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Original Article

Seroprevalence of Middle East Respiratory Syndrome Corona Virus in dromedaries and their traders in upper Egypt

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Abstract

Introduction: Camel trade in Egypt depends mainly on importation. Seemingly healthy imported camels are responsible for the ingress of serious diseases into Egypt. A striking example of this concerning public health globally is the Middle East respiratory coronavirus (MERS-CoV) which causes case fatalities of over 34%. Here, we determined the seroepidemiological situation of the MERS-CoV in imported camels and their traders in Upper Egypt.

Methodology: Sera of sixty-three dromedaries and twenty-eight camel traders were recruited (January 2015-December 2016). The age, gender, and sampling locality of each sampled camel and human were obtained. Semi-quantitative anti-MERS-CoV IgG ELISAs which utilize the purified spike protein domain S1 antigen of MERS coronavirus (MERS-CoV S1) were used to detect specific IgG antibodies against the virus.

Results: The data showed that 58.73% of imported camels and 25% of traders had antibodies specific to MERS-CoV. Interestingly, like seroreactive camels, all seropositive humans were apparently healthy without any history of developing severe respiratory disease in the 14 days prior to sampling. Having specific antibodies among the examined camel sera was significantly different ($P < 0.0001$) in relation to various sampling localities, gender and age groups. In contrast, the seropositivity rate of MERS-CoV IgG in humans did not differ significantly by any of the studied factors.

Conclusions: The current study provides the first serological evidence of occupational exposure of humans to MERS-CoV in Africa. Additionally, it reports that imported camels could be implicated in introducing MERS-CoV into Egypt. Accordingly, application of strict control measures to camel importation is a priority.

Key words: Dromedary camels; MERS-CoV; ELISA; zoonosis.

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Introduction

Middle East respiratory coronavirus (MERS-CoV) is a serious zoonotic pathogen associated with severe respiratory infection, renal failure and multiorgan damage in infected patients [1]. Since its discovery in 2012 and as of the end of September 2019, 2458 MERS-CoV laboratory-confirmed cases in humans were reported across twenty-seven countries worldwide with a case fatality rate exceeding 34% [2].

The single-humped, Arabian dromedary camel (*Camelus dromedarius*) has been strongly implicated as the MERS-CoV reservoir in which the disease is either asymptomatic or manifested as mild respiratory infection [3]. In the past three decades, high seropositivity rates of MERS-CoV were detected in dromedaries from many African and Arabian countries [4–16]. Recent reports indicating the genetic identity between the human and camel isolates of MERS-CoV have provided strong evidence that dromedaries are potential sources of human infection [13,17–21]. Occupational MERS-CoV zoonosis from infected dromedaries was extensively documented in the Arabian Peninsula [21–26] whereas, no zoonotic MERS-CoV cases have been reported to date in Africa [27]. Camel to human transmission of MERS-CoV occurs via direct contact with dromedary camels, consumption of unpasteurized camel milk and raw meat and/or medicinal use of camel urine [28]. In 2013, human-to-human transmission of the virus was documented among healthcare workers in Saudi Arabia [29].

Epidemiological studies regarding the disease have been previously conducted in Egypt [7,9,27,30,31], however, further knowledge regarding the prevalence of MERS-CoV in imported camels and/or the traders involved in their importation is required. Therefore, we lay out the present serological investigation to explore the preliminary seroepidemiological situation of

MERS-CoV in imported dromedary camels and their traders in Upper Egypt.

