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Seroprevalence of MERS-CoV in healthy adults in western Saudi Arabia, 2011–2016

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ABSTRACT

Background: The Middle East respiratory syndrome coronavirus (MERS-CoV) is a newly recognized zoonotic coronavirus. Current evidence confirms the role of dromedaries in primary human infections but does not explain the sporadic community cases. However, asymptomatic or subclinical cases could represent a possible source of infection in the community.
Methods: Archived human sera (7461) collected between 2011 and 2016 from healthy adult blood donors from 50 different nationalities in the western part of Saudi Arabia were obtained for MERS-CoV seroprevalence investigation. Samples were tested for MERS-CoV S1-specific antibodies (Abs) by ELISA and confirmed by testing for neutralizing Abs (nAbs) using both pseudotyped and live virus neutralization assays.
Results: Out of 7461 samples, 174 sera from individuals with 18 different nationalities were ELISA positive (2.3%, 95% CI 2.0–2.7). Presence of nAbs was confirmed in 17 samples (0.23%, 95% CI 0.1–0.4) of which one sample exhibited positivity in both neutralization assays. Confirmed seropositivity was identified in young (15–44 years) men and women from Saudi Arabia, Egypt, Yemen, Pakistan, Palestine, Sudan, and India without significant preference.
Conclusions: An increasing trend of MERS-CoV seroprevalence was observed in the general population in western Saudi Arabia, suggesting that asymptomatic or mild infections might exist and act as an unrecognized source of infection. Seropositivity of individuals from different nationalities underscores the potential MERS exportation outside of the Arabian Peninsula. Thus, enhanced and continuous surveillance is highly warranted.

Introduction

The Middle East respiratory syndrome (MERS) is an emerging respiratory infection associated with a global public health concern. It is caused by a novel lineage C beta coronavirus that first emerged in 2012 in the Arabian Peninsula [1,2]. Typical MERS symptoms include fever, myalgia, cough, chest pain, and shortness of breath with pneumonia, gastrointestinal symptoms, multiple organs failure (MOF), and death being frequent in severe cases especially in the elderly and patients with comorbidities [1–4]. Nonetheless, mild and asymptomatic infections are clearly not uncommon [4–6].

Dromedaries in Saudi Arabia and several other Middle Eastern and African countries are a major MERS-CoV reservoir [7–15]. While some studies have shown some seropositivity in common non-camelid livestock species such as sheep and goats suggesting a possible role of these animals in MERS-CoV transmission,

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especially when they are in contact with infected camels [9,16,17], further studies are required to confirm these findings. Therefore, current epidemiological data highly suggest that infected camels could be the main source of primary zoonotic human cases. However, many sporadic presumably primary cases reported no contact or exposure history with camels, and thus a clear epidemiological link of transmission is not well-established [5,6]. Furthermore, despite the fact that most secondary cases were due to human-to-human spread in household and/or healthcare settings [2,3], there is no evidence of sustained human-to-human transmission, and the virus does not seem to pass easily between humans [18,19].

As of December 2019, more than 2499 laboratory-confirmed human cases have been reported from 27 countries with ~35% mortality [20]. However, this seems to be an overestimation of the true mortality rate as mild and asymptomatic MERS cases might be missed during routine testing and can only be identified via enhanced molecular, serological, and immunological surveillance [4–6,21,22]. Such cases are usually more common among camel handlers as well as household individuals and/or healthcare workers who are in contact with MERS patients [4,5,22–25]. Furthermore, while few early small studies have reported no serological evidence of MERS-CoV in children, abattoir workers, veterinarians, animal handlers and even unexposed individuals in Saudi Arabia [15,26–28], at least one large cross-sectional study has revealed ~0.15% seropositivity in the general Saudi population [5]. Similarly, a study from Qatar showed that 0.21% of the general population were seropositive by ELISA screening of 4719 blood donors however the presence of nAbs was only confirmed in one blood donor (0.02%) [29]. Interestingly, despite the high prevalence of MERS-CoV in camels from different countries such as Kenya, Nigeria, Egypt and Pakistan, confirmed evidence of MERS cases among humans is very limited in these countries except for two seropositive camel handlers in Kenya [10–14,30]. Such data clearly suggest that unknown asymptomatic and subclinical MERS infections or even unrecognized cases might in fact exist in the general population and could represent an underappreciated source of human-to-human transmission. Therefore, it is essential to conduct continuous and enhanced surveillance and epidemiological studies to determine MERS-CoV prevalence especially in endemic regions.

