Letter to the Editor

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

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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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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.

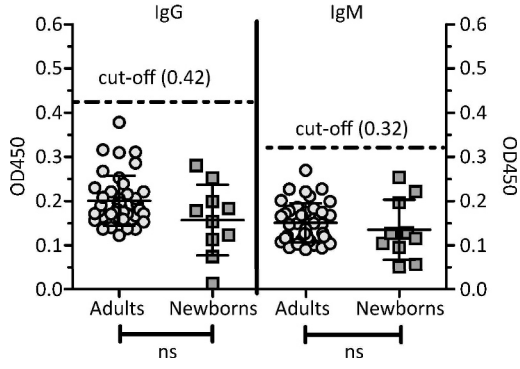

Figure 1. Screening of serum samples from newborns and healthy donors and determination of cut-offs. Serum form 10 newborns and 40 healthy adult donors were diluted 1:80, IgM and IgG were tested by using the inactivated MERS-CoV virion-based ELISA. HRP-labeled goat anti-human IgM or IgG was used as the 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).