Letter to the Editor

Serological Study of An Imported Case of Middle East Respiratory Syndrome and His Close Contacts in China, 2015*

WANG Wen Ling[1,^], WANG Hui Juan[1,^], DENG Yao[1], SONG Tie[2], LAN Jia Ming[1], WU Gui Zhen[1], KE Chang Wen[2], and TAN Wen Jie[1,#]

The first imported Middle East respiratory syndrome (MERS) case in China was identified in May 2015. We determined the kinetics of antibody (IgG and IgM) and neutralizing antibodies against MERS-coronavirus (MERS-CoV) in this case before discharge. Moreover, no seroconversion was found among 53 close contacts by anti-MERS IgG antibody enzyme-linked immunosorbent assay (ELISA) of paired serum samples. These findings suggest that neither community nor nosocomial transmission of MERS-CoV occurred in China.

Case Study

Middle East respiratory syndrome (MERS), caused by the MERS-coronavirus (MERS-CoV), is an emerging respiratory disease of global public health concern with a high mortality rate[1]. On May 29, 2015, a business traveler from the Republic of Korea was identified as the first imported case of MERS-CoV infection in China[2-3]. The World Health Organization (WHO) received notifications of confirmed cases of MERS-CoV infection from the Republic of Korea mid May 2015[4-5]. The WHO was notified later that one close contact of the index MERS case in the Republic of Korea travelled to Guangdong Province, China, by way of Hong Kong. The first MERS-CoV case in China imported from the Republic of Korea was confirmed by The National Health and Family Commission of the People's Republic of China on May 29, 2015[2-3]. The patient was admitted to Huizhou Municipal Central Hospital, Huizhou, Guangdong Province on May 28, 2015 and was discharged after recovery on June 26, 2015. A set of venous blood samples was collected after his admission.

To confirm the specificity of the inactivated MERS-CoV virion-based ELISA and the background of normal sera, anti MERS-CoV IgM and IgG in 10 sera from the newborns and 40 sera from normal healthy adults were detected by ELISA. To evaluate both the serological response of this MERS patient before discharge and the risk of transmission, a set of serum samples from the patient and paired serum samples collected at least 14 d apart from the close contacts of the MERS patient during his trip and hospital admission in China, were tested for MERS-CoV using an inactivated MERS-CoV-based ELISA. Meanwhile to confirm the specificity and sensitivity of inactivated MERS-CoV-based ELISA, a commercially available (EUROIMMUNE AG, Lübeck, Germany) MERS-CoV S1 ELISA was performed as reference. The assay included a calibrator for defining the upper limit of the reference range of non-infected persons (cut-off) recommended by EUROIMMUN. Values above the indicated cut-off are considered as positive, those below as negative. MERS-spike pseudoparticle neutralization test (PPNT) based on Env-defective, a reporter gene of luciferase-expressing HIV-1 genome (pNL4-3R-E-Luc) were performed as previoiusly described[6], neutralizing antibody titers were defined as the highest serum dilutions that resulted at 50% reduction in relative luciferase units.

To strengthen infection control measures and identify possible person-to-person transmission, close contacts of the MERS patient were also sampled. A total of 78 close contacts of the MERS case during his journey were identified and monitored in isolation for 14 d after their last contact with the MERS case, they included hotel staff, company employees, restaurant waiters, bus passengers and plane passengers. Of these close contacts, including those who had face-to-face contact with the patient as well as more distant

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1. Key Laboratory of Medical Virology and Virus Disease, National Health and Family Planning Commission of the People's Republic of China; National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 2. Guangdong Provincial Center for Disease Control and Prevention, Guangzhou 511430, Guangdong, China

contacts, none presented symptoms compatible with MERS, and all throat swab samples from the close contacts were negative for MERS-CoV by real-time RT-PCR[3]. Only 53 paired serum samples were finally available for the close contacts. These paired serum samples from close contacts of the MERS-CoV patient were collected 16 days apart (June 3 and 19, 2015). The blood samples were processed within 24 h of collection, and sera were stored at -80 °C. All serum samples from the close contacts tested negative for MERS-CoV by real time RT-PCR.

Laboratory Findings

We used an inactivated MERS-CoV particle-based ELISA to analyze serum samples from the imported MERS-CoV patient and his close contacts for the presence of IgG against MERS-CoV. All experiments involving live MERS-CoV were conducted according to the standard operating procedure of the biosafety level 3 facilities at the Chinese Center for Disease Control and Prevention.

