parallel with similar efforts in Europe, strategies need to be developed to protect commercial and pet rabbits.

Tracking the spread of RHDV2 in Australia, in competition with existing field strains, highlights the value of Australia's rabbits and their diseases as a model system for emerging infectious diseases. The point releases of both myxoma virus and RHDV into large naive host populations represent a grand experiment in disease emergence and evolution (*10*), which provides a unique opportunity to study the virulence evolution of emerging pathogens as well as their complex interactions with each other. It is notable that since the release of RHDV in Australia in 1995, strains of 1 viral lineage dominate the viral population nationwide despite hundreds of deliberate rereleases of the original virus strain for local rabbit control, which strongly suggests it has a major selective advantage (*7*). That RHDV2 appeared in a wild rabbit is therefore remarkable, particularly because Australian field strains were spreading simultaneously in the same area. Comparing the epidemiology of this strain in Australia to the epidemiology of its well-documented spread in Europe will provide valuable insights into RHDV epidemiology relevant to both continents.

Acknowledgments

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Characteristics of Traveler with Middle East Respiratory Syndrome, China, 2015

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To the Editor: A traveler returning from the Middle East initiated an outbreak of Middle East respiratory syndrome (MERS) in South Korea in 2015, which resulted in 186 cases and 36 deaths (*1–3*). We report a case of

[1]These authors contributed equally to this article.

MERS in a 43-year-old man from South Korea who acquired this disease during this outbreak (online Technical Appendix Figure 1, panel A, http://wwwnc.cdc.gov/EID/article/21/12/15-1232-Techapp1.pdf) (*4*).

The National Health and Family Planning Commission of China determined that collection of data for this patient was part of a public health investigation of an emerging outbreak. Therefore, informed consent was not required. This study was approved by the ethical committee of the First Affiliated Hospital of Guangzhou Medical University.

The patient had been receiving thiamazole for 7 years for hyperthyroidism. He had contact with the index case-patient during the outbreak in South Korea on May 16, 2015. On May 25, the patient traveled to Hong Kong and then to Huizhou, China. He was hospitalized in China on May 28 (day 7 of illness). At admission, he had a high fever (temperature 39.5°C) and a dry cough. Chest radiography on day 7 showed mild bilateral ground glass opacities in the lower lung (online Technical Appendix Figure 1, panel B).

The patient was given oseltamivir (150 mg, 2×/day for 2 days) until identified as being infected with Middle East respiratory syndrome coronavirus (MERS-CoV) on day 8 by real-time reverse transcription PCR. He was given ribavirin (2.0 mg on day 8; 0.6 mg 3×/d on days 9–16; and 0.6 mg 2×/d on days 17–19) and 135 μg of peginterferon αa-2a by intravenous injection on day 8 (online Technical Appendix Table 2). Thrombocytopenia and a decrease in the hemoglobin level developed, which might have been related to use of ribavirin (online Technical Appendix Table 1).

Chest radiography on June 1 (day 11) showed increased bilateral consolidation of the patient's lower lung (online Technical Appendix Figure 1, panel C). He was given intravenous immunoglobulin, antimicrobial drugs, and thymosin α1. His body temperature returned to normal on day 14 (online Technical Appendix Figure 2). Chest radiography on day 35 showed resolution of bilateral lung infiltrations (online Technical Appendix Figure 1, panel D). He was discharged on day 36.

Viral RNA was detected in sputum and fecal specimens up to day 26 of illness. Virus load in sputum specimens collected on days 11–15 were lower than in specimens obtained on days 16–18 (online Technical Appendix Figure 3, panel A). Swab samples collected on days 13 and 15 from the patient's palm, mobile telephone, blanket, and bed railings, and from his hospital room floor were negative for viral RNA.

