ORIGINAL ARTICLE

Transmission of MERS-Coronavirus in Household Contacts

Christian Drosten, M.D., Benjamin Meyer, M.Sc., Marcel A. Müller, Ph.D., Victor M. Corman, M.D., Malak Al-Masri, R.N., Raheela Hossain, M.D., Hosam Madani, M.Sc., Andrea Sieberg, B.Sc., Berend Jan Bosch, Ph.D., Erik Lattwein, Ph.D., Raafat F. Alhakeem, M.D., Abdullah M. Assiri, M.D., Waleed Hajomar, M.Sc., Ali M. Albarrak, M.D., Jaffar A. Al-Tawfiq, M.D., Alimuddin I. Zumla, M.D., and Ziad A. Memish, M.D.

ABSTRACT

BACKGROUND

Strategies to contain the Middle East respiratory syndrome coronavirus (MERS-CoV) depend on knowledge of the rate of human-to-human transmission, including subclinical infections. A lack of serologic tools has hindered targeted studies of transmission.

METHODS

We studied 26 index patients with MERS-CoV infection and their 280 household contacts. The median time from the onset of symptoms in index patients to the latest blood sampling in contact patients was 17.5 days (range, 5 to 216; mean, 34.4). Probable cases of secondary transmission were identified on the basis of reactivity in two reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assays with independent RNA extraction from throat swabs or reactivity on enzyme-linked immunosorbent assay against MERS-CoV S1 antigen, supported by reactivity on recombinant S-protein immunofluorescence and demonstration of neutralization of more than 50% of the infectious virus seed dose on plaque-reduction neutralization testing.

RESULTS

Among the 280 household contacts of the 26 index patients, there were 12 probable cases of secondary transmission (4%; 95% confidence interval, 2 to 7). Of these cases, 7 were identified by means of RT-PCR, all in samples obtained within 14 days after the onset of symptoms in index patients, and 5 were identified by means of serologic analysis, all in samples obtained 13 days or more after symptom onset in index patients. Probable cases of secondary transmission occurred in 6 of 26 clusters (23%). Serologic results in contacts who were sampled 13 days or more after exposure were similar to overall study results for combined RT-PCR and serologic testing.

CONCLUSIONS

The rate of secondary transmission among household contacts of patients with MERS-CoV infection has been approximately 5%. Our data provide insight into the rate of subclinical transmission of MERS-CoV in the home.

From the Institute of Virology, University of Bonn Medical Center, Bonn (C.D., B.M., M.A.M., V.M.C., A.S.), and Euroimmun, Lübeck (E.L.) — both in Germany; Global Center for Mass Gatherings Medicine, Ministry of Health (M.A.-M., R.F.A., A.M. Assiri, A.I.Z., Z.A.M.), Prince Sultan Military Medical City (A.M. Albarrak), and Alfaisal University (Z.A.M.), Riyadh, Johns Hopkins Aramco Healthcare, Dhahran (J.A.A.-T.), and Regional Laboratory, Ministry of Health, Jeddah (R.H., H.M.) and Riyadh (W.H.) — all in Saudi Arabia; Indiana University School of Medicine, Indianapolis (J.A.A.-T.); the Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands (B.J.B.); and the Division of Infection and Immunity, University College London (UCL), and National Institute for Health Research Biomedical Research Centre, UCL Hospitals, London (A.I.Z.). Address reprint requests to Dr. Memish at Alfaisal University, P.O. Box 54146, Riyadh 11514, Saudi Arabia, or at zmemish@yahoo.com; or to Dr. Drosten at the Institute of Virology, University of Bonn Medical Center, 53105 Bonn, Germany, or at drosten@virology-bonn.de.