Methodology

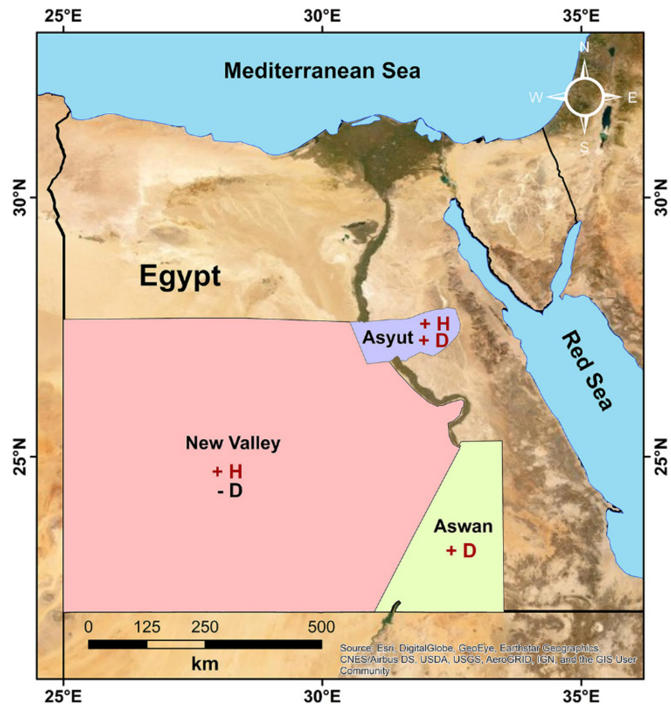
Sample size and sampling criteria

Animal sample size for this study was determined after the equation ($n = Z^2 \times P \times q/d^2$), where "n" is the minimal calculated sample size,"Z" is a constant equal to 1.96 [32]. The MERS-CoV prevalence rate in imported camels in previous studies conducted in Egypt was 88.7–98.2% [7, 9,30]. So, at a confidence level of 95%, the sample size required for the present investigation should fall between 27–154 camels. Sampling protocol was designed to include three different governorates in the southern part of Egypt involving Aswan, Asyut and New Valley (Figure 1). The study was carried out from January 2015 to December 2016. A total of 63 blood samples were randomly collected from imported dromedary camels in the three investigated areas, including Aswan (33), Asyut (14), and New Valley (16). All the sampled camels were adults primarily imported for slaughtering purposes with an age range of 5 to 8 years old and included 35 males and 28 females. Camel samples from Aswan were collected once imported animals arrived from Sudan. Given the tight and continuous contact of camel traders with their animals, they are more likely to contract and transmit the infection to their human contacts. Therefore, we have focused on including this high risk and neglected group in the present work to reveal the extent of MERS-CoV circulation. A sum of 28 camel traders, including 26 males and 2 females with an age range between 20 and 60 years old from Asyut and New Valley Governorates agreed to take part in this study. The age, gender, and sampling locality of each sampled camel and human were recorded. Camels and humans included in the study were apparently healthy with no previous respiratory system infection within 14 days of sampling.

Serum samples

A total of 5 CC blood samples was taken by veinopuncture from camels and humans. The samples were drained directly into plain vacutainer tubes and transferred immediately to the laboratory at the Department of Animal Hygiene and Zoonoses, Assiut University. Blood samples were centrifuged (1500 × *g*, 15 minutes) and sera separated and stored at -20 °C until processed.

Figure 1. Map showing the three sampling sites and distribution of MERS-COV seropositive dromedaries and humans. (+H) stands for MERS CoV-seropositive humans. (+D) stands for MERS CoV-seropositive dromedaries. (-D) stands for MERS CoV-seronegative dromedaries. (from www.mapcruzin.com/download-free-arcgis-shapefiles.htm and processed in the ArcGis environment (ArcGis Desktop 10.5) to extract the map of the study area).