Concentrations of proinflammatory cytokines and chemokines (interferon-α, interferon-inducible protein 10, monocyte chemoattractant protein-1, interleukin 6 [IL-6], IL-10, tumor necrosis factor-α, IL-8, macrophage inflammatory protein-α [MIP-1α], MIP-1β, and IL-1β) were determined for serial serum samples. Interferon-α, interferon-inducible protein 10, monokine induced by interferon-γ, IL-6, monocyte chemoattractant protein-1, and IL-8 were detected on day 11 of illness but levels decreased as the patient clinically improved (online Technical Appendix Figure 3, panel B).

The peginterferon αA2 the patient was given on day 8 might have influenced his plasma interferon-α levels (*6*). However, a previous study also showed increased levels of interferon-α in a patient who survived MERS-CoV infection but not in a person who died of MERS (*7*). Although MERS-CoV evades induction of innate immune responses by cell types, the virus elicits interferon responses in plasmacytoid dendritic cells in vitro (*8*). Levels of tumor necrosis factor-α, MIP-1α, MIP-1β, IL-10, and IL-1β did not increase in any of these specimens.

Peripheral blood mononuclear cells (PBMCs) obtained on day 24 of illness showed a strong specific T-cell response against MERS-CoV spike protein but not against severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein (online Technical Appendix Figure 3, panel C). PBMCs from persons who were infected with SARS-CoV in 2003, as well as healthy persons, showed low-level T-cell responses against MERS-CoV spike protein, although some persons with a history of SARS still had detectable responses to SARS-CoV spike protein. It was reported that T-cell responses to SARS-CoV were directed against spike and nucleocapsid proteins (*9*). We did not have sufficient PBMCs to test T-cell responses against nucleocapsid protein.

Results for MERS-CoV antibody were negative at day 11 of illness by MERS-CoV spike pseudotype assay (MERS-S ppNT), microneutralization, 50% plaque reduction neutralization test ($PRNT_{50}$), and S1 ELISA (EUROIMMUN AG, Lübeck, Germany). The patient showed seroconversion by day 14. MERS-S ppNT and $PRNT_{50}$ provided earlier evidence of seroconversion (day 15) and higher antibody titers than the microneutralization, (day 18) (online Technical Appendix Figure 3, panel D). Potent T-cell responses were elicited to MERS-CoV spike protein. These responses did not show cross-reactivity with SARS-CoV spike protein.

The MERS-S ppNT, which does not require Biosafety Level 3 containment, had sensitivity equivalent with that of $PRNT_{50}$, which requires containment. Thus, MERS-S ppNT is a sensitive and specific assay for detecting neutralizing antibody against MERS-CoV. The sensitivity and specificity of this assay have been well-documented with serum samples from dromedary camels and other animals (*10*).

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Characteristics of Traveler with Middle East Respiratory Syndrome, China, 2015

Technical Appendix

Methods

Detection of Virus in Samples

Throat swab, nasal swab, sputum, urine, serum, fecal, and environmental samples were collected from the patient and placed in viral transport medium. Viral RNA was extracted from samples by using the QIAamp MinElute Virus Spin Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. Middle East respiratory syndrome coronavirus (MERS-CoV) was detected by using real-time reverse transcription PCR with primers upE-Fwd (5′-GCAACGCGCGATTCAGTT-3′) and upE-Rev (5′-GCCTCTACACGGGACCCATA-3′), and probe upE-Prb (6-carboxyfluorescein[FAM]-5′-CTCTTCACATAATCGCCCCGAGCTCG-3′-6-carboxy-N,N,N,N′-tetramethylrhodamine [TAMRA]).

Determination of Cytokine Levels

Levels of interleukin 8 (IL-8), interferon-inducible protein 10, interferon-α, macrophage inflammatory protein-α, macrophage inflammatory protein-β, monocyte chemoattractant protein-1, monokine induced by interferon-γ (IFN-γ), IL-1β, tumor necrosis factor-α, IL-6, and IL-10 in serum samples were determined by using a Cytometric Bead Assay Human Inflammatory Cytokine Kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Data acquisition was performed by using a BD LSR Fortessa Analyzer (BD Biosciences). Data were analyzed by using cytometric bead assay analysis software (BD Biosciences).

