Investigation of Anti–Middle East Respiratory Syndrome Antibodies in Blood Donors and Slaughterhouse Workers in Jeddah and Makkah, Saudi Arabia, Fall 2012

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(See the editorial commentary by Hui and Zumla on pages 173–6, and the major article by Yao et al on pages 236–42.)

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel, potentially zoonotic human coronavirus (HCoV). We investigated MERS-CoV antibodies using a staged approach involving an immunofluorescence assay (IFA), a differential recombinant IFA, and a plaque-reduction serum neutralization assay. In 130 blood donors sampled during 2012 in Jeddah and 226 slaughterhouse workers sampled in October 2012 in Jeddah and Makkah, Saudi Arabia, 8 reactive sera were seen in IFA but were resolved to be specific for established HCoVs by discriminative testing. There is no evidence that MERS-CoV circulated widely in the study region in fall 2012, matching an apparent absence of exported disease during the 2012 Hajj.

Keywords. MERS-Coronavirus; serology; population immunity.

Middle East respiratory syndrome coronavirus (MERS-CoV) is a novel human coronavirus (HCoV) causing cases and case clusters of severe acute respiratory syndrome in countries of the Arabian Peninsula since at least April 2012 [1]. Exported infections have been reported the United Kingdom, Germany, France, Italy, and Tunisia. A total of 102 laboratory-confirmed cases have been reported to date, with 49 fatalities. It is unknown whether the virus enters the human population from a zoonotic source, with ensuing local transmission chains or, alternatively, whether it circulates continuously in humans [2]. Recent analyses based on molecular clock assumptions in phylogeny have estimated that the most recent common ancestor of all presently known viruses existed during mid-2011 [3]. It remains unknown whether this ancestor continues to exist in animals and/or humans and whether the MERS-CoV responsible for the cases identified thus far spreads among humans only. A recent study has provided evidence for virus-neutralizing antibodies in dromedary camels [4]. However, studies on the distribution of virus in either humans or animals have not been performed to date.

In view of the uncertain epidemiology, cross-sectional investigations of existing antibodies in human populations are of interest [2]. Detection of antibodies in large parts of the population would suggest widespread and long-standing circulation of MERS-CoV. In contrast, the absence of antibodies would suggest that large portions of the population are susceptible to infection, increasing the risk of an epidemic. Finally, comparisons of antibody prevalences among humans exposed versus those not exposed to livestock could provide clues to potential sources of zoonotic infection.

The testing of human populations for HCoV antibodies is highly demanding from a technical perspective, because titers are generally low and there is cross-reactivity between HCoVs within and beyond viral genera. In particular, there is only very limited information on the cross-reactivity of antibodies against any of the known HCoVs (HCoV 229E, NL63, OC43, and HKU1) with antibodies against MERS-CoV [5–7]. Because of these uncertainties, we have recently proposed a staged approach for MERS-CoV serology consisting of first-line screening by an immunofluorescence assay (IFA); evaluation with a discriminative recombinant IFA, using recombinant spike proteins from each of the established HCoVs; and then plaque-reduction neutralization tests to confirm specific reactivity against MERS-CoV [6, 8]. Using these 3 methods, we investigate 356 human serum specimens, including 226 from slaughterhouse employees, in Jeddah and Makkah, Saudi Arabia, where one of the first human cases of MERS-CoV infection was diagnosed [9].

METHODS

IFA using full MERS-CoV was performed with a commercially available test kit (Anti-MERS-CoV IIFT, EUROIMMUN AG,

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Lübeck, Germany) exactly as described elsewhere [8]. Discriminatory recombinant IFA followed a described protocol [8] and was extended for the purpose of this study to include full spike proteins (the complete S open reading frame) of HCoV 229E, NL63, OC43, HKU1, and MERS-CoV expressed from pCG1 eukaryotic expression vectors.