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The Middle East respiratory syndrome coronavirus (MERS-CoV) was first isolated in 2012 from a patient with fatal pneumonia in Jeddah, Saudi Arabia.[1] From 2012 through July 2014, at least 834 laboratory-confirmed cases of MERS-CoV infection associated mostly with respiratory tract illness were reported to the World Health Organization; of these cases, 288 were fatal. Known cases have been directly or indirectly linked to countries in the Arabian peninsula.[2] The epidemiologic features of MERS remain poorly defined. Studies that have modeled the reproductive rate of MERS-CoV in humans have been based on notified, clinically apparent cases and auxiliary measurements, such as the viral evolutionary rate.[3,4] However, these studies have emphasized the need to reevaluate estimates of reproductive rates through laboratory-based investigations of the actual rate of transmission, including silent and subclinical infections. Any unnoticed transmission of the virus in the population could explain why newly identified index cases often cannot be linked to zoonotic exposure. However, to date, the lack of serologic tools has hindered in-depth investigation of rates of secondary transmission.

To determine the rate of silent or subclinical secondary infection after exposure to primary cases of MERS-CoV infection, we performed a cross-sectional laboratory investigation of 280 household contacts of 26 index patients, with additional follow-up serologic analysis in 44 contacts. All the contacts were tested for the presence and quantity of viral RNA in throat-swab samples and for antibodies in blood with the use of a staged serologic algorithm that is based on results from enzyme-linked immunosorbent assays (ELISAs), immunofluorescence assays, and plaque-reduction neutralization testing (PRNT).

Methods

Patients

Household contact clusters were associated with the 26 index patients in whom MERS-CoV infection was diagnosed from June 4, 2013, to November 5, 2013, with one cluster per index patient. The median date on which clusters were sampled was September 8, 2013. Diagnoses in index patients were based on hospitalization with bilateral pneumonia and detection of MERS-CoV in respiratory samples on reverse-transcriptase–polymerase chain reaction (RT-PCR). All household contacts of index patients (who were defined as persons living in the same house, regardless of the degree of relation to the index patient) were obliged to participate in the investigation per decree of the Ministry of Health as part of a public health response. Clusters occurred in Riyadh (16 clusters), Dammam (3), Al-Hasa (2), Hafr Al-Batin (2), Jubail (2), and Jeddah (1).

MERS-CoV RT-PCR Assay

We performed RT-PCR assays on RNA that was extracted from upper respiratory tract swabs, with screening for the MERS-CoV genomic region upstream of the envelope gene (upE) and within open reading frame (ORF) 1a, as described previously.[5,6] Confirmation of upE results by ORF1a detection involved the reextraction of RNA from the original samples.

Full-Virus Immunofluorescence Assay

Immunofluorescence assays were performed with slides carrying Vero cells infected with full MERS-CoV, as described previously,[6] and were manufactured into a homogeneous reagent format by an in vitro diagnostics company for improved lot-to-lot consistency (MERS IIFT, Euroimmun). The serum predilution was 1:100.

Recombinant Immunofluorescence Assay

We performed recombinant immunofluorescence assays to determine the specific reactivity against recombinant spike proteins in VeroB4 cells, as described previously.[6] The screening dilution was 1:40. Because the CoV spike protein constitutes the most specific and immunogenic antigen in CoV antibody assays,[7] open reading frames for full spike proteins were cloned from human coronaviruses (HCoV) HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1, as well as for the severe acute respiratory syndrome coronavirus (SARS-CoV).[6,8] Cells for all slides were seeded on chamber slides and transfected in parallel with equal amounts of the respective expression plasmids.[6,8] To compare titers for MERS-CoV with those for different HCoVs, immunofluorescence assays were performed by the same staff member on the same day. Control serum samples for HCoV recombinant immunofluorescence assays were obtained from patients

with recent RT-PCR–confirmed HCoV-229E, HCoV-NL63, HCoV-OC43, or HCoV-HKU1 infection, as seen in routine diagnostic testing. The methods for obtaining control serum samples for testing for the presence of MERS-CoV and SARS-CoV have been described previously.[6,8-10]

IgM Immunofluorescence Assay

Immunofluorescence assay was used to determine the presence of IgM antibodies. All serum samples were depleted of IgG antibodies with the use of Eurosorb reagent (Euroimmun), according to the protocol. The screening dilution was 1:40. Secondary detection was performed with the use of fluorescein isothiocyanate (FITC)–conjugated anti-human IgM antibodies.

