017 was 2040, of which 1672 (82%) were reported by the Kingdom of Saudi Arabia. Since the 5th December 2016, about 190 cases were confirmed in Saudi Arabia, out of which 63 were reported in one outbreak and four different clusters in the Riyadh region.

This review discusses the background of the disease along with its epidemiology, risk factors, clinical features, diagnosis, treatment, vaccination, prevention and control. The review also concludes with some future perspectives.

Key words: Saudi Arabia, MERS-CoV, Worldwide, General review

Background

The Middle East Respiratory Syndrome Corona Virus (MERS-CoV) was initially identified in Middle Eastern countries, particularly Saudi Arabia, in September 2012 (Azhar *et al*, 2014). It belongs to the Nidovirales order, Coronaviridae family and the Coronavirinae subfamily. It is of the Betacoronavirus genus, and the species is Middle East Respiratory Syndrome Coronavirus (MERS-CoV). It was a novel enveloped, positive-sense, the single-stranded RNA genome as a large virus with a helical symmetry of a genomic size ranging from 26 to 32 kilo-bases (Anand *et al*, 2003; Siddell *et al*, 1983).

Globally, since its discovery, more than 2.040 laboratory confirmed cases linked to infectious MERS-CoV were reported to the WHO (2017a), resulting in a high mortality rate (712 deaths). Therefore, serious considerations of the increasing number of infected cases, the high mortality rate as well as the pandemic state of the virus during the Hajj season are essential. Several questions remain open, even though there is invaluable information available about the viral pathophysiology, suggesting an "'animal reservoir", and revealing the intermediate host of the virus (Azhar *et al*, 2014). To provide and review the current knowledge about the infectious MERS-CoV, including its history, the mode of transmission, epidemiology, clinic- al symptoms, diagnostic approaches, treatment options and, finally, future the perspectives the target of the present study.

In September 2012, a novel Corona Virus was isolated and identified by an Egyptian virologist Dr. Ali Mohamed Zaki, then reported on the Program for Monitoring Emerging Diseases (ProMED-mail, 2012). In May the Middle East Respiratory Syndrome Corona Virus (MERS-CoV) was launched by the Corona Virus Study Group of the International Committee on Taxonomy of Viruses (ICTV) (De Groot *et al*, 2013). The isolation of the MERS-CoV in Saudi patients showed that the virus' genomic sequencing belongs to the lineage C of the *Betacoronavirus* genus, together with the bat corona Viruses Ty-BatCoV-HKU4 and Pi-BatCoV-HKU5, which have been detached from two different bats species, respectively: *Tylonycteris pachypus* and *Pipistrellus abramus* (Woo *et al*, 2006; Khalil *et al*, 2013). Prior to 2003, it was assumed that human Corona Viruses were not serious infectious human pathogens, since they were represented by the viruses HCoV-OC43 and HCoV-229E, which can infect the upper respiratory tract system and cause mild flu-like symptoms, known as the common cold (Drosten *et al.*, 2003). However, between 2002 and 2003, the initiation of an unknown infectious human corona virus was reported as the first zoonotic agent introduced by the corona virus into the human population, which was later named Severe Acute Respiratory Syndro-me Corona Virus (SARS-CoV) and led to the first Corona Virus-associated human epidemic and global pandemic viral infection, infecting approximately 8,400 cases and killing 800 people (WHO, 2003). Recently, the MERS-CoV proved to be a highly pathogenic, sporadic, and was ranked the second zoonotic viral disease after SARS-CoV. It was hypothesized that the MERS-CoV originates from natural reservoir animals, such as bats, and an intermediate host, such as palm civet (Guan *et al*, 2003).

There are three existing basic reasons that support the theory that MERS-CoV originated from bats, as follows: 1- it shares a highly similar phylogenetic similarity with the bat *Betacoronaviruses*; BatCoV-HKU4 & BatCoV-HKU5; 2- bats in Asia, Africa, the Americas and Eurasia have been found to possess closely-related Corona Virus sequences; and 3- it enters the cells by using the evolutionary conserved dipeptidyl peptidase-4 (DPP4) protein found in the *Pipistrellus pipistrellus* bats (Lau *et al*, 2010; Raj *et al*, 2013).

Epidemiology: During the summer of year 2012, in Jeddah, Saudi Arabian, an anonymous Corona Virus (CoV) was first isolated from the saliva of a patient who was suffering from acute pneumonia and renal atrophy. The virus was then named MERS-CoV by the ICTV (De Groot *et al.*, 2013). MERS-CoV is known as one of the human corona viruses that consist of a further five strains, namely: SARS-CoV, HCoV-OC43, HCoV-229E, HCoV-NL-63, and HCoV-HKU1. MERS-CoV is considered a zoonotic disease, and seems to have variety of dependent transmission patterns. Moreover, it can be transmitted by droplets or close contact (Fig. 1) between infected camels, chiefly dromedary camels, rhinorrhea and humans, as well as via human to human transmission (Cotten *et al*, 2013; Drosten *et al*, 2014).

On the other hand, the virus can be transmitted by touching contaminated surfaces (Chu *et al*, 2014). MERS have been mainly identified and reported in 27 countries around the world, particularly in the Middle East, Africa, Asia, Europe, and North America. These countries are distributed across four continents and areas, including (Fig. 2): Saudi Arabia, Kuwait, Qatar, Yemen, Oman, Bahrain, Jordan and Lebanon in the Middle East; Tunisia, Egypt and Algeria in Africa; Iran, South Korea, China, Thailand, the Philippines, Malaysia and Indonesia in Asia; Italy, France, Germany, Greece, Austria, Turkey, Netherlands and the United Kingdom in Europe; and in the United States (WHO, 2017b).

Since September 2012, WHO has reported 2,040 laboratory-confirmed cases of MERS-CoV, resulting in at least 712 MERS-CoV-related deaths?

Two thousands forty laboratory confirmed cases of MERS-CoV from 27 countries and the majority of cases (about 82%, e.g. 1672 cases) was reported from Saudi Arabia (Tab.1)

Table 1: No. of laboratory-confirmed MERS-CoV cases reported since September 2012 (WHO, 2017b).