Several serological methods have been proven to be valuable in MERS epidemiological studies where screening assays such as ELISA or immunofluorescence should be confirmed by neutralization assays for increased specificity. Here, we used validated recombinant S1-ELISA (rS1-ELISA) [31] combined with neutralization assays to investigate the seroprevalence rate of MERS-CoV in a large cohort of archived sera collected between 2011 and 2016 from healthy blood donors from 50 different nationalities residing in western Saudi Arabia. All samples that tested positive using the rS1-ELISA were confirmed by both MERS-pseudotyped and live virus neutralization assays, and cases that were positive in either neutralization assay were considered confirmed seropositive for MERS antibodies (Abs).

Materials and methods

Clinical samples

A total of 7461 archived sera collected from healthy blood donors older than 17 years between 2011 and 2016 were retrieved. Demographic data including sex, age and nationality were obtained. Ethical approval was obtained from the Unit of Biomedical Ethics in King Abdulaziz University Hospital. Before starting the study, all samples were anonymized using serial numbers.

Cell line, virus and recombinant protein

African Green monkey kidney-derived Vero E6 cells (ATCC #1568) were cultured and maintained in complete Dulbecco's modified Eagle's medium (DMEM). Human MERS-CoV/Hu/Taif/SA/2015 isolate was propagated and titrated in Vero E6 cells by tissue culture infection dose 50 ($TCID_{50}$) assay.

Recombinant S1-enzyme linked immunosorbent assay (rS1-ELISA)

In-house indirect MERS-CoV S1 subunit protein based ELISA (rS1-ELISA) was used for the initial screening of MERS-CoV Abs in serum samples as previously described [31]. In brief, 1:400 diluted serum samples were incubated on blocked flat bottom microtiter plates (Thermo Scientific, Rochester, NY) coated with 2 μg/ml recombinant MERS-CoV S1 protein (SinoBiological, China) for 1 h at room temperature. Subsequently, 1:2000 diluted peroxidase-conjugated sheep anti-human IgG Abs (Amersham ECL, Pittsburgh, PA) were added, and colorimetric reaction was developed using tetramethylbenzidine (TMB) substrate. TMB BlueSTOP Solution (KPL, Gaithersburg, MD) was used to stop the reaction, and absorbance was read at 650 nm. All samples were tested in duplicates and samples with an optical density (OD) above the cut-off value of 0.26 were considered positive as previously described [31]. This cut-off value was predetermined as the average OD values of known negative serum samples +3 standard deviation (SD), and showed 100% sensitivity and 90% specificity as previously described [31].

MERS-pseudotyped neutralization test (ppNT)

MERS pseudotyped neutralization test (ppNT) was performed as described previously with minor modifications [32]. Heat-inactivated serum samples at 1:20 and 1:40 dilutions were co-incubated with a standard amount of the MERS pseudotyped viral particles (~200,000 RLU) in the presence of Huh7.5 cells (~10,000 cells) per well, and plates were incubated for 48 h at 37 °C. Cells only and cells with MERS pseudotyped (MERSpp) viral particles (with no serum) were included in quadruplicates as controls in all tested plates. Then, cells were lysed, and luciferase activity was measured using Bright-Glo™ Luciferase Assay System (Promega, Madison, WI) according to manufacturer's instructions. Samples were tested in duplicates in two independent experiments, and samples were considered positive with ppNT titer of >20 upon 50% reduction of luciferase activity in all wells in the two experiments compared to cells with MERSpp viral particles control.

Live virus microneutralization test (MNT)

Live virus microneutralization assay was performed as previously described [31]. Briefly, serially diluted (2-fold) heat-inactivated rS1-ELISA positive serum samples were co-incubated with equal DMEM volume containing 100 $TCID_{50}$ of MERS-CoV and tested in quadruplicates to determine the highest dilution that inhibits cytopathic effect (CPE) in confluent Vero E6 cells. Virus control and cell only control were always included in each plate. Neutralizing Ab (nAb) titers were expressed as the reciprocal of the highest dilution of each serum sample that completely prevented CPE in all wells, and MN_{100} titer of ≥ 1:10 dilution was considered positive. Samples were tested twice independently.

