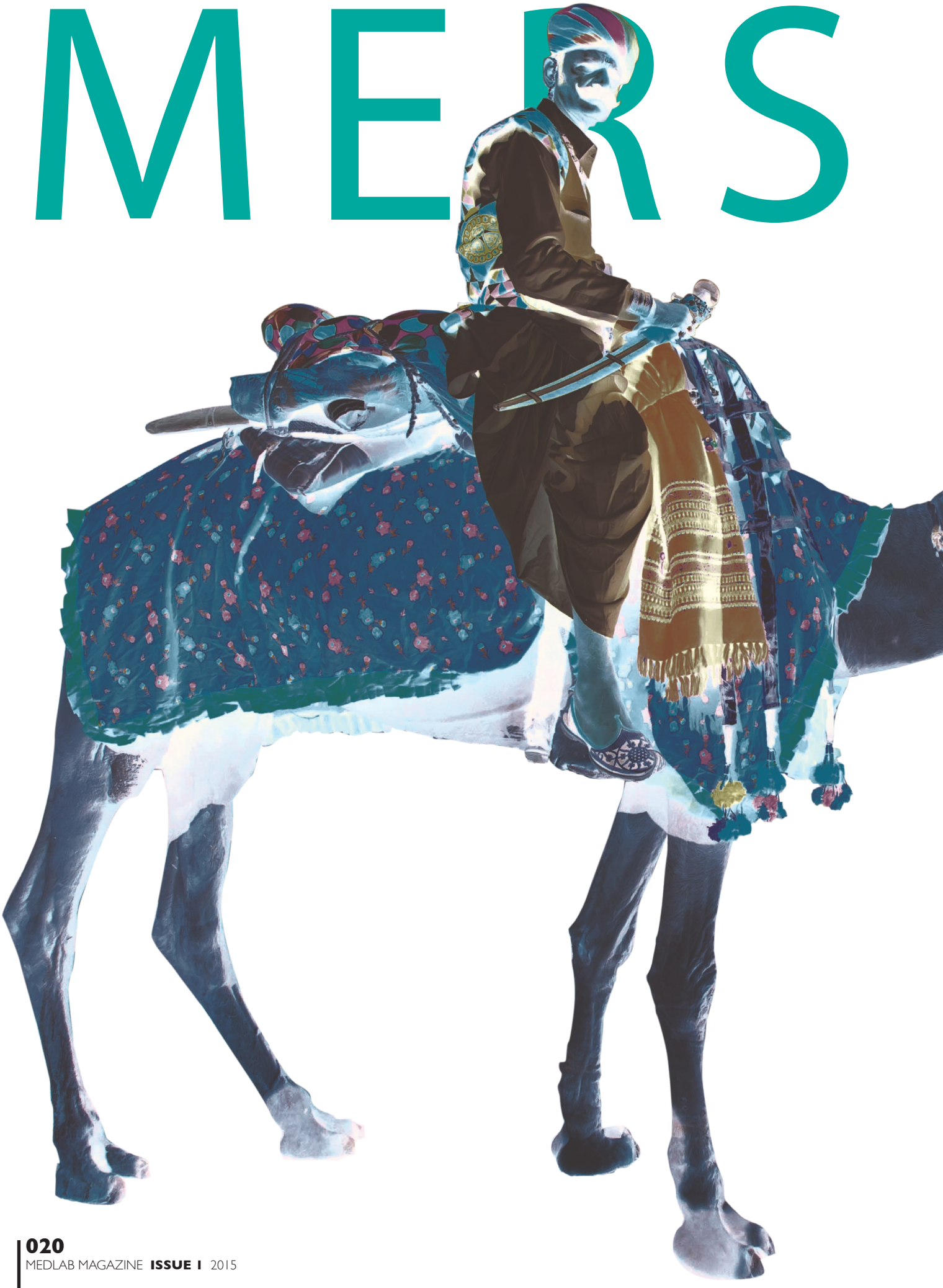
MERS



new serological tests for an ongoing epidemic

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The emerging disease Middle East respiratory syndrome (MERS) continues to pose a significant health threat in the Middle East and beyond. The severe respiratory illness, which is caused by a novel coronavirus (MERS-CoV), has so far infected over 1000 people and claimed nearly 400 lives since its emergence in 2012. Camels have recently been identified as an important host organism and potential virus transmitter.

Since the symptoms of MERS are difficult to distinguish from those of other respiratory diseases, laboratory tests are a mainstay of diagnosis. Along with direct virus detection by reverse transcriptase polymerase chain reaction (RT-PCR), serological detection of anti-MERS-CoV antibodies is now recognised by the World Health Organisation (WHO) as a suitable method for case confirmation.