Serological assay

Semi-quantitative anti-MERS-CoV IgG camel and human ELISAs (Euroimmun, Lübeck, Germany) were used in the present study to detect specific IgG antibodies against MERS-CoV in camel and human sera, respectively. The kits utilize the purified spike protein domain S1 antigen of MERS coronavirus (MERS-CoV S1) based on its high sensitivity and high specificity [11,13,33]. Briefly, the serum samples were diluted (1:101) and the test procedure was then performed as per the manufacturer's instructions. Photometric measurement of the color intensity was read at 450 nm with a reference between 620 nm and 650 nm using Bio Tek Model Elx800 Absorbance Microplate Reader (Fisher Scientific, Lenexa, KS, USA). The result was evaluated semiquantitativly using ratio values as follows: Ratio = Extinction value of the sample/the extinction value of the calibrator. Serum samples with a ratio over 1.1 were considered positive for MERS-CoV infection.

Table 1. Seroprevalence of MERS CoV infection in dromedary camels and their traders.

Species examined	No. examined	Positive No. (%)	Negative No. (%)	Odds Ratio (95% CI)
Camel	63	37 (58.73)	26 (41.27)	4.27 (1.58–11.51)
Human	28	7 (25)	21 (75)	0.23 (0.087–0.63)
Total	91	44 (48.35)	47 (51.65)	P = 0.0034

Statistical analysis

Statistically, categorical variables were computed by Fisher's exact test and Odds ratios (OR) with 95% confidence interval (95% CI) in the GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). P values < 0.05 were considered significant.

Ethical statement

Verbal consent was taken from all participants and they were informed that the obtained information will be strictly confidential. Written consent was not possible because neither literate nor illiterate participants agree to sign such type of consent based on the Egyptian cultural settings especially in field studies. Animal experiments were carried out in accordance with the regulatory rules of Assiut University for animal research. The method of consent and both the animal and human work were approved by the ethics committee of Assiut University, Egypt (Reference no.17300193).

Results

In the present work, the MERS-CoV infection seroprevalence was determined in camels and humans in direct contact. The overall seroprevalence was 48.35% (44 of 91) which accounted for 58.73% (37 of 63) and 25% (7 of 28) in camels and humans, respectively. In this written report, there was a significant relationship between the species and the likelihood of having antibodies against the virus (P = 0.0034) and camels found to be at a greater exposure rate (Odds ratio, OR = 4.27 (95% CI, 1.58–11.51)) compared to humans in contact with camels (OR = 0.23 (95% CI, 0.087– 0.63)) (Table 1).

Statistical analyses of different variables, including locality, gender, and ages of the camels recruited to this subject area, revealed significant influences on the infection rate in camels with P < 0.0001 for each. Regarding locality, a high seroprevalence of MERS-CoV infection of 96.97% was reported among imported camel sera in Aswan Governorate; however, 35.71% and 0% were found to be seroreactive to the MERS-CoV antigen of those collected from Asyut and New Valley Governorates, respectively. Notably, 100% of the examined female dromedaries were found to be seropositive to MERS-CoV infection and had greater odds of exposure to acquire the MERS-CoV infection (OR = 159 (95% CI, 8.81–2870)) compared to males. This group was aged 7–8 years old and were obtained from Aswan Governorate. Moreover, 25.71% (9 of 35) of the examined male camels were found to be seropositive to MERS-CoV infection (Table 2).

In contrast, in the present study, no significant relationship between the age, sampling locality and gender of humans and the MERS-CoV infection rate. Nevertheless, there were higher odds of exposure in male (OR = 1.92 (95% CI, 0.082–44.96)) versus female (OR = 0.52 (95% CI, 0.022–12.16)) participants and in middle adulthood, ages 40–60 years (OR = 1.47 (95% CI, 0.26–8.23)) than in early adulthood, ages 20–39 years (OR = 0.68 (95% CI, 0.12–3.83)). Sampled

Table 2. Seroprevalence of MERS CoV infection among dromedary camels in different localities, age and gender groups in Upper Egypt.