Statistical analysis

Yates corrected two-tail Chi^2 (χ^2) test, Fisher's exact tests, and 95% Mid-P Exact confidence limits (95% Cls) were calculated using OpenEpi (Open Source Epidemiologic Statistics for Public Health).

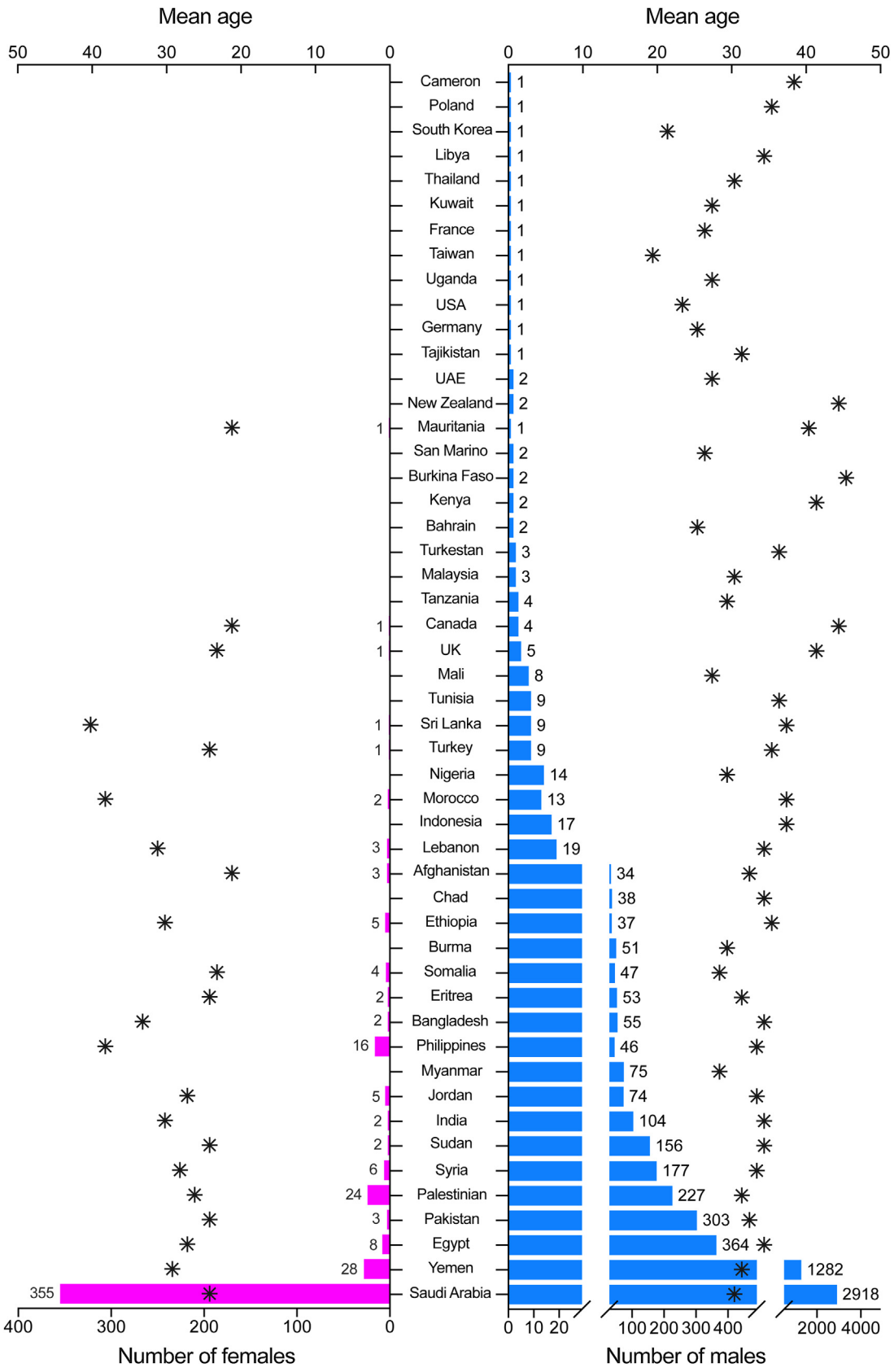

Fig. 1. Demographic information of tested individuals based on country of origin. Asterisks indicate mean age of subjects from each country.