Recombinant ELISA

A recombinant ELISA assay was based on soluble MERS-CoV spike protein S1 domain expressed in HEK-293T cells.[11] This test was developed with the use of samples obtained from small groups of humans and camels in preliminary studies.[7,12] The dilution of human serum samples in this test was 1:100. Additional technical details are provided in Section 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Neutralization Testing

PRNT was performed as described previously.[8] The entry dilution in $\log_2$-dilution series was 1:10. Serum dilutions causing plaque reductions of 90% ($PRNT_{90}$) and 50% ($PRNT_{50}$) were recorded as titers.

Diagnostic Algorithm to Identify Secondary Transmission

We used the above-mentioned RT-PCR method to screen for MERS-CoV in throat swabs. Serum samples were initially screened for antibodies by means of an ELISA. Supportive serologic testing was performed in two stages. Stage 1 seropositivity was defined as reactivity on both an ELISA and a recombinant immunofluorescence assay. Stage 2 seropositivity was defined as stage 1 seropositivity plus reactivity on PRNT. Patients who had dual positive RT-PCR results or positive stage 2 serologic results were determined to have probable cases of secondary transmission. A flow chart summarizing the diagnostic algorithm and overall study outcome is provided in Figure 1.

Results

Study Population

The household clusters we studied included a mean of 11 contacts (range, 2 to 21). The median age of the 26 index patients was 55 years (range, 2 to 83; mean, 54.6); 17 of the patients were male. Of these patients, 24 had coexisting illnesses, 24 required treatment in an intensive care unit, and 18 died. Index patients had stayed at home with symptoms before hospital admission for an average of 6 days (range, 0 to 21).

The median interval from the onset of symptoms in index patients to the collection of the latest blood sample from household contacts was 17.5 days (range, 5 to 216; mean, 34.4). The median age of the 280 household contacts was 29 years (range, 2 to 77; mean, 30.7); 48% of these contacts were female. A total of 59 household contacts were 16 years of age or younger. Information with respect to coexisting illnesses was recorded for 178 household contacts; of these contacts, 12% had coexisting illnesses (Table S1 in the Supplementary Appendix).

Laboratory Testing

Throat swabs from all household contacts were initially tested for MERS-CoV RNA by means of RT-PCR. Of the 280 contacts, 7 (2%) who lived in three clusters had dual positive results on RT-PCR (Fig. 1A). Virus RNA levels in swabs obtained from these contacts ranged from fewer than 500 copies to 80,000 copies per swab sample, with a median level of 2700 copies per sample (Table 1). Only 1 contact with positive results on RT-PCR had mild symptoms at the time of sampling, and 2 contacts had had contact with camels, which have been identified as possible reservoirs for MERS-CoV.[12]

Serologic testing was based on a staged algorithm (Fig. 1B). The algorithm was established after comparative testing of primary serum samples from all 280 household contacts, with one sample per person (Section 3 in the Supplementary Appendix). These combined studies suggested that a recombinant immunofluorescence assay would be an appropriate first-stage diagnostic test for seropositivity for ELISA-based screening results. A total of 6 household contacts had positive ELISA results and positive results on recombinant immunofluorescence assay, fulfilling the