Normal controls comprised serum samples from 40 healthy blood donors; these were used to determine background values and calculate the cut-off level. Anti MERS-CoV IgM and IgG in the 10 sera from newborns and 40 normal control sera were detected at dilution 1:80 by ELISA. The average OD450 values for the newborns and normal adult controls (x) were calculated to be 0.13 (range 0.05-0.25) and 0.15 (range 0.09-0.27) for IgM, with no difference existing between the two groups (*t*-test, *P*>0.05); and 0.16 (range 0.01-0.28) and 0.20 (range 0.12-0.38) for serum IgG, with no difference existing between the two groups (*t*-test, *P*>0.05) (Figure 1), and the cut-off value was determined to be 2.1-fold x, i.e., 0.32 for IgM and 0.42 for IgG.

Anti-MERS-CoV titers were determined in serial serum samples from the MERS patient. His serum IgM and IgG level on the day following admission (May 29, 2015) was markedly lower than the cut-off value, exceeded the cut-off value 11 d after admission and plateaued 15 days after admission (Figure 2A). The serum IgM and IgG titer increased to 0.62 and 0.77 immediately before discharge (June 24, 2015)(Figure 2A). The IgG titer of the patient shortly after admission were both 1:40, compared with 1:320 (8-fold higher) and 1:640 (16-fold higher) before discharge (data not shown), which demonstrated MERS-CoV seroconversion. MERS S1-based ELISA results showed that the OD450 value for the calibrator recommended by EUROIMMUN was 0.43 which was defined as the cut-off value, consequently, anti-MERS S1IgG in the MERS patient exceeded the cut-off value 8 days after admission (Figure 2B), consistent with the results reported by Guan et al.[7]. Although seroconversion of anti-MERS-CoV S1 IgG appeared two days earlier than that of inactivated MERS-CoV, similar antibody kinetics based on MERS S1 and inactivated MERS-CoV appeared and there was high correlation coeffieient of 0.9331 between them (Figure 2C).

A lentivirus-based MERS-CoV pseudovirus neutralization test was performed to confirm the presence of MERS-CoV-specific antibodies in serum samples from the patient. The neutralizing antibody titer was tested as 1:141 6 days after admission and peaked 15 d after admission (Figure 2D). The results of the neutralization tests correlated well with the ELISA results.

All 53 paired serum samples from the close contacts were below the cut-off value, negative for MERS-CoV by ELISA (Figure 3). The average OD450 values of the serum samples collected on June 3 and 19, 2015 were 0.18 and 0.19, respectively. No seroconversion was detected in the 53 close contacts.

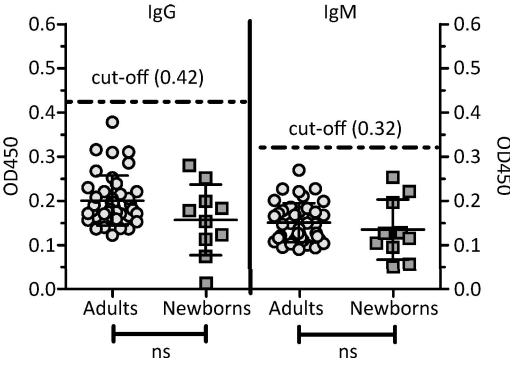

Figure 1. Screening of serum samples from newborns and healthy donors and determination of cut-offs. Serum form 10 newborns and 40 healthy adult donors were diluted 1:80, IgM and IgG were tested by using the inactivated MERS-CoV virion-based ELISA. HRP-labeled goat anti-human IgM or IgG was used as the secondary antibody, with 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate, and the absorbance was determined at 450 nm. The cutoff values were calculated as the mean absorbance readings of the serum samples from 40 blood donors multiply 2.1. *t*-test was used to analyze the difference between the newborn group and healthy adult group (ns, not significant).