No. of Countries	Countries reporting	No. of laboratory-confirmed MERS cases
1	Algeria	2
2	Austria	2
3	Bahrain	1
4	China	1
5	Egypt	1
6	France	2
7	Germany	3
8	Greece	1
9	Iran	6
10	Italy	1
11	Jordan	28
12	Kuwait	4
13	Lebanon	2
14	Malaysia	1
15	Netherlands	2
16	Oman	8
17	Philippines	2
18	Qatar	19
19	Republic of Korea	185
20	Saudi Arabia	1672
21	Thailand	3
22	Tunisia	3
23	Turkey	1
24	United Kingdom	4
25	United Arab Emirates	83
26	United States of America	2
27	Yemen	1
Total		2,040

Furthermore, it is worthy to mention that since the 5th December 2016; about 190 cases were confirmed from Saudi Arabia, of whom 63 were reported from one outbreak and four different clusters in the Riyadh Region (Tab. 2).

Table 2: Outbreaks and clusters of MERS-CoV from the Kingdom of Saudi Arabia since 5th December 2016 (WHO, 2017b).

City reporting	Data	No. of laboratory-confirmed MERS cases
Wadi-Aldwaser (a cluster at one hospital)	March 2017	10
Wadi-Aldwaser (a cluster at same hospital)	April 2017	5
Riyadh (a cluster at health care facility)	June 2017	5
Riyadh (a cluster at 1 hospital)	June 2017	9
Riyadh (an outbreak at a hospital)	1 June 2016 - 3 July 2017	34
Total		63

The case mortality rate is exceedingly high and the fatality rate reaches up to 40% among older people (WHO, 2017b). MERS-CoV has an incubation period ranging between two days to two weeks (Lee, 2015). The human respiratory tract is considered the primary infection site for MERS-CoV. It was verified that MERS-CoV could apparently infect, target and robustly replicate within human respiratory epithelium tissues. MERS-CoV uses its Spike protein receptor to bind its tropism receptors, such as metalloprotease amino-peptidase N and dipeptidyl peptidase 4 (DPP4), in the surface molecules cells, which are mainly non-ciliated bronchial epithelial cells, alveolar epithelial cells, bronchiolar epithelial cells, and endothelial cells of the pulmonary vessels (Zhou *et al*, 2015). As the first line of host immune defense, human primary respiratory epithelial cells, human lung tissue, and respiratory epithelial cell lines are stimulated to produce antiviral and pro-inflammatory chemokines and cytokines to eradicate the invading virus. Also, MERS-CoV infection fails to elicit a strong pro-inflammatory cytokines response in human primary respiratory epithelial cells and respiratory tissues. Copulatively

MERS-CoV may have evolved various antagonistic mechanisms to attenuate or diminish the host immune defense, which might contribute to the high pathogenicity in humans (Zhou *et al,* 2015).

Risk factors conducive to MERS-CoV infections: Farmers, shepherds, abattoir workers or dromedary owners who are in close or direct contact with camels or dealing with animals or visiting farms, camel pens, barn areas or market environments where camels are sold are at a high risk of encountering the MERS-CoV (WHO, 2014). Moreover, persons who are at high risk of severe pattern of MERS-CoV include the immunocompromised hosts who are undergoing chemotherapy or radiation, smokers, the elderly and people who suffer from diabetes, chronic lung disease, renal failure or heart failure and/or hypertension (WHO, 2014).

It is also noticed from Saudi Arabia that workers at health-care settings are at high risk of encountering the MERS-CoV infection. This is in line with the report of the WHO (2017b) which stated that around 20% of the total confirmed cases from 2012 up to date have been among health-care practitioners.

Clinical features: People infected with the MERS-CoV present symptoms for a duration ranging from 2 to 14 days. It is unknown whether patients are infectious and capable of spreading the virus during this incubation period (IDSA, 2014). Clinically, MERS-CoV can infect the upper and lower respiratory systems and cause disease ranging from mild to severe clinical symptoms (IDSA, 2014). Therefore, the majority of infected cases remain asymptomatic, without the appearance of any severe symptoms, or normally present with signs and symptoms such as a high fever, chill, cough, shortness of breath, chest pain, headache, myalgia, sore throat and arthralgia (Al-Abdallat *et al,* 2014). Within one week, a few cases display dyspnea, which rapidly develops into pneumonia and shock (Arabi *et al,* 2014). Gastrointestinal symptoms such as abdominal pain, anorexia, vomiting, and severe diarrhea are present in some cases (Assiri *et al,* 2013). Co-infections, mainly related to nosocomial bacterial, fungi, and respiratory viruses, appear to be very common (Memish *et al,* 2014). Immunocompromised patients or those with "other co-morbidities" such as chemotherapy, radiotherapy, obesity, hypertension, diabetes, lung disease, cardiac disease, acute renal failure (ARF), and organ transplantation display high severity symptoms (Assiri *et al.,* 2013). MERS-CoV is a highly fatal disease, particularly in lethal appearances during severe pneumonia with acute respiratory distress syndrome (ARDS) as well as in ARF due to kidney infection and renal tissue hypoxia. Additionally, severe neurologic syndrome associated with MERS-CoV was identified in three patients, resulting in a changed level of consciousness, confusion, ataxia, focal neurological deficits, and coma (Arabi *et al,* 2015). Besides, laboratory abnormality data recognized with MERS-CoV included disseminated intravascular coagulation (DIC), leukopenia, lymphopenia, thrombocytopenia, elevated serum level of lactate dehydrogenase (LDH), liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine levels (Assiri *et al,* 2013).

Diagnosis: A reliable diagnosis of MERS-CoV is a prerequisite for treating and preventing the infection. Therefore, MERS-CoV samples can be collected from either the upper respiratory tract system (such as by a nasopharyngeal swab taken from the nasopharynx or an oropharyngeal swab), or lower respiratory tract system (such as from sputum, bronchoalveolar lavage, lung tissue and tracheal aspirates), which constitute a significant body of evidence about the highest viral loads (Drosten *et al,* 2013; Guery *et al,* 2013). Whole blood and serum specimens are able to provide strong evidence during virus and immunity detection (Kraaij-Dirkzwager *et al,* 2014). A low level of viral load has been detected in urine and stool samples that were considered less sensitive regarding viral detection than upper and lower respiratory tract system samples (Drosten *et al,* 2013; Guery *et al,* 2013). It is impossible to identify the existence of MERS-CoV from semen samples. Several diagnostic techniques, as the chest radiography, electron microscope, Immunofluorescence microscopy, cell culture, enzyme linked immunosorrbent assay (ELISA), and real time-polymerase chain reaction (RT-PCR) were utilized in viral identification and quantification (Corman *et al*, 2012a; Drosten *et al,* 2015).