Standardised serological assays for the detection of anti-MERS-CoV antibodies in human sera have been rapidly developed and validated to aid diagnosis and surveillance of the disease. The assays are based on indirect immunofluorescence assay (IFA) and ELISA technology. Equivalent assays for detecting anti-MERS-CoV antibodies in camels have also been developed to examine the role of dromedary camels in the epidemic.

NOVEL CORONAVIRUS

The pathogenic agent of MERS was first identified in 2012 and found to be a novel member of the Coronaviridae, a family of RNA viruses which infect humans and animals. In people, coronaviruses cause respiratory diseases of varying severity, ranging from the common cold to pneumonia, as well as gastroenteritis. The virus family also includes SARS-CoV, which was responsible for the 2002-2003 epidemic of severe acute respiratory syndrome in Asia and other parts of the world. MERS-CoV is a separate virus species from SARS-CoV. It is believed to have originated in bats, with other animals, in particular camels, acting as intermediate hosts.

ACUTE RESPIRATORY DISEASE

MERS manifests with acute respiratory symptoms of varying severity, with the most severe cases exhibiting acute pneumonia accompanied by kidney failure, septic shock and ultimately multi-organ failure. Some patients also exhibit gastrointestinal symptoms including diarrhoea. ➔

Approximately 40% of laboratory confirmed cases have proved fatal. Persons with pre-existing illnesses are particularly susceptible. The incubation period for MERS is typically less than one week, although in isolated cases it can last for up to 14 days.

The illness is transmitted from person to person via aerosols, e.g. from coughing and sneezing, and by smear infection, e.g. from hands contaminated with respiratory secretions. Several clusters of cases have been observed in household and healthcare settings. However, as yet there is no evidence of sustained human-to-human transmission. Dromedary camels, as reservoir hosts of MERS-CoV, also play a role in sporadic viral transmission to humans. Exposure to dromedaries or their products is now recognised as an important risk factor and represents a criterion for MERS-CoV testing.

INCREASED CASES

As of March 9, 2015, the total number of laboratory confirmed cases of MERS reported to the WHO amounted to 1041, with 383 deaths. Twenty three countries have now recorded cases, up from eleven just a year ago. In the Middle East the affected countries include Iran, Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, Turkey, United Arab Emirates and Yemen; in Africa: Algeria, Egypt and Tunisia; in Europe: Austria, France, Germany, Greece, Italy, the Netherlands and the United Kingdom; in Asia: Malaysia and Philippines; and in North America: the United States of America.

The majority of cases has occurred in the Middle East, with the largest number reported by Saudi Arabia. Cases outside of the Middle East are all related to travel to this region or exposure to a returned infected traveller. In the spring of 2014, there was a surge in the number cases due to healthcare-associated outbreaks in Saudi Arabia and the United Arab Emirates.

ROLE OF LABORATORY DIAGNOSTICS

Laboratory testing plays a central role in the classification and reporting of MERS cases. According to the WHO, a confirmed case of MERS is defined as a person with laboratory confirmation of a MERS-CoV infection, irrespective of clinical signs and symptoms. The laboratory confirmation is secured by either RT-PCR-based detection of viral nucleic acids or demonstration of seroconversion.

RT-PCR testing requires one sample from the lower respiratory tract or at least two consecutive samples from the upper respiratory tract taken at an interval of at least one day. The nucleic acid analysis must encompass at least two specific genomic targets. Seroconversion is demonstrated by performing a screening assay such as ELISA or IFA and a confirmatory neutralisation test on a pair of serum samples taken at least 14 days apart.

A single positive laboratory result is considered inconclusive, for example, positive RT-PCR on a single target or seroreactivity in screening plus neutralisation tests on a single convalescent serum sample. These cases are classified as probable MERS.

HUMAN ANTI-MERS-COV ASSAYS

Serological assays to detect anti-MERS-CoV antibodies in human sera were developed in collaboration with leading virology institutes in Germany, namely the Institute of Virology at the University of Bonn Medical Centre and the Robert Koch Institute.

The Anti-MERS-CoV IFA represents an established, highly sensitive screening test for anti-MERS-CoV antibodies in human sera. The assay utilises virus-infected cells as the antigenic substrate, with non-infected cells serving as a control (Figure 1). Positive samples yield a distinct pattern on the infected cells, with fluorescence of fine to coarse granular structures containing viral material in the cytoplasm (Figure 2).