IFN-γ ELISPOT Assay

Whole blood samples from healthy donors, health care workers who had been infected with SARS-CoV, and the MERS patient were collected into anticoagulant tubes containing EDTA, and the peripheral blood mononuclear cells (PBMCs) were

isolated by using standard density gradient centrifugation. An IFN-γ ELISPOT assay was performed as follows. A 96-well plate containing Immobilon-P membrane (MSIPS4510; Millipore, Billerica, MA, USA) was incubated with monoclonal anti-monkey IFN-γ (CT610–10; U-Cytech, Utrecht, the Netherlands) at 4°C overnight and blocked with RPMI medium 1640 (no. 11875–093; Gibco, Grand Island, NY, USA) containing 0.05 mmol/L 2-mercaptoethanol, 1 mmol/L sodium pyruvate, 2 mmol/L L-glutamine, 10 mmol/L HEPES, and 10% fetal bovine serum (no. SH30070; HyClone, Logan, UT, USA) at 37°C for 2 h.

PBMCs were plated at a density of 2.5×10^5 cells/well. Recombinant MERS-CoV spike protein (no. 40069-V08B; Sino Biologic Inc., North Wales, PA, USA) and SARS-CoV spike S1 subunit (no. 40150-V08B1; Sino Biologic Inc.) were added to the wells at a final concentration of 10 μg/mL to stimulate PBMCs. Phosphate-buffered saline was used for mock stimulation. At 24-h poststimulation, IFN-γ was detected by using biotinylated anti-monkey IFN-γ (CT610–10; U-Cytech), and color was developed by using nitro-blue tetrazolium and 5-bromo-4-chloro-3'-indolyphosphate (Pierce, Rockford, IL, USA). Spots were counted by using an ELISPOT reader (Bioreader 4000; BIOSYS, Germany), and the results were reported as number of spot-forming cells/million PBMCs.

Microneutralization Test

Serum samples were heat-inactivated (56°C for 30 min) and mixed with equal volumes of two hundred 50% tissue cultures infectious doses of MERS-CoV. After 1 h of incubation at 37°C, 35 μL of virus/serum mixture was added in quadruplicate to Vero cell monolayers in 96-well microtiter plates. After 1 h of adsorption, the virus/serum mixture was removed, 150 μL of culture medium was added to each well, and plates were incubated for 3 days at 37°C in an atmosphere of 5% CO_2 in a humidified incubator. Cytopathic effect was read at day 3 postinfection. The highest serum dilution that completely protected the cells from a cytopathic effect in half of the wells was taken as the neutralizing antibody titer and was estimated by using the Reed-Muench method (*1*).

MERS-CoV Spike Pseudoparticle Neutralization Assay

MERS CoV pseudoparticles were incubated for 1 h with an equal volume of serum dilution at 4°C. After incubation for 1 h, serum/virus mixture was added to Vero cell monolayers in triplicate in a 96-well format. At day 3 posttransduction,

luciferase activity was measured by using a Microbeta Luminometer (PerkinElmer, Waltham, MA, USA). The highest serum dilution giving 90% reduction of luciferase activity was used as the antibody titer (*2*).

MERS CoV S1 ELISA

A commercially available (EUROIMMUN AG, Lübeck, Germany) MERS CoV S1 ELISA was used. In brief, serum dilutions, a calibrator, and controls were added to MERS CoV–coated plates and incubated for 30 min. Plates were washed 3 times and peroxidase labeled anti-human IgG was added and incubated for 30 min. Substrate solution was added and incubated for 15 min at room temperature. Reactions were stopped, and absorbance was measured at 450 nm using a reference wave length of 650 nm. The ratio between the extinction values of control/sample and calibrator was calculated. A ratio >0.8 was considered a positive result (*3*).