Table 1
Socio-demographic characteristics of tested blood donors.[a]

Characteristic	Categories	n (%)
Sex	Male	6183 (92.9)
	Female	475 (7.1)
Age group (years)	15–24	1743 (26.2)
	25–34	2916 (43.8)
	35–44	1486 (22.3)
	45–54	448 (6.7)
	≥55	65 (1.0)
Nationality	Saudi	3273 (49.2)
	Non-Saudi	3385 (50.8)
Total		6658

[a] Socio-demographic data were only available for 6658 samples (89.2%) out of the 7461 tested archived serum samples.

Results

Between 2011 and 2016, 7461 archived sera from healthy blood donors were obtained to evaluate the burden of MERS-CoV in the western region of Saudi Arabia. Specifically, 90, 1360, 999, and 5012 archived serum samples were retrieved from the years 2011, 2012, 2013, and 2016, respectively. Demographic data were available for 6658 individuals (89.2%) of the total archived samples (Table 1). The mean age of the tested individuals with available demographic information was 30.8 ± 8.5 years (median age 30 years, range 17–65 years including 6183 males (92.9%) and 475 females (7.1%). The mean age was 31.3 ± 8.4 years for males (median age 30 years, range 17–65 years), and 25 ± 7.6 years for females (median age 22 years, range 17–59 years). Tested samples were from individuals from 50 different countries (Fig. 1) in which Saudis represented the majority (3273, 43.9%) followed by Yemenis (1310, 17.5%) and Egyptians (372, 5.0%).

Out of the 7461 tested serum samples, 174 samples (2.3%, 95% CI 2.0–2.7) were found positive for anti-MERS binding IgG Abs with OD values above the cut-off value. While 1.1% (1/90) in 2011 and 0.7% (10/1360) in 2012 of the samples were positive for MERS-CoV IgG, higher seroprevalence of 1.5% (15/999) and 3.0% (148/5012) were observed in 2013 and 2016, respectively, suggesting possible increase in seropositivity in the general population in the western region of Saudi Arabia (Table 2). To confirm MERS seropositivity, samples were subjected to ppNT and MNT assays and samples that are positive in either test were considered confirmed positive. Out of the 174 rS1-ELISA positive sera, 9.8% (i.e. 17 specimens, 0.23%, 95% CI 0.1–0.4) were confirmed to have nAbs (Fig. 2 and Table 2). All these confirmed positive samples were obtained in 2016 in which only one sample was positive in both ppNT and MNT assays with a MNT_{100} titer of 20 (Table 2). Notably, testing a large number of rS1-ELISA negative samples by these confirmatory assays showed no false negative results further confirming the validity of our in-house developed indirect rS1-ELISA with a 100% sensitivity and even higher specificity (97.9%) compared to our previous report [31].

Cases were confirmed in individuals between the age of 15–44 years with highest rate in the 25–34 age group, however there was no statistical differences between the groups (Table 3). Based on confirmed cases, no significant difference was observed between men (16 [0.26%] of 6183 and women 1 [0.21%] of 475) using Fisher's exact test. Furthermore, confirmed seropositivity was identified in individuals from Saudi Arabia, Egypt, Yemen, Pakistan, Palestine, Sudan, and India without significant preference (Table 4). While proportion of confirmed infected cases was higher in this study

Table 4
Seropositivity based on country of origin.