Variable	No. examined	Positive No. (%)	Negative No. (%)	Odds Ratio (95% CI)
Sampling locality				
Aswan	33	32 (96.97)	1 (3.03)	57.60 (5.943–558.3)
Asyut	14	5 (35.71)	9 (64.29)	Reference value
New Valley	16	0 (0.00)	16 (100)	0.052 (0.003–1.06)
Total	63	37 (58.73)	26 (41.27)	P < 0.0001
Gender				
Male	35	9 (25.71)	26 (74.29)	0.006 (0.0003–0.11)
Female	28	28 (100)	0 (0.00)	159 (8.81–2870)
Total	63	37 (58.73)	26 (41.27)	P < 0.0001
Age				
5 – 6 years	35	9 (25.71)	26 (74.29)	0.006 (0.0003–0.11)
7 – 8 years	28	28 (100)	0 (0.00)	159 (8.81–2870)
Total	63	37 (58.73)	26 (41.27)	P < 0.0001

Table 3. MERS CoV seroconversion rate among humans adjusted by sampling locality, gender and age.

Variable	No. examined	Positive No. (%)	Negative No. (%)	Odds Ratio (95% CI)
Sampling locality				
Asyut	12	3 (25)	9 (75)	1.000 (0.18–5.64)
New Valley	16	4 (25)	12 (75)	1.000 (0.18–5.64)
Total	28	7 (25)	21 (75)	P = 1.0000
Gender				
Male	26	7 (26.92)	19 (73.08)	1.92 (0.082–44.96)
Female	2	0 (0.00)	2 (100)	0.52 (0.022–12.16)
Total	28	7 (25)	21 (75)	P = 1.0000
Age				
20 –39 years	14	3 (21.43)	11 (78.57)	0.68 (0.12–3.83)
40 – 60 years	14	4 (28.57)	10 (71.43)	1.47 (0.26–8.23)
Total	28	7 (25)	21 (75)	P = 1.0000

humans either from Asyut or New Valley were at equal risk of getting the virus (Table 3).

Discussion

Recently, the consumption of camel meat drastically increased in Egypt as it provides an affordable alternative to beef. Given that local camel breeds are reared by smallholders, the production does not cover the increasing demands and this shortage is compensated by importation of 1.2 million heads of single-humped camels per annum from Sudan and East African countries, primarily Ethiopia [7]. Depending on their possible role in the ingress of multiple exotic diseases into the Egyptian setting, imported camel associated epidemiological studies have to be extensively conducted. In this study, we have investigated a random sample of imported camels from different localities in Upper Egypt including Aswan, Asyut and New Valley Governorates, as well as some camel traders for the circulation of specific antibodies against MERS-CoV.

The present study used EUROIMMUN ELISA based on the purified spike protein domain S1 antigen for determining the seropositivity rate in camels and humans. The performance of this kit for testing MERS-CoV infection has been previously evaluated and it was stated that using a confirmatory test after initial screening of samples with this ELISA is not mandatory [33]. It has been also successfully used by other authors with 99% specificity compared to microneutalization test [11]. Recently, the specificity and sensitivity of the assay were found as comparable as the neutralization test for detecting the virus in camel serum which accounted for 100% and 95.98%, respectively [13].

The overall prevalence of MERS-CoV detected among the imported camels recruited to this study was 58.73%. Compared to previous Egyptian studies conducted on imported camels reporting seroprevalence rates of 89.5%, 88.7%, 98.2% and 86.5 [7,9,27,30], respectively, our study reported the lowest prevalence.