Plaque Reduction Neutralization Test

Equal volumes of each serum dilution and 40–60 PFU of MERS CoV/24-well plate were mixed and incubated for 1 h at 37°C in an atmosphere of 5% CO_2 in a humidified incubator. After incubation, virus/serum mixture was added to Vero cell monolayers in a 24-well format in duplicate. Plates were incubated for 1 h to enable absorption before overlaying with agarose. After 3 days, cells were fixed with 10% paraformaldehyde and stained. Titers are expressed as serum dilutions resulting in plaque inhibition $\geq$90%.

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Technical Appendix Table 1. Biochemical parameters for a 43-year-old traveler with Middle East respiratory syndrome, China, 2015*

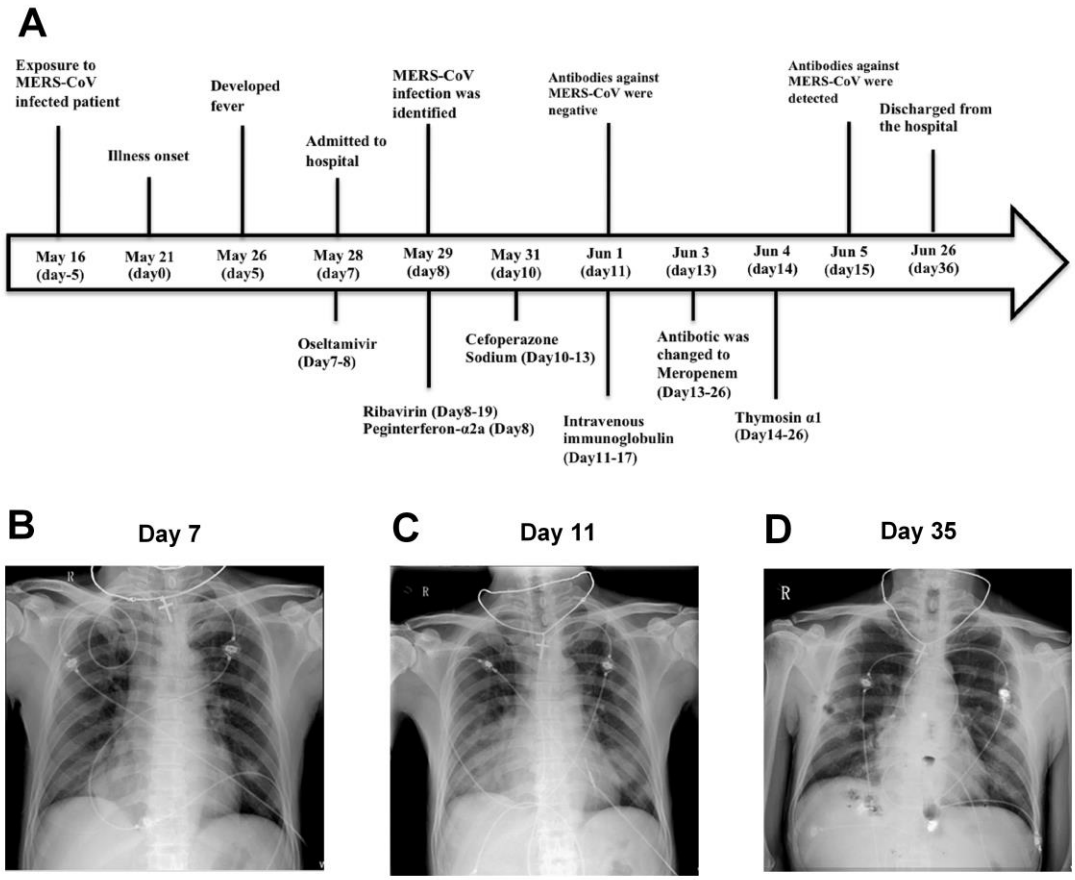
Parameter	Reference range	Days after illness onset						
		9	11	16	21	23	29	33
Total leukocytes, × 10^9 cells/L	4.0–10.0	3.22	3.06	3.6	4.9	4.8	4.6	7.3
Neutrophils, × 10^9 cells/L	1.8–6.3	2.35	1.71	2.0	2.6	2.5	2.4	4.6
Lymphocytes, × 10^9 cells/L	1.1–3.2	0.65	1.02	1.0	1.7	1.7	1.7	2.0
Platelets, × 10^9/L	125–350	81	113	253	378	362	274	263
Hemoglobin, g/dL	130–175	142	149	117	119	119	109	106
Aspartate aminotransferase, U/L	17–59	24	26	–	35	34	35	44
Creatinine, μmol/L	71–133	54	65	–	52	51	54	59
Creatine kinase, U/L	55–170	–	–	–	21	41	29	41
Creatine kinase isoenzyme, U/L	0–25	8	4	–	6	6	6	4

