

# Still Learning From the Earliest Known MERS Outbreak, Zarqa, Jordan, April 2012

Daniel R. Lucey

Department of Microbiology and Immunology, Georgetown University Medical Center, Washington, D.C.

(See the Major Article by Al-Abdallat et al on pages 1225–33.)

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In this issue of *Clinical Infectious Diseases*, Mohammad Al-Abdallat and colleagues from the Jordan Ministry of Health (MOH) and the US Centers for Disease Control and Prevention (CDC) provide the most comprehensive report to date on the earliest known outbreak of the Middle East respiratory syndrome (MERS) coronavirus (CoV) [1]. This retrospectively diagnosed intensive care unit/critical care unit (ICU/CCU)-associated outbreak occurred in April 2012 in a large public hospital in Zarqa, Jordan, located 25 km northeast of the Jordanian capital of Amman [2]. The authors, using 3 antibody assays developed by the CDC, are the first to provide serologic data on this outbreak. These assays advance the ability to conduct wider population-based serosurveys for MERS as well as to assist in the diagnosis of individual patients.

Importantly, these serologic assays should be compared collaboratively with

other MERS serologic tests published in 2012 and 2013, particularly by European investigators in the Netherlands and Germany [3–5]. More generally, this earliest known MERS outbreak in the Middle East affords an opportunity to create international consensus guidelines for laboratory testing when explicitly searching for a novel infectious disease agent.

The persistent investigative response by the Jordan MOH to this April 2012 outbreak is fascinating and instructive and is an exemplary model of outbreak response. The MOH undertook at least 3 major epidemiologic investigations. The first in April 2012 stopped the outbreak of the unknown and likely contagious hospital-associated pneumonia that involved 13 persons, most of whom were healthcare personnel. Importantly, patient samples from at least 2 of the fatal cases were obtained and stored. The second major investigation was with the World Health Organization (WHO) in November–December 2012 after the laboratory-confirmed diagnosis of MERS had been made retrospectively on the stored specimens from the 2 fatal cases [2, 6, 7]. The third was in collaboration with the CDC in May 2013. This study added 7 survivors who, 13 months after the outbreak, had at least 2 serologically positive tests for anti-MERS-CoV antibodies [1].

A more detailed summary of the timeline for this outbreak in Zarqa is instructive in illustrating the efforts made to identify the cause of the outbreak. The initial outbreak communication response by the Jordan MOH occurred on 19 April 2012 when they closed the ICU at Zarqa Public Hospital and notified the press that 1 healthcare worker had died due to this outbreak of “pneumonia.” On 20 April the *Jordan Times* published the first of a series of articles on the outbreak [8]. Notably, the European Centre for Disease Control and Prevention (ECDC) published an epidemiological summary and assessment in its 29 April 2012–5 May 2012 Communicable Disease Threats Report. ECDC noted that 1 nurse had died and that at least 7 nurses and 1 doctor had a febrile lower respiratory illness. Citing the Jordan MOH, it was reported that the cause was likely to be viral; however, no laboratory tests were available. ECDC stated that due to the severity and “unusualness” of the illness in healthcare personnel, they contacted WHO and the CDC and that both organizations also “are following this event” [9]. Meanwhile, the outbreak was stopped, in part, by creating a case definition that focused on unexplained pneumonia and transferring suspected patients to 2 hospitals in Amman where strict infection control measures were undertaken (unlike in the

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Correspondence: Daniel R. Lucey, MD, MPH, Suite 321 SW Medical Dental Building, Department of Microbiology and Immunology, Georgetown University Medical Center, 3900 Reservoir Road NW, Washington, DC 20057 (drl23@georgetown.edu).

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ICU/CCU at Zarqa Hospital). The onset of illness for the last patient was on 26 April [2].

In a subsequent WHO update (30 November 2012) on the outbreak, it was noted that in April Jordan MOH made a request for a team from the WHO Collaborating Centre for Emerging and Re-emerging Infectious Diseases (NAMRU-3) in Cairo, Egypt, to help with the laboratory investigation [6]. This NAMRU-3 team traveled to Jordan, and on 24 April, promptly reported to the MOH that all patient specimens had tested negative for respiratory viruses including known CoVs. The specific laboratory assays performed were not identified nor were any attempts made to culture a virus using cell lines [6].

The clue that a novel CoV, later named the MERS-CoV, was the cause of the outbreak in Zarqa came with the report of a single patient with pneumonia from Bisha, Saudi Arabia, who had died in the Dr Soliman Fakeeh Hospital in Jeddah in June 2012 [10]. This report first appeared on 20 September 2012 on the Program for Monitoring Emerging Diseases (ProMED) website. It was posted by the astute microbiologist, Dr Ali Zaki, who reported that this virus grew easily on Vero cells [11]. Testing with a pan-CoV reverse-transcription polymerase chain reaction (RT-PCR) was consistent with a CoV and with molecular identification in the Erasmus Medical Center, Netherlands, which showed that it was a novel beta CoV [10, 11]. Later studies from the University of Hong Kong also showed that the virus could grow on a broad range of cell lines [12]. On 25 September 2012 ProMED posted an inquiry from Dr Iren Lai from Sydney, Australia, asking if this novel CoV could be the cause of the outbreak in Zarqa, Jordan [13].