Chest X-rays are easy to perform, fast, and fairly inexpensive, producing data that were available in electronic format and so easily be archived, stored, and made widely available to hospitals and clinicians (Delrue *et al,* 2011). However, excessive exposure to radiation can cause malignancy in general and birth defects among pregnant women. Moreover, this technique cannot distinguish between different viral infections, and the interpretation of chest X-ray reports remains challenging (Delrue *et al*, 2011).

Electron microscope, meanwhile, relies on the isolation of the novel coronavirus from classic tissue-culture that is used to amplify the viral pathogen for the purpose of etiological identification. It is a widely-used, highly sensitive, and versatile technique that offers several advantages, including the used in clinical virology and histopathology research, not requiring specific reagents, and being able to recognize viral morphology's "unique nature" and distinguish among different microorganisms in the tested samples to help to connect them to the disease (Johnson *et al,* 1977; Hazelton and Gelderblom, 2003). Nevertheless, this technique requires expertise, it's availability was only in reference labs, very expensive, cannot classify a pathogen based on its appearance and morphogenesis, and is unsuitable for investigating a large number of samples during outbreaks (Hazelton and Gelderblom, 2003; Biel *et al,* 2004).

Moreover, an alternative diagnostic method is to use immunofluorescence microscopy to detect the antibody response to MERS-CoV. This labour- and materials-intensive approach has numerous applications, including genetically modifying and fluorescently staining biological molecules, a routinely feasible approach, detecting difficult, uncultured microbes, investigating outbreaks of respiratory disease, detecting antigens in the absence of viral infectivity, examining post-mortem specimens, controlling cross-infection, and diagnosing infectious agents of a distance samples from laboratories (Jenson *et al,* 1998). However, the specificity of this assay is doubtful, due to the cross reactivity between the Betacorona viruses genus, the ability to identify the infection only during the convalescent phase, the temporary nature of fluorescence dye activity, the occurrence of photo-bleaching, the necessity for the chemical fixation of fluorescence fadesAntibody-labeled as well as treatment with detergents to prepare cell membrane permeability (Gardner, 1984).

Cell culture is generally a useful, less technically-demanding method that was effective for isolating a variety of viruses (Leland and Ginocchio, 2007). Notwithstanding, its disadvantages included the time-consuming incubation period and observation of the cytopathic effect (CPE), high cost, need for substantially skilled, experienced staff for the interpretations, and the high risk of viral contamination and danger of viral transmission (Hematian *et al,* 2016)

Another approach is ELISA, the greatest advantage of which is that it provides results faster than any of the previous techniques and utilized not only to quantify antibodies and discriminate between the early and end phases but also to detect antigens (WordPress, 2016). ELISA is a widely-distributed technique in developing and developed countries, and has been used as a sensitive and specific serological diagnosis approach for routinely detecting and monitoring several microbiological organisms in different medical fields (Izumi *et al,* 1991; WordPress, 2016). Also, due to being more cost effective, not requiring any skilled personnel and being easy to use, apply and prepare for large population screening, it is favored over other alternative methods (Balsam *et al,* 2013). Despite the benefits of using ELISA methods, they suffered from several drawbacks, including: the immobilization of antigens is not specific, cross-reactivity, time-consuming and less flexibility because of using a secondary antibody as well as the need for an inimitably conjugated primary antibody, respectively, to detect each protein of interest (Koenig, 1981; Oswald, 2014).

To sum up the technical aspects of MERS-CoV diagnosis at present, real-time reverse-transcription polymerase chain reaction (RT-PCR) assays are suitable for the quantitative and qualitative detection of the new infectious agents, (Corman *et al,* 2012b) because the quantitative technique can help to evaluate the height and period duration of the virus secretion, and can also be functional as an early, robust parameter for monitoring treatment outcomes, its benefits, and understanding the viral pathogenesis (Peiris *et al,* 2003; Corman *et al,* 2012b). Nucleic acid amplification methods also offer certain advantages, including rapid identification and clear differentiation between coronavirus sp. and disease levels (Sampath *et al,* 2005). PCR technique provides results very rapidly (within 60 minutes), which facilitated coronavirus monitoring and enabled avoidance of its clinical complications and pathogen distribution (Vos *et al,* 1995; Van de Pol *et al,* 2006). The method can be utilized to examine a high number of several types of specimens in a single run that decreased time required for viral detection and analysis (Cupić *et al.,* 2006). Additional advantages of PCR include its higher sensitivity and specificity over conventional techniques, its ability to identify and detect difficult and uncultured viral and atypical pathogens, and its greater cost-effectiveness (Gruteke *et al,* 2004; Syrmis *et al,* 2004). Finally, without cross-reactivity is in a well-designed PCR with consequence of 100% primer sensitivity and specificity that led to reliable, accurate diagnoses (Thai *et al,* 2004).

Treatment and Vaccination: Two main clinical complications followed MERS-CoV (respiratory & renal atrophy), inspiring physicians, pharmacists and researchers to seek a drug treatment that is capable of eradicating the viral infection. Although no effective antiviral agents are suggested for treating patients with MERS-CoV infection, primarily supportive medicine, focusing on supporting the organs and reducing the virus transmission, is considered the current clinical management for MERS-CoV disease (De Wilde *et al,* 2013). The use of extracorporeal membrane oxygenation (ECMO) is recommended due to its characteristic ability to reduce fertility rates during acute respiratory failure (Zampieri *et al,* 2013). For renal atrophy, however, the use of continuous veno-venous hemofiltration as well as a reduction in immunosuppressant drugs was thought to increase infected life expectancy (Villa *et al,* 2017). For secondary bacterial infection and other viral agents, beta-lactam broad-spectrum, such as Amoxicillin and Ampicillin, and antivirals against influenza infection, mainly zanamivir and ribavirin, have been endorsed (Akers *et al,* 2017; Bakaletz, 2017). Several potential therapeutic agents were assessed for targeting and curing MERS-CoV infection (Tab.3).