*–, data not collected.

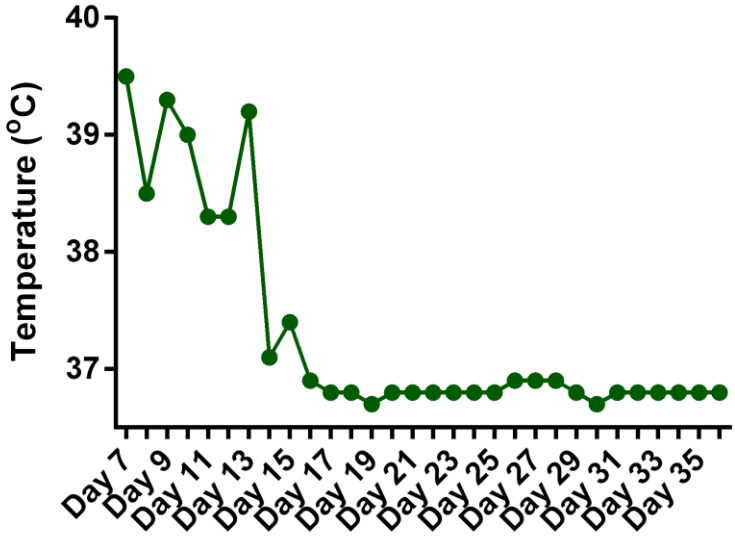
Technical Appendix Table 2. Clinical characteristics for a 43-year-old traveler with Middle East respiratory syndrome, China, 2015*

Characteristic	Result or value
Clinical features	
Fever	Yes
Highest temperature, °C	39.5
Cough	No
Sputum production	Yes
Shortness of breath	Yes
Chills	No
Complications	
Pneumonia	Yes
Acute respiratory distress syndrome	Yes
Shock	No
Bacterial co-infection	No
Treatment	
Oxygen therapy	Yes, inhalation oxygen with nasal catheter
Mechanical ventilation	No
Antivirus	Oseltamivir (150 mg, 2×/d on days 7 and 8); ribavirin (2.0 mg/d on day 8; 0.6 mg 3×/d on days 8–16; 0.6 mg 2×/d on days 17–19); peginterferon-α 2a (135 μg/d iv on day 8)
Antimicrobial drug	
Ceftriaxone sodium	2.0 g/d iv on days 10–13
Meropenem	2.0 g 3×/d iv on days 13–26
Glucocorticoids	No
Immune regulation therapy	
Immune globulin	20 g/d iv on days 11–17
Thymosin alpha-1	1.6 mg/d iv on days 14–18; 1.6 mg 2×/d iv on days 19–26
Clinical outcome	Discharged

*iv, intravenous.



Technical Appendix Figure 1. A) Timeline of clinical course of a 43-year-old traveler with Middle East respiratory syndrome, China, 2015. MERS-CoV, Middle East respiratory syndrome coronavirus. B–D) Chest radiographs on days 7, 11, and 35.