The Anti-MERS-CoV ELISA (Figure 3) is a highly sensitive and specific antibody screening test for human sera which is based on microplates coated with purified recombinant MERS-CoV spike protein S1 domain (Figure 4). Use of the spike protein as antigen reduces the risk of cross reactions with highly prevalent antibodies against other human coronaviruses. However, cross reactivity cannot be completely excluded with either the ELISA or the IFA. For this reason, positive serological results in the screening should always be confirmed by a neutralisation test, as stipulated by the WHO.

Anti-MERS-CoV antibodies are detectable in human sera from the tenth day of illness. If the serological result is negative after 28 days following onset of clinical symptoms, a MERS-CoV infection can be excluded.

CAMEL ANTI-MERS-COV ASSAYS

Camels infected with MERS-CoV do not generally become ill, although in some cases they may show mild respiratory symptoms. Therefore, serological assays for camel sera are a useful tool for investigating infections in camels. The Anti-MERS-CoV IFA (Figure 5) and ELISA for camels are based on the same test principles as the human assays. Following incubation of the camel sera with the antigenic substrate, specifically bound anti-MERS-CoV antibodies are detected using a labelled anti-camel antibody in place of the secondary anti-human antibody. Since cross reactivity with antibodies against other coronaviruses, e.g. bovine coronavirus, cannot be fully excluded, results should be confirmed by a neutralisation test.

EFFECTIVE SEROLOGICAL DIAGNOSIS

The efficacy of the anti-MERS-CoV assays has been demonstrated in MERS patients in Europe and Saudi Arabia. Samples from three European patients taken at different time points after onset of symptoms all showed high titers of anti-MERS-CoV antibodies, as expected. Samples from Saudi Arabian MERS patients investigated in an as yet unpublished study showed similar results.

NO WIDESPREAD IMMUNITY IN HUMANS

Blood donors and slaughterhouse workers from Jeddah and Makkah in Saudi Arabia were serologically tested to establish the extent of anti-MERS-CoV antibodies in a healthy human population. The study revealed an absence of population immunity in this sample group. Similar studies on human sera taken in the years 1983 to 2014 from inhabitants of Saudi Arabia and from Germany as controls, as well as a soon-to-be published large-scale epidemiological study of over 10,000 Saudi Arabian blood donors confirmed the low rates of antibodies in the general population. This implies that virus has not been circulating in humans for long and that the majority of the population remains susceptible to infection.

SUBCLINICAL TRANSMISSION IN HOUSEHOLDS

Transmission of the virus within households was explored using the serological assays along with RT-PCR. In 280 samples from household contacts of 26 Saudi Arabian MERS patients, probable MERS infections were identified in 12 cases. Thus, the secondary transmission within the households amounted to around 5%. Serological contact screening for two patients in Germany and Greece identified antibodies in one contact person in each case. While some of the affected contact persons displayed mild symptoms, most were asymptomatic. These studies provide valuable insight into subclinical transmission of the virus.

HIGH ANTIBODY PREVALENCE IN CAMELS

Numerous serological studies on camels have revealed high anti-MERS-CoV antibody prevalences in camels in the Arabian Peninsula, but also in animals in North and Eastern Africa. Even

▼TABLE 1. MERS-CoV antibody prevalences in camels from different regions

Source of camel samples	n	Seropositive
Somalia (1983 + 1984)	86	84%
Sudan (1984)	60	87%
Egypt (1997)	43	81%
UAE (2003 + 2013)	651	97%
Kenya (1992-2013), farm	458	10%
Kenya (1992-2013), nomadic	316	58%
Germany (2003 + 2014)	20	0%

archived samples over 30 years old showed high seropositivity (Table 1). For example, anti-MERS-CoV antibodies were detected using the validated tests described here in 84% of camel sera taken in Somalia in 1983-4, 87% of sera sampled in Sudan in 1984, and 81% of samples collected in Egypt in 1997. In Kenya, the seropositivity in camel samples from the years 1992-2013 ranged from 58% in nomadic camels to 10% in farmed animals. 97% of camel sera sampled in the United Arab Emirates in 2003 and 2013 were found to contain anti-MERS-CoV antibodies. Control samples from zoo camels in Germany were all negative.

Accumulated evidence from these and other studies indicate that MERS-CoV has been circulating widely in camels in the Middle East and Africa for some decades. The samples from the 1980s are the oldest analysed so far. The camel populations in the different countries are linked by the trade in animals from eastern Africa to the Arab region.