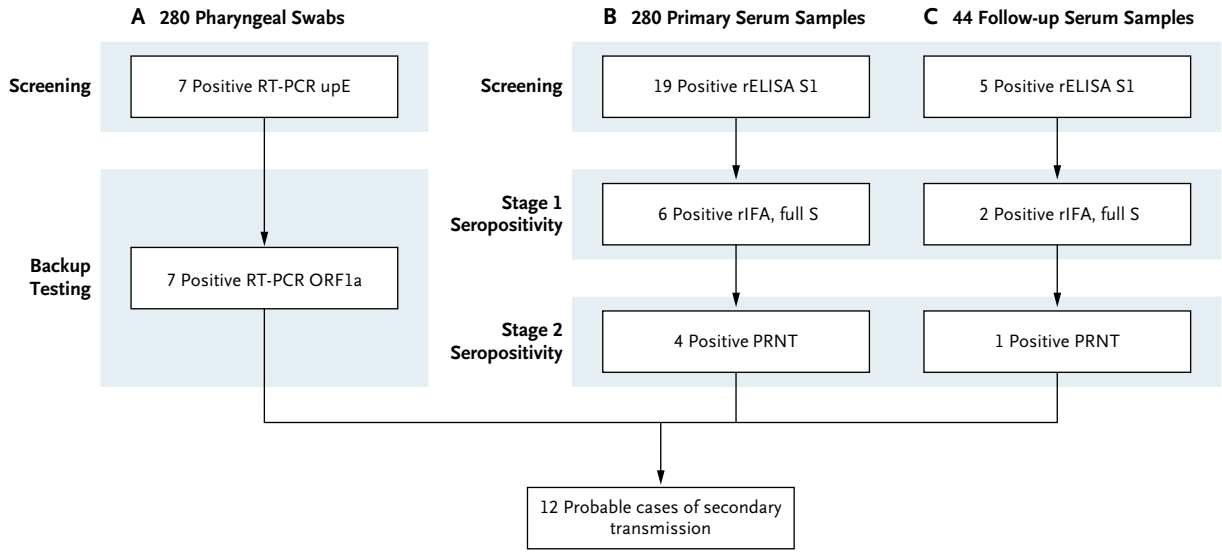

Figure 1. Diagnostic Testing Performed in the Study.

Throat swabs (column A) and serum samples (column B) were obtained from 280 household contacts of 26 index patients with MERS-CoV infection. A total of 44 household contacts provided a second serum sample for follow-up (column C). Screening involved testing of all throat swabs by means of a reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay and testing of all serum samples by means of recombinant enzyme-linked immunosorbent assay (rELISA) with the use of the S1 domain of the MERS-CoV spike protein. RT-PCR backup testing was based on a second RT-PCR assay, including re-extraction from the original samples. Eluted RNA was screened for the MERS-CoV genomic region upstream of the envelope gene (upE) and within open reading frame (ORF) 1a. Testing for stage 1 seropositivity involved the use of a recombinant immunofluorescence assay (rIFA) with the full MERS-CoV spike protein (full S) for all serum samples with a positive result on ELISA screening. Testing for stage 2 seropositivity was performed on serum samples with stage 1 seropositivity by means of plaque-reduction neutralization testing (PRNT), with plaque reduction of 50% as the criterion for neutralizing activity. Among the 4 samples with positive results on PRNT in column B, 2 were also determined to be positive on IgM testing. In the analysis of the 44 follow-up serum samples for stage 1 seropositivity, one case of MERS-CoV infection had already been confirmed in the primary serum sample, and one case was identified as a new seroconversion.

definition of stage 1 seropositivity. These household contacts included none of those with positive results on RT-PCR. Serum from the RT-PCR–positive contacts had been obtained 4 to 14 days after the onset of symptoms in the index patients, which suggested that these contacts were still in the period before seroconversion.

We asked all the household contacts for permission to obtain a second blood sample after 2 to 6 months for additional testing. Only 44 contacts in nine clusters agreed to provide a second sample. Of these contacts, 5 had positive results on ELISA; of these 5 contacts, 2 were seropositive on recombinant immunofluorescence assays (stage 1 seropositivity) (Fig. 1C). One of these 2 contacts had stage 1 seropositivity on the basis of his first serum sample, and the other (Contact 180) underwent seroconversion sometime between the first and second serum sample (Table S6 in the Supplementary Appendix).

Stage 2 seropositivity was tested on the basis of the above-mentioned PRNT cutoffs ($PRNT_{90}$ and $PRNT_{50}$). $PRNT_{90}$ titers were positive in an index patient (who was used as a control in the analysis of household contacts) and in samples obtained from 2 of 7 household contacts with stage 1 seropositivity. Three additional samples had positive $PRNT_{50}$ titers (Table 2). To correlate serum neutralizing activities with specific antibody titers, we performed differential recombinant immunofluorescence assays against all common HCoVs in all samples with confirmed stage 1 seropositivity (Table 2). In the serum pair for Contact 180, results on differential recombinant immunofluorescence assay indicated a significant rise in titer against MERS-CoV but not against any of the other HCoVs tested. It was concluded that Contact 180 represented a specific MERS-CoV seroconversion. Since this seroconversion was detected on $PRNT_{50}$ but not