For serum neutralization tests, Vero B4 cells were grown to subconfluence in 24-well plates. Preincubation reactions contained 25 plaque-forming units of MERS-CoV (EMC strain) in 100 μL of medium, mixed 1:1 with patients' serum specimens prediluted in medium. The starting dilution was 1:10. After 1 hour of incubation at 37°C, each well was infected for 1 hour at 37°C, using the total 200-μL preincubation reaction. Supernatants were removed and overlaid with Avicell resin exactly as described by Herzog et al [10]. Assays were terminated and stained by immersion in formaldehyde/crystal violet solution after 3 days, thereby inactivating the test virus [10]. Neutralization titers were defined as the serum dilution reducing the number of plaques in 4 parallel wells in summary by >90%.

All enrolled slaughterhouse workers gave blood voluntarily and provided written informed consent to have their blood samples tested for MERS-CoV antibodies.

RESULTS

For a first and orienting assessment of antibodies in the local population, an anonymized panel of serum specimens from 130 healthy blood donors was assembled. These individuals had been submitted for blood donor eligibility screening at King Abdulaziz University Hospital, Jeddah, between January and December 2012. Samples were tested at a predilution of 1:40 by IFA as described elsewhere [8], yielding no specific fluorescence signal in any case. Of note, in a previous study, IFAs of serum specimens from blood donors did not cross-react at this dilution [8].

Because of reports on potential exposures with livestock animals in MERS cases, workers from 2 slaughterhouses in Jeddah and 3 slaughterhouses in Makkah were investigated in October 2012. Slaughtered animals included cattle, camels, and sheep. The workers were aged 16–61 years, had worked in slaughterhouses for intervals ranging from 2 months to 36 years, and were from various countries, including Bangladesh (n = 74), Burkina Faso (n = 1), Egypt (n = 24), India (n = 9), Mali (n = 12), Niger (n = 10), Pakistan (n = 21), Saudi Arabia (n = 9), Sudan (n = 10), and Yemen (n = 58). Workers had lived in Saudi Arabia for durations ranging from 3 months to 36 years. Upon initial screening using IFA slides containing cells infected with full MERS-CoV, 8 of 226 serum specimens yielded specific IFA patterns. There were no apparent differences in detection rates between individual study sites (Table 1).

All 8 samples were further investigated using discriminative recombinant IFA. As summarized in Table 2, all 8 specimens showed dominant titers against recombinant spike proteins of HCoVs other than MERS-CoV. Only 2 of the 8 specimens reacted with MERS-CoV. In contrast, specimens from 2 subjects with laboratory-confirmed MERS showed clear reactivity with recombinant MERS-CoV spike proteins. These patients' specimens had concomitant reactivity against spike proteins from other HCoVs, as well, which was to be expected because of the recency of MERS-CoV infection in these patients, involving low-avidity antibodies (both patients were anti–MERS-CoV immunoglobulin M positive) [3].

All 8 samples were additionally tested for the presence of neutralizing antibodies. To detect serum neutralization, serum specimens were incubated with 25 plaque-forming units of MERS-CoV strain EMC/2012 for 1 hour before they were adsorbed onto Vero cells for another 1 hour. Cells were then overlaid with Avicell resin and incubated at 37°C for 3 days. Serum dilutions affecting >90% reduction of the cumulative number of resulting plaques in 4 replicate assays were recorded as serum neutralization titers (NT_{90}). Because serum dilutions of 1:5 appeared as NT_{90} in a small fraction of blood samples from healthy German adults who did not have anti–MERS-CoV antibodies, a dilution of 1:10 was chosen as the titration limit (ie, starting dilution) in neutralization assays. As summarized in Table 2, none of these 8 specimens had serum neutralization titers >2-fold the titration limit, whereas serum specimens from 2 patients with laboratory-confirmed MERS-CoV infection had clear neutralization titers.