Country	Total samples n (%)	rS1-ELISA positive n (%) (95% CI)	Confirmed cases n (%) (95% CI)
Saudi Arabia	3273 (43.9)	83 (2.5; 2.0–3.1)	9 (0.3) (0.1–0.5)
Yemen	1310 (17.5)	32 (2.4) (1.7–3.4)	3 (0.2) (0.1–0.6)
Egypt	372 (5.0)	8 (2.2) (1.0–4.1)	1 (0.3) (0.0–1.3)
Pakistan	306 (4.1)	8 (2.6) (1.2–4.9)	1 (0.3) (0.0–1.6)
Palestine	251 (3.4)	4 (1.6) (0.5–3.8)	1 (0.4) (0.0–1.9)
Syria	183 (2.5)	2 (1.1) (0.2–3.5)	0 (0.0) (0.0–1.6)
Sudan	158 (2.1)	5 (3.2) (1.2–6.8)	1 (0.6) (0.0–3.1)
India	106 (1.4)	6 (5.7) (2.3–11.4)	1 (0.9) (0.0–4.6)
Jordan	79 (1.1)	3 (3.8) (1.0–9.9)	0 (0.0) (0.0–3.7)
Myanmar	75 (1.0)	1 (1.3) (0.1–6.4)	0 (0.0) (0.0–3.9)
Bangladesh	57 (0.8)	1 (1.8) (0.1–8.3)	0 (0.0) (0.0–5.1)
Eritrea	55 (0.8)	2 (3.6; 0.6–11.5)	0 (0.0) (0.0–5.3)
Chad	38 (0.7)	2 (5.3) (0.9–16.3)	0 (0.0) (0.0–7.6)
Afghanistan	37 (0.5)	1 (2.7) (0.1–12.6)	0 (0.0) (0.0–7.8)
Nigeria	14 (0.2)	1 (7.1) (0.4–32.5)	0 (0.0) (0.0–20.6)
Mali	8 (0.1)	1 (12.5) (0.6–48.0)	0 (0.0) (0.0–31.2)
UAE	2 (0.0)	1 (50.0) (2.5–97.5)	0 (0.0) (0.0–77.6)
USA	1 (0.0)	1 (100) (5–100)	0 (0.0) (0.0–95.0)
Other countries[a]	333 (4.5)	0 (0.0) (0.0–0.9)	0 (0.0) (0.0–0.9)
Unknown[b]	803 (10.8)	12 (0.5) (0.8–2.5)	0 (0.0) (0.0–0.4)
Total	7461	174 (2.3) (2.0–2.7)	17 (0.2) (0.1–0.4)

[a] Countries where no individuals had seropositivity in rS1-ELISA screening assay.
[b] Individuals with unknown country of origin.

Table 2
Seroprevalence of MERS-CoV in blood donors based on year of collection.

Year	Total samples n (%)	rS1-ELISA positive n (%) (95% CI)	$ppNT_{50}$ positive n (%) (95% CI)	MNT_{100} positive n (%) (95% CI)
2011	90 (1.2)	1 (1.1) (0.1–5.4)	0 (0.0) (0.0–3.3)	0 (0.0) (0.0–3.3)
2012	1360 (18.2)	10 (0.7) (0.4–1.3)	0 (0.0) (0.0–0.2)	0 (0.0) (0.0–0.2)
2013	999 (13.4)	15 (1.5) (0.9–2.4)	0 (0.0) (0.0–0.3)	0 (0.0) (0.0–0.3)
2016	5012 (67.2)	148 (3.0) (2.5–3.4)	17 (0.3) (0.2–0.5)	1 (0.0) (0.0–0.1)
Total	7461	174 (2.3) (2.0–2.7)	17 (0.2) (0.1–0.4)	1 (0.0) (0.0–0.1)

Table 3
Seroprevalence of MERS-CoV in blood donors based on age group.

Age group	Total samples n (%)	rS1-ELISA positive n (%) (95% CI)	$ppNT_{50}$ positive n (%) (95% CI)	MNT_{100} positive n (%) (95% CI)
15–24	1743 (23.4)	46 (2.6) (2.0–3.5)	1 (0.1) (0.0–0.3)	0 (0.0) (0.0–0.2)
25–34	2916 (39.1)	72 (2.5) (2.0–3.1)	11 (0.4) (0.2–0.7)	1 (0.0) (0.0–0.2)
35–44	1486 (19.9)	32 (2.2) (1.5–3.0)	5 (0.3) (0.1–0.7)	0 (0.0) (0.0–0.2)
45–54	448 (6.0)	7 (1.6) (0.7–3.1)	0 (0.0) (0.0–0.7)	0 (0.0) (0.0–0.7)
≥55	65 (0.9)	5 (7.7) (2.9–16.2)	0 (0.0) (0.0–0.5)	0 (0.0) (0.0–0.5)
Unknown[a]	803 (10.8)	12 (0.5) (0.8–2.5)	0 (0.0) (0.0–0.4)	0 (0.0) (0.0–0.4)
Total	7461	174 (2.3) (2.0–2.7)	17 (0.2) (0.1–0.4)	1 (0.0) (0.0–0.1)

[a] Individuals with unknown age information.