Table 1. Laboratory Data and History of 12 Household Contacts Identified as Having Probable Secondary Infection with MERS-CoV.*

Contact No.	Age	Sex	Latency Period†	Serologic Analysis	Results of RT-PCR Assay‡	Symptoms	Coexisting Illnesses	Animal Contact
	yr		*days*		*RNA copies/sample*			
52	28	F	14	ELISA, 0.11 (negative); IFA, negative	Positive§	None	Unknown	None
278	7	F	4	ELISA, 0.06 (negative); IFA, negative	38,500	None	None	Camels
279	15	M	4	ELISA, 0.10 (negative); IFA, negative	2700	None	None	Camels
280	26	M	4	ELISA, 0.07 (negative); IFA, negative	38,500	None	None	None
257	74	F	8	ELISA, 0.06 (negative); IFA, negative	80,000	Pharyngitis¶	None	None
258	3	F	8	ELISA, 0.05 (negative); IFA, negative	<500	None	None	None
259	18	M	8	ELISA, 0.07 (negative); IFA, negative	<500	None	None	None
99	37	M	19	ELISA, 1.82 (reactive); IFA, 1:1280; PRNT, 1:160; IgM, 1:40	Negative	None	None	None
104	24	M	19	ELISA, 1.84 (reactive); IFA, 1:2560; PRNT, 1:160; IgM, 1:40	Negative	None	None	None
102	31	M	19	ELISA, 0.45 (reactive); IFA, 1:320; PRNT, 1:40; IgM, negative	Negative	None	Unknown	None
180‖	26	F	23	ELISA, 0.9 (reactive); IFA, 1:1280; PRNT, 1:40; IgM, negative	Negative	None	Unknown	None
193	31	M	13	ELISA, 1.57; (reactive); IFA, 1:40; PRNT, 1:40; IgM, negative	Negative	None	None	None

* Secondary infection was defined as dual positivity on RT-PCR assay or stage 2 seropositivity. ELISA denotes enzyme-linked immunosorbent assay, IFA immunofluorescence assay, and PRNT plaque-reduction neutralization testing.
† The latency period was the number of days between the onset of symptoms in the index patient and sampling of the household contact.
‡ The result was based on positive outcome of two independent RT-PCR assays in the upE and ORF1a target genes after reextraction from the original sample. The approximate concentration is based on the upE target gene assay.
§ This sample was tested in a local laboratory by means of RT-PCR assays in the upE and ORF1a target genes without recording of values for the threshold cycle (RNA concentration unknown). The original sample was not retrievable.
¶ This symptom was recorded on the day of sampling. Respiratory failure and death occurred in this contact soon after testing.
‖ For this household contact, laboratory results are based on a second follow-up serum sample.

$PRNT_{90}$, we chose $PRNT_{50}$ as a criterion to define stage 2 seropositivity in our diagnostic algorithm. Application of this criterion resulted in the identification of 5 household contacts with stage 2 seropositivity.

Since it is unknown whether the development of neutralizing antibodies as measured on PRNT can lag behind the development of reactivity on ELISA and immunofluorescence assay, we evaluated IgM antibodies as an additional potential indicator of recent infection. However, IgM titers were detected in serum samples that had positive $PRNT_{90}$ titers but not in samples with isolated $PRNT_{50}$ titers, which suggests that testing for IgM antibodies in an immunofluorescence assay–based format provides low sensitivity overall (Table 2). Table 3 summarizes the final study results, including the results of testing of second serum samples when they were available.

DISCUSSION

We report the results of a targeted investigation of silent and mild infections in the 280 household contacts of 26 index patients in whom MERS-CoV infection had been confirmed on RT-PCR assay with corroborating clinical findings. Seven household contacts carried MERS-CoV in their upper respiratory tract, as shown on RT-PCR assays. Pharyngeal RNA concentrations were low in most contacts, a finding that matches observations in a study on SARS in Taiwan in which asymptomatic or mildly symptomatic health care workers were shown to carry low pharyngeal levels of viral RNA after exposure to patients infected with SARS-CoV.[13] Our findings suggest that persons in the first few decades of life without coexisting illnesses may be able to carry low levels of MERS-CoV RNA with-