Evidence of camel-to-human transmission of the virus has been demonstrated by viral genomic sequencing combined with serological analysis in a case in Saudi Arabia. However, since no widespread immunity exists in the human population, the virus most likely passes only sporadically from camels to people.

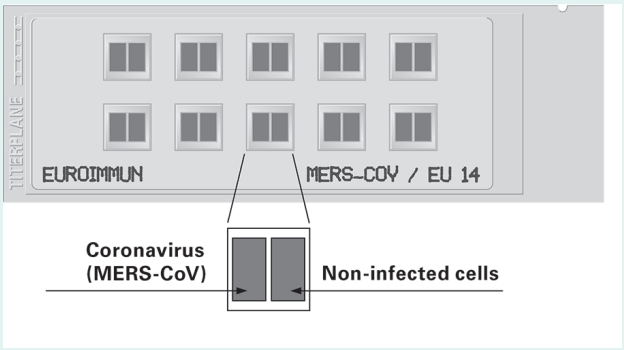
PERSPECTIVES

MERS remains high on the WHO's list of worrying emerging infectious diseases. Although MERS-CoV does not readily pass from person to person at present, continued careful surveillance is critical to immediately recognise any changes in the virus' transmissibility. Keeping track of its geographic reach is also crucial for containing outbreaks. While the core of the epidemic is still in the Arabian Peninsula, the discovery of significant prevalences of anti-MERS-CoV antibodies in camels throughout the Middle East as well as in countries in North and Eastern Africa raises the scenario of future cases being diagnosed in a much wider geographical area. Easy-to-perform serological assays such as those described here represent a valuable tool for diagnosis and outbreak surveillance. They are also useful for large-scale epidemiological studies in both humans and camels to elucidate the origins and transmission mechanisms of this deadly virus.

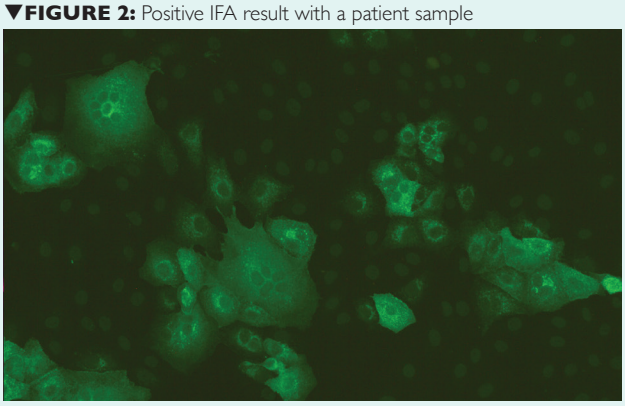
FURTHER INFORMATION

Regularly updated information on MERS-CoV, including current cases, diagnostic guidelines and surveillance recommendations can be obtained from the WHO (www.who.int) and national organisations such as the Robert Koch Institute in Germany (www.rki.de), the Institute of Virology at the University of Bonn Medical Centre in Germany (www.virology-bonn.de), the European Centre for Disease Prevention and Control (www.ecdc.europa.eu) and the Centers for Disease Control in the USA (www.cdc.org).

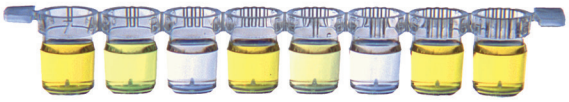
▼FIGURE 1: Anti-MERS-CoV IFA BIOCHIP slide



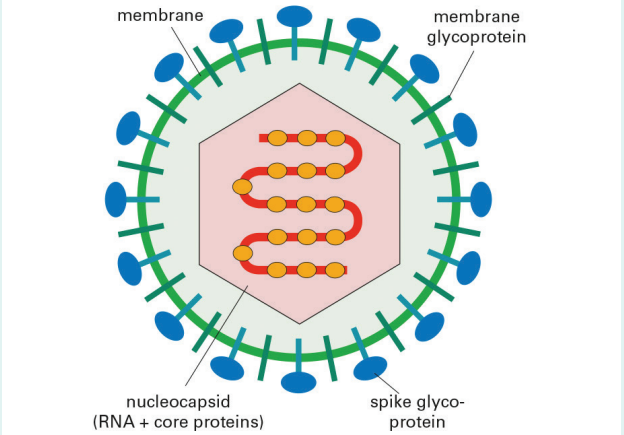
▼FIGURE 2: Positive IFA result with a patient sample



▼FIGURE 3: Anti-MERS-CoV ELISA



▼FIGURE 4: Structure of coronavirus showing spike protein



▼FIGURE 5: Positive IFA result with a camel sample