In October 2012 stored patient specimens were sent from Jordan MOH to NAMRU-3. By November, testing had identified this novel CoV in the bronchoalveolar lavage fluid and nasal swab extract from the 40-year-old nurse who

died and in the serum of the 25-year-old student who died [2, 6, 7]. Thus, the second major investigation began when the MOH requested that WHO personnel travel to Amman from 28 November 2012 to 7 December 2012 and assist with another epidemiological investigation of the April outbreak, strengthening the sentinel surveillance system for severe acute respiratory infections. On 21 December 2012 the WHO summarized the results of this investigation. Notable findings included that the index case “could not be determined” and “there was no history of travel or contact with animals among confirmed or probable cases” [7]. The more detailed epidemiological results of this Jordan MOH–WHO outbreak investigation were published in early 2013 as part of a special issue of the *Eastern Mediterranean Health Journal* devoted to the novel CoV [2].

The third major investigation of the Zarqa outbreak by the Jordan MOH, and the only one that involved serologic data, took place in May 2013 and is reported in this issue of *Clinical Infectious Diseases*. The design of the study and the conclusions are strengthened by the inclusion of 124 volunteers including household contacts, healthcare personnel at the 3 hospitals that cared for the patients, and MOH field investigators. Six of the serologically positive cases were among the suspected patients during the original outbreak. The seventh case was a close family member. Thus, the serological testing did not identify anyone without a potential exposure history.

Several methodological points emphasized here. Very few specimens from different patients were available to obtain either the MERS-CoV or convalescent sera-containing antibody from virologically confirmed patients. In this context, the WHO has stated that “there is no clear consensus on interpretation of serological test results in individual patients. Given that currently available assays have been validated using only a limited number of convalescent sera . . . cases where the testing

laboratory has reported positive serological test results in the absence of PCR testing or sequencing, are considered *probable* cases of MERS-CoV infection . . .” [14].

WHO also recommends “that any positive result by a single serological assay be confirmed with a neutralization assay” [14]. In the current study by Al-Abdallat and colleagues, a positive serology was defined as a positive screening enzyme-linked immunosorbent assay (ELISA) plus either a positive indirect fluorescent antibody (IFA) or microneutralization test. In fact, only 1 of the 7 serologically positive patients did not have neutralizing antibodies detected (“outbreak member 11”). She had only a positive ELISA and IFA serology. A chest X-ray for this 41-year-old patient was “not documented (ND)” and she had the shortest hospital stay (4 days) of the 7 hospitalized patients [1]. Possibly she had a mild infection with the MERS-CoV and had lost her neutralizing antibody by 13 months after the infection. On the other hand, the CDC in May 2014 announced that a business associate in Illinois who had met with an Indiana MERS patient was not infected with the virus because he had a negative neutralizing antibody test even though he had a positive ELISA and IFA serology [15].

The 25-year-old Jordanian student with MERS who died on 25 April 2012 did not have anti-MERS-CoV antibody detected in any of 3 assays. A possible explanation is the fact that he was still viremic before he died. A small number of other patients with MERS have been found to be viremic as well, thereby raising the concern of a risk to the blood supply or of maternal–fetal transmission of MERS-CoV. In fact, 1 of the serologically positive cases in this study, household contact “HHM-303,” was a 39-year-old woman who had a stillbirth at 5 months gestation during the time she had an acute respiratory illness. She had been exposed to 2 family members who would later be diagnosed with MERS within 7 days of the onset of her illness and spontaneous delivery of a stillborn infant.

However, no testing of the fetus for MERS-CoV was performed [16].

Despite the 3 comprehensive investigations of the MERS-CoV outbreak in Zarqa Public Hospital, it remains unclear who the index case was and how the virus entered the hospital. Moreover, despite patient specimens having been tested during the April 2012 outbreak in laboratories in Jordan and at the regional WHO collaborating Centre for Emerging and Re-emerging Infectious Diseases in Cairo, a novel virus was not identified. In such situations, it is prudent to search explicitly not only for known pathogens but also for novel pathogens.

It was the outbreak of severe acute respiratory syndrome-CoV among healthcare workers in Hong Kong, Guangdong, and Hanoi that led the WHO to issue its global alert for this “atypical pneumonia” on 12 March 2003 [17]. In 2001 an unexplained outbreak involving several dozen persons in a hospital in Siliguri, India, near Bangladesh was later attributed to Nipah virus [18]. Other hospital-associated infections with novel viruses, such as Ebola in 1976, have been well recognized.

One major benefit of the accumulated epidemiologic, serologic, and clinical data on the then-unidentified MERS outbreak in April 2012 in Zarqa Public Hospital would be if an international consensus is reached for specific laboratory testing when explicitly searching for an unknown infectious disease agent, for example, in a healthcare facility. Obtaining and preserving patient specimens as comprehensively as possible would be an essential start. The specific types of laboratory assays, ranging from contemporary molecular to traditional culture techniques, and electron microscopy would likely be included. Acute and convalescent serum for serologic tests and for assessing the pathogen in blood would be important. Where possible, preservation

of peripheral blood mononuclear cells could also be helpful when testing for a reservoir for the pathogen and cell-mediated immune responses.

If such international consensus laboratory guidelines were formulated and applied to future unexplained outbreaks in healthcare facilities, then all the efforts devoted to the sequential studies of the MERS outbreak in Zarqa Public Hospital would leave an important diagnostic legacy when searching for unexplained outbreak pathogens. This legacy might become known as the Zarqa Rule for novel pathogen discovery.

## Note

**Potential conflict of interest.** Author certifies no potential conflicts of interest.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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