Table 2. Differential Serologic and Neutralization Analyses for MERS and Other Coronaviruses in 7 Household Contacts with Stage 1 Seropositivity and 1 Index Patient.*

Cluster	Contact No.	ELISA	IgG Titer on Recombinant IFA						IgM Titer on Recombinant IFA	PRNT MERS†	
			MERS	SARS	OC43	HKU1	229E	NL63	MERS	$PRNT_{90}$	$PRNT_{50}$
F	Index patient	4.05	5120	160	1,280	1,280	1280	320	160	40	160
J	99	1.82	1280	Negative	2,560	1,280	640	160	40	80	160
J	102	0.45	320	1280	5,120	5,120	640	1280	Negative	<20	40
J	104	1.83	2560	640	20,480	20,480	1280	2560	40	40	160
RIII	180‡	0.05	Negative	Negative	2,560	640	1280	640	Negative	<20	<20
	180§	0.90	1280	Negative	1,280	320	640	320	Negative	<20	40
RIII	187‡	0.31	80	Negative	1,280	1,280	640	1280	Negative	<20	<20
	187§	0.29	160	Negative	1,280	640	640	640	Negative	<20	<20
RIV	193	1.57	40	160	320	640	1280	640	Negative	<20	40
TII	274	0.46	160	Negative	2,560	1,280	160	160	Negative	<20	<20

* SARS denotes severe acute respiratory syndrome.
† Stage 2 seropositivity was tested by means of PRNT with cutoff values of 90% ($PRNT_{90}$) and 50% ($PRNT_{50}$). A titer of less than 1:20 indicates a negative result.
‡ This sample was obtained on day 23.
§ This sample was obtained on day 178 during follow-up.

out obvious symptoms. Asymptomatic, RT-PCR–positive health care workers were identified in a recent outbreak of MERS in Jeddah.

However, positive RT-PCR findings in persons with subclinical infection who are tested soon after exposure should be complemented by serologic analysis. In three earlier cross-sectional serologic studies, none of the 3024 participants showed evidence of previous infection with MERS-CoV.[1,14,15] In our study, the detection of antibodies was most likely due to our focus on household contacts of index patients. Differences in assay sensitivity may play an additional role. Our staged serologic algorithm is based on earlier experience with current assays or their technical precursors[6,8,12,14,16-20] and was developed as much as possible by a comparison of methods in the study samples available. Although validation of this algorithm will be necessary, our results suggest that ELISA is an appropriate screening assay and that recombinant immunofluorescence assay is an appropriate first-stage confirmatory test, owing to its higher specificity without an apparent substantial lack of sensitivity. Differential serologic testing by means of recombinant immunofluorescence assay cannot be used to rule out MERS-CoV infection, since anti–MERS-CoV titers did not always predominate in probable cases of secondary transmission. It is unknown whether MERS-CoV infection may cause a boost in preexisting titers against related HCoVs acquired earlier in life, such as is seen with many other infectious diseases. There are conserved regions between S proteins in MERS-CoV and those in various HCoVs that might explain the existence of cross-reactive serum antibodies (Fig. S2 in the Supplementary Appendix). Our data suggest that testing for stage 2 seropositivity may have to rely on PRNT as long as no better information regarding antibody cross-reactivity between MERS and HCoVs is available. We consider PRNT to be the most specific test because of its reliance on functionality of measured antibodies, but we cannot rule out cross-reactivity for results obtained.

In our study population, we encountered household contacts in whom serum neutralizing activity became apparent only when we used a 50% reduction in the test virus as a more sensitive diagnostic criterion than 90% reduction. This suggests that neutralizing antibodies are produced at low levels and are potentially short-lived after mild or asymptomatic infection, which puts the predictive value of cross-sectional serologic studies in perspective if such studies are not

Table 3. Summary of Serologic Results in Household Contacts, According to Timing of Sampling. *