These candidate drugs' activities promised to reduce or suppress viral replication, cytopathic effect formation, viral genomic transcription and translation, and viral progeny release, and so alleviate the symptoms and complications associated with MERS-CoV disease (De Wilde *et al,* 2013; Giere *et al,* 2013). Although various drug measures and control procedures have been followed to prevent/reduce the virus' effectiveness and dissemination, vaccinations remain the most powerful way to cure the disease (Hajj Hussein *et al,* 2015). Hence, numerous mechanisms to develop effective vaccines to eradicate viral respiratory disease are ongoing, these mechanisms include neutralizing the antibody pathway, signaling processes, inducing protective immunity and stimulating immune response cells (Loutfy *et al,* 2003; Pulendran *et al,* 2011).

Table 3: Therapeutic agents evaluated as promising drugs and vaccines candidates for opposing MERS-CoV infection (Doulkeridou, 2013).

Drug	Mechanisms	Study
INF-α	Reduction of MERS-CoV replication in pseudo-stratified HAE cultures	Kindler *et al.* (2013)
pegylated IFN-α	Inhibition of MERS-CoV-induced CPE and reduction of viral RNA levels in human lung epithelial and monkey kidney cell lines	De Wilde *et al.* (2013)
INF-β	Reduction of viral load in MERS-CoV-infected human lung epithelial and monkey kidney cell lines	Zielecki *et al.* (2013)
INF-λ3	Reduction of MERS-CoV replication in pseudo-stratified HAE cultures	Kindler *et al.* (2013)
INF-α2b	Reduction of MERS-CoV-induced CPE & viral protein levels in monkey kidney cell lines (more efficient combined with Ribavirin)	Falzarano *et al.* (2013)
Ribavirin	Reduction of MERS-CoV-induced CPE & viral protein levels in monkey kidney cell lines (more efficient combined with INF-α2b)	Falzarano *et al.* (2013)
Corticosteroids	Significant improvement in respiratory condition of a MERS-CoV patient (no direct effect proved)	Guberina *et al.* (2014)
Cyclosporin A	Inhibition of MERS-CoV-induced CPE in monkey kidney & human liver cell lines	De Wilde *et al.* (2013)
SB203580	Reduction of viral load in a human lung epithelial cell line	Josset *et al.* (2013)
ADS-J1	Inhibition of MERS-CoV pseudo-virus infection in human liver & mink lung cell lines	Zhao *et al.* (2013)
HP-HAS	Inhibition of MERS-CoV pseudo-virus infection in human liver & mink lung cell lines	Zhao *et al.* (2013)
MDL28170	Inhibition of MERS-CoV-S-mediated transduction of a human fetal lung fibroblast cell line	Gierer *et al.* (2013)
NH_4Cl	Inhibition of MERS-CoV-S-mediated transduction of a human fetal lung fibroblast cell line	Burkard *et al.* (2014)
Camostat	Inhibition of MERS-CoV-S-mediated transduction of a human colon cell line	Gierer *et al.* (2013)
N3	Inhibition of proteolytic activity of MERS-CoV 3CLpro	Ren *et al.* (2013)
CE-10	Inhibition of proteolytic activity of MERS-CoV 3CLpro	Kilianski *et al.* (2013)
MERS-CoV RBD	Reduction of viral load in a MERS-CoV-infected monkey kidney cell line	Chen *et al.* (2013)
Chloroquine/ Hydrocholoroquine	Prevent binding to receptor Endosome	Savarino *et al,* (2003)
Lopinavir/ Ritonavir/ Nelfinavir	Inhibition of viral replication	Groneberg *et al,* (2005)
Interferon alfacon-1	Counteracting antiviral signaling by altering intracellular environment to restrict viral replication	Loutfy *et al,* (2003)
MERS-CoV S protein, Novavax	Inducing virus neutralizing antibodies (NAbs)	Excler *et al,* (2016)

Prevention and control: Infection prevention and control are considered essential ways of preventing the transmitting of infection among patients, health care workers, visitors, accompaniers, livestock, shepherd and slaughterhouses workers. Also, preparing health care facilities for early identification, avoiding antimicrobial dissemination or resistance and minimizing the negative impact of infectious agents are other essential aspects of disease prevention. Recommendations for disease prevention and control for patients with suspected or confirmed MERS-CoV infection in outbreaks have been implemented by the World Health Organization (WHO, 2017c). According to the WHO, while caring for patients with acute or chronic respiratory tract infection, the standard and droplet precautions must be followed to prevent viral transmission (WHO, 2017c). Consequently, several procedures in hospitals to avoid the spread of infectious agents include airborne and contact precautions, eye protection, medical masks, negative pressure rooms, hand hygiene, and remaining at least 1 metre away from the patient when controlling and managing probable and identified MERS-CoV cases as well as raising public awareness of MERS-CoV (WHO, 2017c).

WHO recommend that patients who are recovering from MERS-CoV infection and did not require hospitalization, isolated and cared at their home (WHO, 2017c). Besides, several risk factors associated with MERS-CoV must be considered, mainly avoiding camel contact, particularly dromedary camels, during two weeks before symptoms onset were recommended by WHO. Other recommendations included avoiding contact with animals such as bats and goats, following good hand hygiene through frequent hand-washing before and after touching animals, protective clothing and washing soiled clothing, plastic gloves, shoes and other items. Avoiding contact with camel urine or drinking raw camel milk, and only eating camel meat that has been cooked thoroughly. Eating food that may be contaminated with animal secretions or products should be avoided unless these have been appropriately cleaned, peeled or cooked (WHO, 2014). Also, heightening bio-security measures at farms, sharing information around camels, probable introduction of certificate/passports for racing camels and trade, engagement of private sector, e.g. breeding enterprises, meat packing operations and racing associations and finally closer coordination to avoid risks are compulsory for controlling of virus infections and diseases (Younan *et al,* 2016). Likewise, travelling to endemic countries must be avoided to reduce virus distribution and risk of infection becoming pandemic (Al-Tawfiq *et al,* 2014).