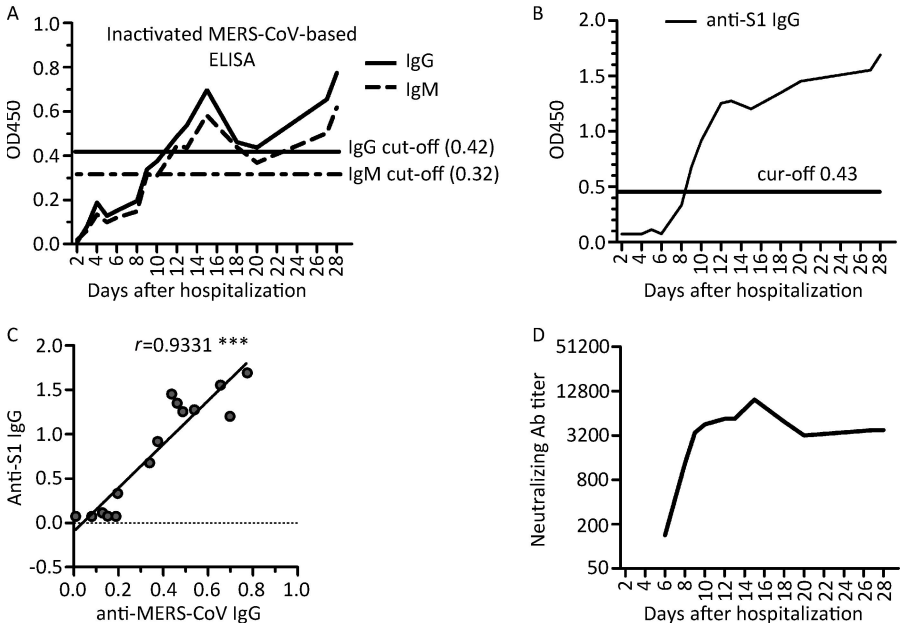

Figure 2. Kinetics of serum MERS-CoV-specific antibodies of the first imported MERS-CoV patient in China. A. ELISA plates were coated with inactivated MERS-CoV particles. Serum samples obtained from the imported MERS-CoV patient after admission were diluted 1:80. HRP-labeled goat anti-human IgM or IgG was used as the secondary antibody, with 3,3',5,5'-tetramethylbenzidine (TMB) as the substrate, and the absorbance was determined at 450 nm. B. MERS-CoV S1 ELISA was performed and a series of sera samples of the imported MERS-CoV in China were detected following the instruction book. C. Correlations between anti-MERS-CoV IgG and anti MERS S1 IgG based on data from Figure 2A and Figure 2B. ***, $P<0.001$. D. Lentivirus-based MERS-CoV pseudovirus was pre-incubated with serially diluted sera from the imported MERS-CoV patient at 37 °C for 1 h before addition to cells. After 24 h of incubation, fresh medium was added to the culture, followed by incubation for a further 48 h. Cells were washed with PBS and lysed using the lysis reagent included in the luciferase kit (Promega). Aliquots of cell lysates were transferred to 96-well Costar flat-bottom luminometer plates (Corning Costar), followed by addition of luciferase substrate (Promega). RFI values were determined immediately using a Gaomax luminometer (Promega). A 50% RFI reduction was used to calculate MERS-CoV-specific neutralizing antibody titers in serum samples.

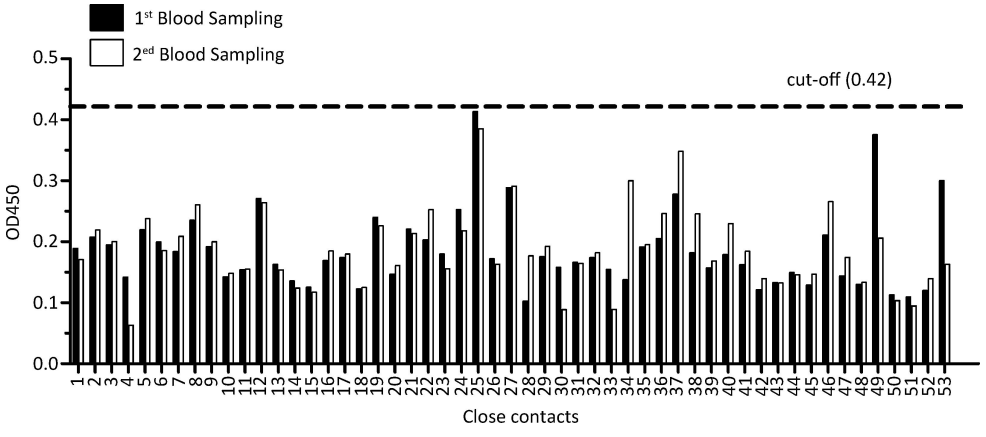

Figure 3. Screening of serum samples from close contacts of the first imported MERS-CoV patient in China. Fifty-three paired serum samples from 53 close contacts that may have been exposed to the imported MERS-CoV patient were collected at an interval of at least 14 d. All of the sera were diluted 1:80 and subjected to inactivated MERS-CoV-based ELISA. HRP-labeled goat anti-human IgG was used as the secondary antibody, with TMB as the substrate, and the absorbance was read at 450 nm. OD450 values higher than the cut-off value were considered to be indicative of MERS-CoV positive.