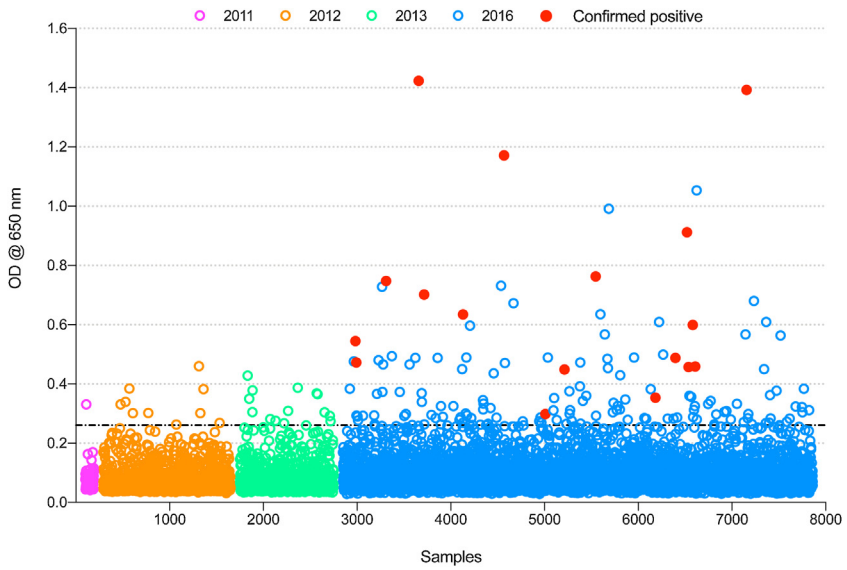

Fig. 2. Overall seroprevalence result of samples for the period between 2011–2016. 174 positive samples with OD value above the cut-off in rS1-ELISA. Only 17 samples were confirmed to have nAbs by either ppNT or MNT assays from 2016, and are shown in red. Dotted black line indicates the cut-off of the rS1-ELISA.

Table 5
MERS-CoV Abs in the general population of the western region of Saudi Arabia vs the whole country.

	Total number	rS1-ELISA positive		Confirmed positive	
Study		n (%; 95% CI)	Chi^2 test[a]	n (%; 95% CI)	Chi^2 test[a]
Western region[b]	7461	174 (2.3) (2.0–2.7)	–	17 (0.2) (0.1–0.4)	–
Western region[c]	1513	19 (1.3) (0.8–1.9)	$P = 0.0113$	0 (0.0) (0.0–0.2)	$P = 0.1250$
Nation-wide[c]	10009	152 (1.5) (1.3–1.8)	$P = 0.0001$	15 (0.2) (0.1–0.2)	$P = 0.3128$

[a] Yates corrected Chi^2 test of positive cases in each cohort vs cases this study.
[b] This study.
[c] Results from Ref. [5] on samples from 2013.

(0.23%) compared to the previously reported 0.00% rate in a cohort from the western region of Saudi Arabia in 2013 from a nation-wide study [5], we observed no statistical differences between the examined cohorts (Table 5). Data summary of positive cases are shown in Supplementary data.

Discussion

MERS-CoV is reminiscent of the severe acute respiratory syndrome-coronavirus (SARS-CoV) which emerged in 2002, and thus has a potential to spread globally as seen in 2015 in South Korea. Several epidemiological studies have proven MERS-CoV endemicity in dromedaries, which can be in close contact with humans in the Arabian Peninsula and Africa. While contact with MERS-CoV shedding camels could be the main cause of primary cases in the Arabian Peninsula, such transmission cannot explain all laboratory confirmed infections in humans. Thus, it was proposed that asymptomatic or mild cases in the general population could act as an unrecognized source of infection in these endemic regions [4–6,22]. Nonetheless, only limited number of reports have investigated MERS-CoV seroprevalence in the general populations especially in endemic regions [5,25,29]. Therefore, active and enhanced surveillance is pivotal in order to better understand the true burden of MERS-CoV.