Future perspectives: MERS-CoV is a recently-discovered virus, about which further research and investigation is required. The environmental reservoir of MERS has been unclear for some time. It appears to have been transmitted from bats, camels and goats, but its precise origin remains unclear, as are the environmental factors are foster its sustained growth. So, the mode of transmission and risk factors among humans, camel herds and other animals such as bats remain questionable and require further research to decrease the global health threat posed by MERS-CoV. Also, scientific research must be conducted to uncover the exact modes of transmission in health-care settings. To date, older males constitute the majority of patients who have been reported as having MERS-CoV. Although MERS-CoV infection in humans has numerous risk factors, they are still not completely understood and require further investigation. The incubation period is a maximum of two weeks, but the delivery of the virus during the infectious stage remains unknown. To date, our understanding of the MERS-CoV pathogenesis remains unclear due to the lack of surgical and pathological details about the infected people. Actual mechanism and receptors binding MERS-CoV, humans and other susceptible hosts require further investigation. In spite of the ability of RT-PCR to detect and quantify MERS-CoV, this is not an optimal approach because of its high cost and considerable laboratory equipment demand. Nowadays, effective treatments or vaccines against MERS-CoV are not available, thus, more research are demanded.

References

Akers, IE, Weber, R, Sax, H, Böni, J, Trkola, A, *et al,* 2017: Influence of time to diagnosis of severe influenza on antibiotic use, length of stay, isolation precautions and mortality: a retrospective study. Influenza Other Respir. Viruses 11, 4:337-44.

Al-Abdallat, MM, Payne, DC, Alqasrawi, S, Rha, B, Tohme, RA, *et al,* 2014: Hospital-associated outbreak of Middle East respiratory syndrome coronavirus: a serologic, epidemiologic, and clinical description. Clin. Infect. Dis. 359, 9:1225-33.

Al-Tawfiq, JA, Zumla, A, Memish, ZA, 2014: Travel implications of emerging coronaviruses: SARS and MERS-CoV. Travel Med. Infect. Dis. 12, 5:422-8.

Anand, K, Ziebuhr, J, Wadhwani, P, Mesters, JR, Hilgenfeld, R, 2003: Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science 300, 5626:1763-7.

Arabi, YM, Arifi, AA, Balkhy, HH, Najm, H, Aldawood, AS, *et al,* 2014: Clinical course and outcomes of critically ill patients with Middle East respiratory syndrome coronavirus infection. Ann. Int. Med.160, 6:389-97.

Arabi, YM, Harthi, A, Hussein, J, Bouchama, A, Johani, S, *et al,* 2015: Severe neurologic syndrome associated with Middle East respiratory syndrome coronavirus (MERS-CoV). Infection 43, 4: 495-501.

Assiri, A, Al-Tawfiq, JA, Al-Rabeeah, AA, Al-Rabiah, FA, Al-Hajjar, S, *et al,* 2013: Epidemio-logical, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. The Lancet Infect. Dis. 13, 9:752-61.

Azhar, EI, El-Kafrawy, SA, Farraj, SA, Hassan, AM, Al-Saeed, MS, *et al,* 2014: Evidence

for camel-to-human transmission of MERS coronavirus. N. Engl. J. Med. 370, 26:2499-505.
Bakaletz, LO, 2017: Viral–bacterial co-infections in the respiratory tract. Curr. Opin. Microbiol. 35:30-5.
Balsam, J, Ossandon, M, Bruck, HA, Lubensky, I, Rasooly, A, 2013: Low-cost technologies for medical diagnostics in low-resource settings. Expert Opin. Med. Diagnos.7, 3:243-55.
Biel, SS, Nitsche, A, Kurth, A, Siegert, W, Özel, M, *et al,* 2004: Detection of human polyomaviruses in urine from bone marrow transplant-patients: Comparison of electron microscopy with PCR. Clin. Chemis. 50, 2:306-12.
Burkard, C, Verheije, MH, Wicht, O, van Kasteren, SI, van Kuppeveld, FJ, 2014: Coronavirus cell entry occurs through the endo. Lysosomal Pathway 10, 11:e1004502-17
Chen, Y, Rajashankar, KR, Yang, Y, Agnihothram, SS, Liu, C, *et al,* 2013: Crystal structure of the receptor-binding domain from newly emerged Middle East respiratory syndrome coronavirus. J. Virol. 87, 19:10777-83.
Chu, DK, Poon, LL, Gomaa, MM, Shehata, MM, Perera, RA, *et al,* 2014: MERS coronaviruses in dromedary camels, Egypt. Emerg. Infect. Dis. 20, 6:104-8.
Corman, V, Müller, M, Costabel, U, Timm, J, Binger, T, *et al,* 2012a: Assays for laboratory confirmation of novel human coronavirus (hCoV-EMC) infections. Eurosurveillance 17, 49: 20334-8.
Corman, V, Eckerle, I, Bleicker, T, Zaki, A, Landt, O, *et al,* 2012b: Detection of a novel human coronavirus by real-time reverse-transcription polymerase chain reaction. Eurosurveillance 17, 39:1-6.
Cotten, M, Watson, SJ, Kellam, P, Al-Rabeeah, AA, Makhdoom, HQ, *et al,* 2013: Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: A descriptive genomic study. Lancet 382, 9909: 1993-2002.
Cupić, M, Lazarević, I, Knezević, A, Stanojević, M, 2006: Conventional and molecular methods in diagnosis and monitoring of viral infection. Serbian Arch. Med. 135, 9/10:589-93.
De Groot, RJ, Baker, SC, Baric, RS, Brown, CS, Drosten, C, *et al,* 2013: Middle East respiratory syndrome coronavirus (MERS-CoV): Announcement of the coronavirus study group. J. Virol. 87, 14:7790-2.
Delrue, L, Gosselin, R, Ilsen, B, Van Landeghem, A, de Mey, J, *et al,* 2011: Difficulties in the interpretation of chest radiography. In: Comparative Interpretation of CT and Standard Radiography of the Chest.
De Wilde, AH, Raj, VS, Oudshoorn, D, Bestebroer, TM, van Nieuwkoop, S, *et al,* 2013: MERS-coronavirus replication Induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon α treatment. J. Gen. Virol. 94, 8:1749-60.
Doulkeridou, S, 2013: Middle East Respiratory Syndrome coronavirus (MERS-CoV): A review, Utrecht: Utrecht University..
Drosten, C, Günther, S, Preiser, W, Van Der Werf, S, Brodt, HR, *et al,* 2003: Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N. Engl. J. Med. 348, 20:1967-76.
Drosten, C, Seilmaier, M, Corman, VM, Hartmann, W, Scheible, G, *et al,* 2013: Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection. The Lancet Infect. Dis. 13, 9:745-51.
Drosten, C, Kellam, P, Memish, ZA, 2014: Evidence for camel-to-human transmission of MERS coronavirus. N. Engl. J. Med. 371, 14:2-9.
Drosten, C, Muth, D, Corman, VM, Hussain, R, Al Masri, M, *et al,* 2015: An observational, laboratory-based study of outbreaks of Middle East respiratory syndrome coronavirus in Jeddah and Riyadh, Kingdom of Saudi Arabia, 2014. Clin. Infect. Dis. 60, 3:369-77.
Excler, JL, Delvecchio, CJ, Wiley, RE, Williams, M, Yoon, IK, *et al,* 2016: Toward developing a preventive MERS-CoV vaccine: Report from a Workshop Organized by the Saudi Arabia Ministry of Health and the International Vaccine Institute, Riyadh, Saudi Arabia, November 14-15, 2015. Emerg. Infect. Dis. 22, 8: e160229.
Falzarano, D, de Wit, E, Martellaro, C, Callison, J, Munster, VJ, *et al,* 2013: Inhibition of novel β coronavirus replication by a combination of interferon-α2b and ribavirin. Scientific Reports 3:1686.
Gardner, PS, 1984: Viral diagnosis by immunofluorescence. In: Recent Advances in Virus Diagnosis. Springer; Netherlands.
Gierer, S, Bertram, S. Kaup, F, Wrensch, F, Heurich, A, *et al,* 2013: The spike protein of the emerging betacoronavirus EMC uses a novel coronavirus receptor for entry, can be activated by TMPRSS2, and is targeted by neutralizing anti-