Variable	Time from Onset of Symptoms in Index Patient until Sampling of Contact			
	Any Time	<2 Wk	2–3 Wk	>3 Wk†
No. of household contacts	280	127	45	108
No. of clusters	26	10	3	13
Positive results — no. (%)				
RT-PCR	7 (2)	7 (6)	0	0
ELISA	20 (7)	8 (6)	3 (7)	11 (10)
IFA	7 (2)	1 (1)	3 (7)	3 (3)
IFA plus PRNT	5 (2)	1 (1)	3 (7)	1 (1)
Household contacts with possible or probable secondary infection — no. (%)				
Possible on basis of RT-PCR or stage 1 seropositivity	14 (5)	8 (6)‡	3 (7)	3 (3)
Positive RT-PCR assay	7 (2)	7 (6)	0	0
Stage 1 seropositivity	7 (2)	1 (1)	3 (7)	3 (3)
Probable on basis of dual positive RT-PCR assays or stage 2 seropositivity	12 (4)	8 (6)	3 (7)	1 (1)
Dual positive RT-PCR assays	7 (2)	7 (6)	0	0
Stage 2 seropositivity	5 (2)	1 (1)	3 (7)	1 (1)
Clusters of household contacts with possible or probable secondary infection — no. (%)				
Possible on basis of RT-PCR or stage 1 seropositivity‡	6 (23)	4 (40)	1 (33)	2 (15)
Positive RT-PCR assay	3 (12)	3 (30)	0	0
Stage 1 seropositivity	4 (15)	1 (10)	1 (33)	2 (15)
Probable on basis of dual positive RT-PCR assays or stage 2 seropositivity	6 (23)	4 (40)	1 (33)	1 (8)
Dual positive RT-PCR assays	3 (12)	3 (30)	0	0
Stage 2 seropositivity	3 (12)	1 (10)	1 (33)	1 (8)

* Percentages may not sum to the overall total in a category because of rounding.
† For household contacts who provided serum samples more than 3 weeks after exposure, the indicated test results are based on second samples if they were available.
‡ One cluster included a contact with positive results on RT-PCR and another with positive serologic results.

specifically targeted at persons with recent exposure.

An important limitation of our study was the low rate of household contacts from whom we could obtain a second blood sample. Although the first serologic investigation was obligatory, the participation in follow-up investigations was voluntary. The sentiment in Saudi Arabia at the time that we conducted the study was dominated by stigmatization of patients with MERS and discrimination against affected families. There was a widespread belief that medical care facilities act as sources of infection, triggered by media reports focusing on hospital outbreaks, such as that in Al-Hasa.[21] For the combination of these reasons, household contacts would widely refuse to participate in any research activity connected with MERS.

Because of the limited number of follow-up samples and the variable timing of contact-cluster investigations, the timing of serologic testing after the onset of symptoms in the index patients might have influenced the diagnostic usefulness of the various tests that we used. Positive RT-PCR findings in our study were identified only during the first 14 days after exposure, whereas stage 2 seropositivity was identified only 13 days or more after exposure (Table 1). This matches expected patterns of laboratory results when infections are acquired around the time of or shortly after exposure (defined as the time of symptom onset in index patients). In par-

ticular, the timing of the first antibody results for stage 2 seropositivity matches the typical timing for the development of an IgG response. If we had evaluated only household contacts who had provided serum samples 13 days or more after exposure and considered only serologic results, we would have identified cases of probable transmission in 5 of 172 contacts (3%; 95% confidence interval [CI], 1 to 7) in 4 of 17 clusters (24%). These data from limited testing are consistent with the overall study results on the basis of RT-PCR and serologic analysis (i.e., rates of secondary infection of 4% [95% CI, 2 to 7] among household contacts, in 23% of the clusters). Therefore, although RT-PCR testing may have compensated for a lack of serologic follow-up in household contacts who provided serum samples only during the first 2 weeks after exposure, we cannot determine whether we may have missed contacts who had delayed seroconversion. We also could not evaluate possible common sources of exposure for index patients and their household contacts.

Finally, it is relevant to note that in the whole study, we identified only 12 household contacts who may have been infected by 26 index cases. Although rates of secondary transmission may be higher in other situations, such as nosocomial outbreaks or social circumstances in which there may be a more closed or intimate environment, these data may inform approaches to estimating the epidemiologic reach of MERS-CoV in humans.[3,4]

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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