In the present study, we investigated MERS-CoV seroprevalence in archived human sera collected in the western region of Saudi Arabia. These samples were collected from healthy individuals from 50 different nationalities who donated blood at a major tertiary hospital between 2011 and 2016. Our data showed an evidence of MERS-CoV S1-specific binding Abs in 2.3% of the tested cohort (174/7461) in which seroprevalence increased over the years. Interestingly, binding Abs were detected in individuals from 18 different countries, suggesting that such individuals could be responsible for MERS-CoV exportation outside the Arabian Peninsula. Consistent with previous reports [4,5,29], nAbs were only confirmed in 9.8% of these rS1-ELISA positive serum samples (17/174) obtained mostly from Saudi men and resulting in a confirmed seroprevalence of 0.23% in the general population in the western region of Saudi Arabia. This confirmed seroprevalence is higher than the previously reported rates in the general Saudi population (0.15%) as well as the population in the western region of the country (0.00%) in 2013, although no statistical significances were observed between the two studies.

It is important to note that the possibility of asymptomatic or mild infections in the remaining non-confirmed rS1-ELISA 157 individuals cannot be overlooked. This is mostly because of the high specificity of ppNT and MNT assays [16,33] that could result in reduced sensitivity in the testing algorithm as well as the high sensitivity of rS1-ELISA (at least 10-fold more sensitive) and so could actually be detecting S1-binding but non-neutralizing Abs. Furthermore, while cross-reactive low-affinity IgG against other coronaviruses cannot be completely excluded in ELISA [29], MERS-CoV S1 has low homology and cross-reactivity with S1 subunit from other known coronaviruses [33,34]. Thus, it could be postulated that some of these young and healthy individuals between the age of 15–44 years might have been indeed exposed to MERS-CoV but only suffered from subclinical infections and mounted transient and weak nAb responses that might wane quickly resulting in false negative results by neutralization assays. This is partially true as it was recently demonstrated that not all individuals with

history of MERS infection including high-risk groups could elicit detectable nAbs [21,22]. Another possibility is that antigenically diverse MERS-CoV strains are circulating and thus not all binding Abs are cross-neutralizing. Therefore, prospective seroepidemiological studies should combine serological and immunological methods in order to determine the actual disease burden caused by asymptomatic or subclinical cases acting as an unidentified intermediate source for virus transmission in the population.

By simple comparison to the previously published nation-wide seroprevalence study in Saudi Arabia [5], it is clear that the seroprevalence of S1-binding Abs from the 2013 cohort in this current study (1.5%; 95% CI 0.9–2.4) is comparable to that reported by Müller et al. in the cohort collected from the same region in 2013 (1.3%; 95% CI 0.8–1.9) in which no nAbs were detected in both reports. Nonetheless, we observed higher overall rate of confirmed seropositivity (0.23; 95% CI 0.1–0.4) compared to the previously reported nation-wide seroprevalence rate (0.15; 95% CI 0.1–0.2) in which cases seem to increase over the years [5]. Similarly, extrapolation of our results also indicates that there could be around 19,000 seropositive individuals out of the total population of 8,325,304 in the western region alone in 2016. Such number is of huge concern especially that this region hosts one of the largest mass gathering events (Hajj and Umrah), where the continuous travel of people and movement of livestock to and from the western part of Saudi Arabia could represent a possible way for MERS-CoV infection and exportation by human-to-human or zoonotic transmission.

Summary

Screening of 7461 serum samples collected from healthy blood donors from 50 different nationalities residing in western Saudi Arabia showed an increased seropositivity rate (0.23%) in young and healthy individuals aged between 15–44 years from 7 different countries.

Contributors

AAD did the laboratory tests, analyzed data, and generated figures. SSA, AMHass, and MM did the laboratory tests and coordinated sample collection and data retrieval. AA did the laboratory tests. SA and RYA searched the published work. AAM analyzed data. SIH arranged sample collection and data retrieval. NKA did the laboratory tests and analyzed data. EIA organized and supervised sample logistics and the study. AMHash designed and coordinated the study, searched the published work, wrote the manuscript, analyzed data, and generated figures. All authors revised and approved the final version of the manuscript.

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Competing interest

None declared. The sponsors had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jiph.2020.01.001.

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