bodies. J. Virol. 87, 10:5502-11.
Groneberg, DA, Poutanen, SM, Low, DE, Lode, H, Welte, T, *et al*, 2005: Treatment and vaccines for severe acute respiratory syndrome. The Lancet Infect. Dis. 5, 3:147-55.
Gruteke, P, Glas, AS, Dierdorp, M, Vreede, WB, Pilon, JW, *et al*, 2004: Practical implementation of a multiplex PCR for acute respiratory tract infections in children. J. Clin. Microbiol. 42, 12:5596-603.
Guan, Y, Zheng, BJ, He, YQ, Liu, XL, Zhuang, ZX, *et al*, 2003: Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 302, 5643:276-8**.**
Guberina, H, Witzke, O, Timm, J, Dittmer, U, Müller, MA, *et al*, 2014: A patient with severe respiratory failure caused by novel human coronavirus. Infection 42, 1:203-26.
Guery, B, Poissy, J, el Mansouf, L, Séjourné, C, Ettahar, N, *et al*, 2013: Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. Lancet 381, 9885:2265-72.
Hajj Hussein, I, Chams, N, Chams, S, El Sayegh, S, Badran, R, *et al*, 2015: Vaccines through centuries: major cornerstones of global health. Frontiers Publ. Hlth. 3:269-74.
Hazelton, PR, Gelderblom, HR, 2003: Electron microscopy for rapid diagnosis of infectious agents in emergent situations.1-Synopsis. Emerg. Infect. Dis. 9, 3:294-304.
Hematian, A, Sadeghifard, N, Mohebi, A, Taherikalani, M, Nasrolahi, A, *et al*, 2016: Traditional and modern cell culture in virus diagnosis. Osong Publ. Hlth. Res. Perspect. 7, 2:77-82.
IDSA, 2014: MERS clinical update from the IDSA. Available: http://www.magnetmail.net/actions/ email_web_version.cfm?recipient_ id=124961864&message_id=4470910&user_id=IDSociety&group_id=1191715&jobid=18802511.
Izumi, Y, Fukazawa, T, Sugiyama, Y, Yagami, KI, Urano, T, *et al*, 1991: The advantage of enzyme-linked immunosorbent assay (ELISA) as a method of microbiological monitoring for rat virus (RV). Exp. Anim. 40, 3:367-3.
Jenson, HB, Grant, GM, Ench, Y, Heard, P, Thomas, CA, *et al*, 1998: Immunofluorescence microscopy and flow cytometry characterization of chemical induction of latent Epstein-Barr virus. Clin. Diagnos. Laborat. Immunol. 5, 1:91-7.
Johnson, KM, Lange, JV, Webb, PA, Murphy, FA, 1977: Isolation and partial characterisation of a new virus causing acute haemorrhagic fever in Zaire. Lancet 309, 8011:569-571.
Josset, L, Menachery, VD, Gralinski, LE, Agnihothram, S, Sova, P, *et al*, 2013: Cell host response to infection with novel human coronavirus EMC predicts potential antivirals and important differences with SARS coronavirus. MBio. 4, 3:e00165-13.
Khalil, HM, Kandeel, HZ, Morsy, AT, 2013: Severe acute respiratory syndrome (SARS) coronavirus: possible re-emergence of the Asian-global novel threat. J. Egypt. Soc. Parasitol. 43, 2: 555-60.
Kilianski, A, Mielech, A, Deng, X, Baker, SC, 2013: Assessing activity and inhibition of rMERS-CoV papain-like and 3C-like proteases using luciferase-based biosensors. J. Virol. 10: 02105-13.
Kindler, E, Jónsdóttir, HR, Muth, D, Hamming, OJ, Hartmann, R, *et al*, 2013: Efficient replication of the novel human betacoronavirus EMC on primary human epithelium highlights its zoonotic potential. MBio, 4, 1:e00611-12.
Koenig, R, 1981: Indirect ELISA methods for the broad specificity detection of plant viruses. J. Gen. Virol. 55, 1:53-62.
Kraaij-Dirkzwager, M, Timen, A, Dirksen, K, Gelinck, L, Leyten, EM, *et al*, 2014: Middle East respiratory syndrome coronavirus (MERS-CoV) infections in two returning travellers in the Netherlands. Eurosurveillance 19, 21:1-6.
Lau, SK, Li, KS, Huang, Y, Shek, CT, Tse, H, *et al*, 2010: Ecoepidemiology and complete genome comparison of different strains of severe acute respiratory syndrome-related *Rhinolophus* bat coronavirus in China reveal bats as a reservoir for acute, self-limiting infection that allows recombination events. J. Virol. 84, 6:2808-12.
Lee, J, 2015: Better understanding on MERS corona virus outbreak in Korea. J. Korean Med. Sci. 30, 7:835-6.
Leland, DS, Ginocchio, CC, 2007: Role of cell culture for virus detection in the age of technology. Clin. Microbiol. Rev. 20, 1:49-78.
Loutfy, MR, Blatt, LM, Siminovitch, K, Ward, S, Wolff, B, *et al*, 2003: Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. JAMA 290, 24: 3222-8.
Memish, ZA, Almasri, M, Turkestani, A, Al-Shangiti, AM, Yezli, S, 2014: Etiology of severe community-acquired pneumonia during the