Discussion

The Korean outbreak showed that MERS-CoV can result in an epidemic despite its lack of apparent mutations[2], and thus it represents a global threat to public health. Many laboratory tests-such as immunofluorescence assays, ELISA, microneutralization assays and real-time RT-PCR assays-have been used to confirm MERS-CoV infection in MERS patients and their close contacts[8]. However, data regarding the dynamics of anti-MERS-CoV IgG and MERS-CoV neutralizing antibodies are lacking. In this study, we developed methods to detect antibodies to MERS-CoV using an inactivated MERS-CoV particle-based indirect enzyme-linked immunosorbent assay (ELISA) and lentivirus-based MERS-CoV pseudovirus assay. The serum anti-MERS-CoV IgG level of the MERS patient immediately improved 16-fold before discharge, demonstrating seroconversion. His serum IgM and IgG level reached a plateau at 15 d after admission. We further determined the kinetics of MERS-CoV neutralizing antibody; the titer was highest (approximately 1:10,000) at 15 d after admission, in agreement with the IgG curve, and then declined gradually to approximately 1:4500 before discharge.

South Korea experienced a MERS outbreak representing the largest cluster of MERS cases outside the Middle East. As of October 31, 2015, 186 cases (including the MERS case exported to China) of MERS-CoV infection have been associated with the South Korea outbreak, 36 of whom died[4-5]. There have been several reports of household or nosocomial transmission of MERS-CoV to healthcare workers in hospitals with MERS-CoV patients and/or delayed implementation of effective infection control practices[5,8]. When the imported MERS-CoV patient in China was admitted on June 28, he was suffering from high fever, and real-time RT-PCR indicated a high viral load in serum and throat swab samples. Seroconversion often occurs 10-14 d after exposure, so we performed serological detection on paired serum samples to minimize the possibility of missing asymptomatic infection. However, none of the 53 close contacts were positive for MERS-CoV, which suggest that none community transmission of MERS-CoV occurred in China. The lack of transmission of MERS-CoV from the patient to the close contacts in this study may have been due to several factors: 1) the short duration of exposure, and more importantly, 2) implementation by China of strict infection prevention and control practices immediately after being notified of importation of the MERS-CoV patient. These findings are similar to the results of an investigation of healthcare workers following importation of the first MERS case into the USA[9].

In conclusion, we first determined the kinetics of antibody (IgG and IgM) and neutralizing antibodies against MERS-CoV for the first imported MERS case in China. The data from the close contacts investigation indicated that the ability is limited for human-to-human transmission of MERS-CoV. However, every year approximately 10,000 residents of China travel to and return from Mecca, Saudi Arabia for the Ramadan period, so travel-associated importation and transmission of MERS-CoV remains a possibility. To prevent MERS-CoV infection, early screening and routine supervision are required, especially for confirmed and suspected MERS cases and close contacts thereof[10-11].

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Conflict of interest None declared

Authors' contributions TAN Wen Jie designed the experiment. WANG Wen Ling, WANG Hui Juan, DENG Yao, LU Rou Jian, and LAN Jia Ming perfomed the detection. SONG Tie and KE Chang Wen provided the information and samples from the imported MERS case in China and close contacts. WU Gui Zhen coordinated matters regarding samples and information and guaranteed the BSL-3 facilities. WANG Wen Ling and TAN Wen Jie analysed the results and drafted the manuscript.

^WANG Wen Ling and WANG Hui Juan contributed equally to this work.

#Corresponding should be addressed to TAN Wen Jie, MD, PhD, E-mail: tanwj28@163.com

Biographical notes of the first authors: WANG Wen Ling, female, born in 1973, PhD, Researcher, majoring in pathogen biology; WANG Hui Juan, female, born in 1972, Bachelor, Associate Chief Technician, majoring in pathogen biology.

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