In the present investigation, the MERS-CoV seropositivity rate was found to be higher in Aswan (96.97%) than in Asyut (35.71%) and none of the examined camels from the New Valley Governorate were found to be seropositive. The difference in the reported rates of MERS-CoV infection in different localities was found to be statistically significant ($P < 0.0001$). Keeping in mind that the samples were collected at different times during 2015-2016, we could attribute such high and significant variation between the three localities involved in the study either to the absence of infection in the seronegative camels or to the fact that antibodies against MERS-CoV typically wane rapidly (two weeks) as documented elsewhere [7]. Additionally, the high seropositivity rate detected among Aswan camels (96.97%), could be due to the spread of the infection among camels during the animal movement for long distances as a result of the transport stress as well as the close proximity of the animals to each other [9]. As noted above, the onset of sampling from Aswan was undertaken as soon as the animals were imported, indicating that the animals likely acquired the infection from Sudan. Compared to our report, two previous Egyptian studies on imported Sudanese camels revealed somewhat comparable seropositivity rate accounted for 91.4% and 92.3% [9,31], respectively. However, a very recent study documented lower MERS CoV seroprevalence (88.2%) in imported camels sampled from Aswan [27]. Taken together, this underscores the need for employing strict regulations for the importation of camels from possible MERS-CoV-enzootic countries.

In agreement with previous findings [7], the present investigation also showed that all imported female camels were seropositive for MERS-CoV antibody.

Thus, the present study revealed a significant relationship between the camel gender and MERS-CoV infection rate, with female animals more likely to acquire infection than males. The factors contributing to this variation among genders is still unclear; nevertheless, the fact the majority of the Aswan samples (which had the highest infection rate; 96.97%) were adult females of age ranged between 7 to 8 years old, suggests that age may contribute to susceptibility. Moreover, the close association of the cows and their highly susceptible calves in the exporting country could put them at a repeated exposure to the virus as previously demonstrated [7].

As seen in our study, all the sampled camels were adults and no juveniles were included. Statistically, a significant association between the age of the examined camels and the MERS-CoV infection rate was found ($P < 0.0001$). Older age group (7-8 years) had a greater odds of exposure compared to the younger cohort (5-6 years). This finding indicates that the seropositivity rate for MERS-CoV infection not only differs between adult and juvenile animals as documented [7,34] but also varies within adults themselves which may be explained by the repeated exposure of animals to the infection over time [35].

This report documented the presence of specific antibodies to the MERS-CoV in 25% of the camel traders examined. Sampled camels had a significantly higher seroprevalence ($P = 0.0034$) and exposure rate compared to humans in contact. Four of the 16 tested camel traders who agreed to participate in this study from New Valley reacted positively to MERS-CoV despite the absence of the infection in the contact camels in that region at the sampling time. However, this finding was not surprising since the animal traders were known to have daily occupational contact with different camels from diverse sources.

Under the Egyptian setting, a previous epidemiological study in Cairo and Nile Delta region revealed the absence of antibody reactivity to MERS-CoV in 815 human sera recruited from participants with no previous contact with dromedaries [30]. In the following year, a study tested sera from 179 camel abattoir workers in Giza, Egypt and found that none of the samples were seropositive to the MERS-CoV [31]. In the African context, in addition to the lack of any reported MERS-CoV infected cases among camel traders in the main exporting countries of camels [9], neither 760 individuals with household exposure to seropositive camels in Kenya in 2013 nor 311 camel abattoir workers exposed to seropositive animals in Kano, Nigeria during 2016 had evidence of MERS-CoV antibody [36,37]. Unlike the previous Egyptian and African studies, here we report the possibility of occupational exposure of the Egyptian camel traders to MERS-CoV infection. Since genetic and phenotypic traits of different MERS-COV strains are region-dependent and might be associated with differences in the zoonotic potential [38], thus, one possible explanation for the occupational exposure of the camel traders to the MERS-CoV in our study is that the strains circulating among camels in Upper Egypt might be genetically and phenotypically identical to those in the Arabian Peninsula camels. However, further studies are needed to compare different strains of MERS-CoV circulating in camels and humans in Upper Egypt. The zoonotic nature of the MERS CoV could be linked to the viral strain type as noted earlier [38], however, the presence of a genetic predisposition among different humans might be a contributing factor. Thus possible host-derived factors such as genetic predispostion remain to be studied.