2013 Hajj-part of the MERS-CoV surveillance program. Int. J. Infect. Dis. 25:186-90.

Miguel, E, Chevalier, V, Ayelet, G, Bencheikh, MNB, Boussini, H, *et al*, 2015: Risk factors for MERS coronavirus infection in dromedary camels in Burkina Faso, Ethiopia, and Morocco, 2015. Eurosurveillance 22, 13:1-10.

Oswald, N, 2014: Let me introduce you to ELISA. No, not the girl. The assay. Available at: http://bitesizebio.com/19584/let-me-introduce-you-to-elisa-no-not-the-girl-the-assay.

Peiris, JSM, Chu, CM, Cheng, VCC, Chan, K S, Hung, IFN, *et al*, 2003: Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet, 361, 9371:1767-72.

ProMED-mail, 2012: Novel coronavirus-Saudi Arabia: human isolate, 20 September Available: at<http://www.promedmail. org/ direct.php ?id= 20120920.1302733[Accessed1/3 2017).

Pulendran, B, Ahmed, R, 2011: Immunological mechanisms of vaccination. Nature Immunol. 12, 6:509-17.

Raj, VS, Mou, H, Smits, SL, Dekkers, DH, Müller, MA, *et al*, 2013: Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature 495, 7440: 251-4.

Ren, Z, Yan, L, Zhang, N, Guo, Y, Yang, C, *et al*, 2013: The newly emerged SARS-like coronavirus HCoV-EMC also has an" Achilles' heel": current effective inhibitor targeting a 3C-like protease. Protein Cell 4, 4:248-52.

Sampath, R, Hofstadler, SA, Blyn, LB, Eshoo, MW, Hall, TA, *et al*, 2005: Rapid identification of emerging pathogens: Coronavirus. Emerg. Infect. Dis. 11, 3:373-9.

Savarino, A, Boelaert, JR, Cassone, A, Majori, G, Cauda, R, 2003: Effects of chloroquine on viral infections: an old drug against today's diseases. The Lancet Infect. Dis. 3, 11:722-7.

Siddell, SG, Anderson, R, Cavanagh, D, Fujiwara, K, Klenk, HD, *et al*, 1983: Coronaviridae. Intervirology 20, 4:181-9.

Syrmis, MW, Whiley, DM, Thomas, M, Mackay, IM, Williamson, J, *et al*, 2004: A sensitive, specific, and cost-effective multiplex reverse transcriptase-PCR assay for the detection of seven common respiratory viruses in respiratory samples. J, Molecul. diagnos. 6, 2:125-131.

Thai, HTC, Le, MQ, Vuong, CD, Parida, M, Mnekawa, H, *et al*, 2004: Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. J. Clin. Microbiol.42, 5:1956-61.

Van de Pol, AC, Wolfs, TF, Jansen, NJ, van Loon, AM, Rossen, JW, 2006: Diagnostic value of real-time polymerase chain reaction to detect viruses in young children admitted to the paediatric intensive care unit with lower respiratory tract infection. Crit. Care 10, 2:1-7.

Villa, G, Chelazzi, C, Morettini, E, Zamidei, L, Valente, S, *et al*, 2017: Organ dysfunction during continuous veno-venous high cut-off hemodialysis in patients with septic acute kidney injury: A prospective observational study. PloS one 12, 2:e0172039.

Vos, P, Hogers, R, Bleeker, M, Reijans, M, Van de Lee, T, *et al*, 1995: AFLP: a new technique for DNA fingerprinting. Nucl. Acids Res. 23, 21:4407-14.

Woo, PC, Lau, SK, Li, KS, Poon, RW, Wong, BH, *et al*, 2006: Molecular diversity of coronaviruses in bats. Virology 351, 1:180-7.

WHO, 2003: Consensus document on epidemiology of severe acute respiratory syndrome (SARS). Available: http://www.who.int/csr/sars/en/WHOconsensus.pdf. [Accessed 29/5/2014].

WHO, 2014: Middle East Respiratory Syndrome Coronavirus (MERS-CoV)-13th June 2014. Update on MERS-CoV Transmission from Animals to Humans, and Interim Recommendations for at-risk Groups. Available at: http:// www.who.int/csr/disease/coronavirus_infections/MERS_CoV_RA_20140613.pdf?ua=1.

WHO, 2017a: Emergencies preparedness, response, Middle East respiratory syndrome coronavirus (MERS-CoV) - Saudi Arabia. [Online] Available at: http://www.who.int /csr/ don/10-february-2017-mers-saudi-arabia/en//

WHO, 2017b: Middle East respiratory syndrome coronavirus (MERS-CoV). [Online] Available at: http://www.who.int/emergencies/mers-cov/en/. [Accessed 25 6 2017].