Table 1. Results of First-Line Immunofluorescence Assays to Detect Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Antibodies in Serum Specimens From Subjects in Saudi Arabia

Sample Origin	No. Positive/No. Tested (%)
Blood donors	0/130 (0.0)
SH workers	
Jeddah	
SH 1	3/52 (5.8)
SH 2	1/44 (2.3)
Makkah	
SH 1	0/24 (0.0)
SH 2	2/65 (3.1)
SH 3	2/41 (4.9)
Total	8/356 (2.2)

MERS-CoV–infected cells were used as viral antigen.

Abbreviation: SH, slaughterhouse.

DISCUSSION

Our investigation yields 2 major conclusions. First, we demonstrated that immunofluorescence analysis using virus-infected

Table 2. Serological Findings for Human Pathogenic Coronaviruses (CoVs) for Saudi Arabian Subjects With Reactive First-Line Immunofluorescence Assays (IFAs) and German Control Subjects With Middle East Respiratory Syndrome[a]

	Discriminative Recombinant IFA Reactivity							
		Recombinant CoV Spike Proteins						
Sample Origin, Identifier	Full MERS-CoV	229E	NL63	OC43	HKU1	SARS	MERS	MERS-CoV $PRNT_{90}$ Titer
Saudi Arabia								
Jeddah								
SH 1, JS1–21	±	–	–	–	+	–	–	<1:10
SH 1, JS1–43	+	–	–	–	+	–	–	1:20
SH 1, JS1–54	+	±	–	–	+	–	±	1:20
SH 2, JS2–9	±	–	–	–	+	–	–	<1:10
Makkah								
SH 2, MS2–19	+	±	–	+	+	–	–	<1:10
SH 2, MS2–39	+	+	±	+	++	–	–	<1:10
SH 3, MS3–30	+	–	–	–	+	–	±	1:20
SH 3, MS3–34	+	+	±	±	+	–	–	<1:10
Germany[a]								
Essen (ex Qatar), SE 001/13	+++	+	++	++	+++	+	+++	1:640
Munich (ex UAE), 13/07439	+++	–	±	++	++	+	++	1:320

Abbreviations: IFA, immunofluorescence assay; MERS-CoV, Middle East respiratory syndrome coronavirus; $PRNT_{90}$, plaque-reduction neutralization assay; SH, slaughterhouse; UAE, United Arab Emirates; –, negative; ±, borderline reactivity; +, weakly positive; ++, positive; +++, strongly positive.

[a] Both patients from whom control samples were obtained have been described elsewhere [3].

cells will, if performed alone rather than as part of a set of analyses, return false-positive MERS-CoV antibody results for a fraction of patients. Of note, IFA is considered a highly robust assay because of the possibility to inspect the specific fluorescence staining patterns in virus-infected cells, providing an additional diagnostic criterion that is not available in other serology formats, such as enzyme immunoassays (EIA). The same propensity for false-positive results is therefore to be expected with EIA that use full virus as the test antigen. Future studies should take this into account to avoid any false-positive end points. Exact delineation of specific antigenic epitopes within the MERS-CoV spike protein, which could then be used as a recombinant antigen in EIA, could be useful in the development of a more-reliable protocol for serological detection of MERS-CoV.

Second, we found no significant rates of antibodies against MERS-CoV in individuals living in the Jeddah and Makkah regions. However, the number of samples analyzed in this preliminary study is limited, making it impossible from a statistical perspective to exclude the presence of antibodies in the population. Furthermore, although we know that IFA will readily detect antibodies in clinically severe cases of MERS-CoV infection, it is conceivable that lower levels of antibodies could exist in mild or asymptomatic cases. Nevertheless, we believe that our data suggest that long-term circulation of MERS-CoV at the population level is not occurring in the area from which our target population was sampled. The population should therefore be largely susceptible to MERS-CoV infection. Of note, the investigated geographical area overlaps with the destination for the Hajj and the Umrah, where millions of pilgrims gather each year and respiratory viruses are known to be acquired and transmitted at high rates [11, 12]. The absence of antibodies in our study is consistent with the absence of reports of any MERS-CoV infection in pilgrims returning from the Hajj in 2012 [11, 13].

Notes

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