In the Arabian Peninsula, there are mixed reports regarding occupational exposure to the MERS-CoV infection. For instance, 226 slaughterhouse workers in Jeddah and Makkah, Saudi Arabia were found to be seronegative to MERS-CoV [39]. Similarly, no serological evidence of MERS-CoV infection among 300 animal workers from which 17% were in daily contact with dromedaries in Southern Saudi Arabia during 2012 [40]. Also, it was stated that the zoonotic origin of MERS-CoV is presumably scarce, even in those in frequent contact with dromedaries [41,42]. On the other hand, between Dec 2012 and Dec 2013, a total of 15 confirmed MERS-CoV human cases were documented in a nationwide epidemiological study in Saudi Arabia, in which the disease seroprevalence in shepherds and slaughterhouse workers was found to be 15 and 23 times higher compared to the general population, respectively [22]. Moreover, 5 (41.7%) of 12 confirmed community-acquired MERS-CoV human cases in Hafr Al-Batin, Saudi Arabia had close contact with camels [23]. Furthermore, a recent study showed high occupational exposure of camel workers in Riyadh, Saudi Arabia to the virus with an infection rate of 53.3% [24]. The occupational exposure to the MERS-CoV infection from dromedaries was also reported in 7 (6.42%) of 109 slaughterhouse workers in Doha, Qatar during 2013-2014 with contact with dromedaries [25]. In Unites Arab Emirates (UAE), two asymptomatic human cases possibly linked to infected dromedaries were reported in May 2015 [21]. Additionally, 6-17% of 100-235 live animal markets and slaughterhouse workers sera were recently found seropositive to MERS-CoV antibodies in Abu Dhabi, UAE [26].

Interestingly, all the seven MERS-CoV-seropositive camel traders documented in our study were apparently healthy without any history of developing severe respiratory disease in the 14 days prior to sampling. In addition to the current report, several published studies documented the presence of asymptomatic cases associated with MERS-CoV infection in humans [21,24,44–49]. Taken together, we postulate that the number of MERS-CoV cases reported worldwide (n = 2458) since 2012 and so far [2] is underestimated and does not represent the true global burden of the disease due to underreporting of the subclinical cases. A possible reason behind the increasing reports of asymptomatic MERS-CoV cases in humans is that the individuals with perpetual contact with camels are less likely to present with serious illness due to the building of a functional immunity against the virus [21,28].

It was worthy to note that the number of women examined in this study was limited because camel trading is usually a male activity in the Egyptian setting. Thus, based on such a small sample size and the low involvement of females in the trading economy, it was plausible to find that none of the two sampled women reacted positively to MERS-CoV and all seropositive humans were exclusively men. Generally, several reports have previously studied MERS-CoV in the community and/or healthcare settings and demonstrated that men had a higher frequency of acquiring MERS-CoV infection than women [21,49]. The remarkable disparity among both human genders could be a result of differentiated exposure rather than a biological gender variation in vulnerability [28].

Concerning human age, there was no significant impact on acquiring MERS-CoV infection; However, individuals within the middle adulthood cohort (40–60 years) were at a slightly higher odds of infection compared to those in early adulthood (20–39 years). This low variation could be attributed to the difference in immune response between both age groups. Our result is in agreement with [49] who found that the age groups 45–59 years and above had a higher infection rate compared to younger age groups.

Despite the importance of the current investigation in highlighting the preliminary epidemiological situation of the disease in Upper Egypt, a more comprehensive study comprising a larger sample size as well as a molecular-based investigation is necessary to characterize MERS-CoV isolates from Upper Egypt.

Conclusions

The present study documented the detection of specific antibodies against MERS-CoV in imported camels, confirming that they could play an essential role in introducing such serious zoonosis into Egypt. This underscores the urgent need for applying strict measures to camel importation. Moreover, the study provided a serological correlation linking MERS-CoV human infections to one-humped camels. Furthermore, both seropositive animal and human cases documented in this study could present a niche for the virus with a possibility of camel to human and subsequent interhuman transmission and spread.

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