WHO, 2017c: Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Summary and Literature. [Online] Available at: from: http:// www. who. int/ csr/disease/corona-virus_ infections/MERS_CoV_Update_09_May_2014. pdf?

WordPress, 2016: Advantages of ELISA test and its Application in Disease Viruses Diagnosttic. Available at: http:// rapidtest. com/blog/tag/ advantages-of-elisa-test/ [Accessed 7 4 2017].

Younan, M, Bornstein, S, Gluecks, IV, 2016: MERS and the dromedary camel trade between Africa and the Middle East. Trop.Anim. Hlth.

Product. 48, 6:1277-82.

Zampieri, FG, Mendes, PV, Ranzani, OT, Taniguchi, LU, Azevedo, LCP, *et al*, 2013: Extracorporeal membrane oxygenation for severe respiratory failure in adult patients: a systematic review and meta-analysis of current evidence. J. Crit. Care 28, 6:998-1005.

Zhao, G, Du, L, Ma, C, Li, Y, Li, L, *et al*, 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, 1:266-9.

Zhou, J, Chu, H, Chan, JF, Yuen, KY, 2015: Middle East respiratory syndrome coronavirus infection: virus-host cell interactions and implications on pathogenesis. Virol. J. 12, 1: 218-21.

Zielecki, F, Weber, M, Eickmann, M, Spiegelberg, L, Zaki, AM, *et al*, 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, 9:5300-4.

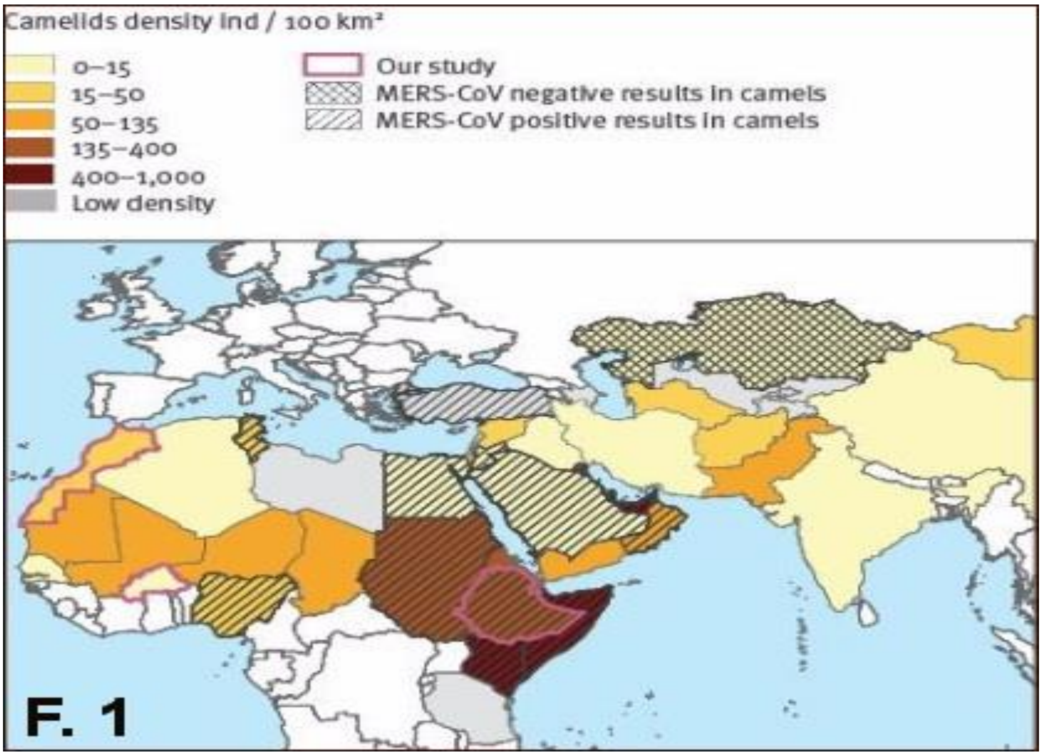

Fig. 1: Worldwide density of camel livestock with/without MERS-CoV (Miguel *et al,* 2015).

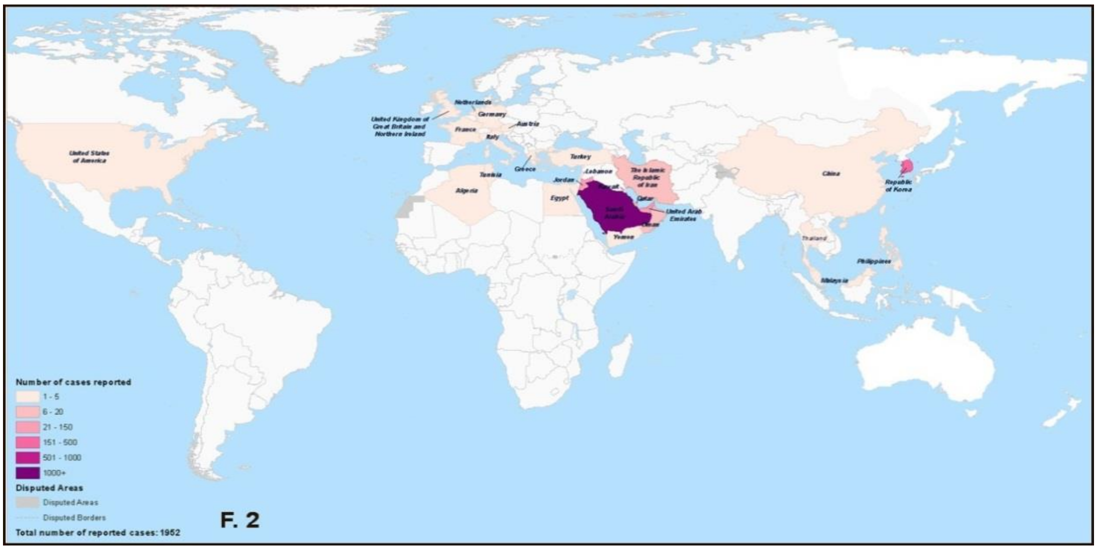

Fig. 2: Confirmed Global Cases of MERS-CoV (2012 - 2017)