Technical Appendix Figure 2. Body temperature of 43-year-old traveler with Middle East respiratory syndrome, by day of hospitalization, China, 2015.

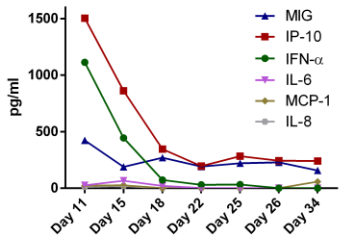
A

The viral load detected from varies collection of samples during hospitalization

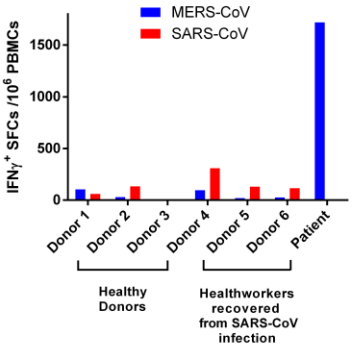
	MERS-CoV RNA copy numbers (log10)												
Day of onset	11	12	13	14	15	16	17	18	19	22	25	26	36
Sputum on demand	3.0	3.9	3.6	2.0	2.4	N.C.	N.C.	N.C.	2.8	2.2	N.C.	N.C.	-
Expectorated sputum*	N.C.	N.C.	N.C.	N.C.	N.C.	6.0	6.1	2.5	N.C.	N.C.	2.0	3.0	N.C.
Throat Swab	-	-	-	-	-	-	-	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Nasal Swab	-	-	N.C.	-	-	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Serum	-	N.C.	N.C.	-	-	-	N.C.	-	N.C.	-	N.C.	-	N.C.
Stool	N.C.	N.C.	N.C.	2.5	N.C.	N.C.	N.C.	N.C.	3.5	N.C.	-	2.1	N.C.
Urine	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	-	-	N.C.	-	N.C.	N.C.
Environmental samples	N.C.	N.C.	-	N.C.	-	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.

Samples with threshold cycle lower than 40 were regarded as negative; N.C. : Not collected; - : Negative detection; Environmental samples : Palm, Pass Box, Bed railings(Day 13); Palm, Bed railings, Blanket, mobile phone, Floor (Day 15); *: A sputum cup was provided for the patient to collect sputum when he coughed. The sputum cup was collected in the morning for analysis.

B



C



D

Detection of antibodies against MERS-CoV by using different assays

	MERS-CoV antibody				
Days after onset of illness	ppNT titer+	Micro-neutralization titer	PRNT# (90% reduction)	PRNT (50% reduction)	S1-ELISA* (Calibrator/sample extinction ratio)
11	<1:10	<1:10	<1:10	<1:10	0.03 (Negative)
15	1:80	1:10	1:40	1:80	1.32 (Positive)
18	1:320	1:40	1:80	1:160	2.25 (Positive)
22	1:320	1:40	1:80	1:160	2.53 (Positive)
25	1:320	1:40	-	-	2.46 (Positive)
26	1:320	1:40	1:160	1:320	2.81 (Positive)
34	1:640	1:80	1:160	1:320	3.1 (Positive)

*ELISA optical density cut-off for positive result = 0.8; + ppNT: MERS-CoV spike pseudotype assay; #PRNT: Plaque reduction neutralization titer.

Technical Appendix Figure 3. Clinical parameters for a 43-year-old traveler with Middle East respiratory syndrome, China, 2015. A) Virus load. MERS-CoV, Middle East respiratory syndrome coronavirus. B) Cytokine levels and antibody titers. MIG, monokine induced by interferon-γ; IP10, interferon-inducible protein 10; IFN-α, interferon-α; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein-1. C) T-cell response of peripheral blood mononuclear cells (PBMCs) after challenge with MERS-CoV spike protein. SARS-CoV, severe acute respiratory syndrome coronavirus; IFNγ, interferon-γ; SFCs, spot-forming cells. D) Detection of antibodies against MERS-CoV by different assays.