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SEROPREVALENCE AND MOLECULAR DETECTION OF MIDDLE EAST RESPIRATORY SYNDROME CAUSED BY CORONA VIRUS IN SOME CAMELES IN EGYPT

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ABSTRACT

This study was carried out to investigate seroprevalence and molecular detection of Middle East Respiratory Syndrome – Corona virus (MERS-CoV) in some camels in Egypt. For this purpose, a total of 177 blood samples were collected from camels at different abattoirs and localities in Egypt (Nahya, 20,Alwarraque, 27,Belbais, 12, Kerdasa, 20, Kerdasa camel houe hold, 6, Banha, 7, camel studies and developing center farm in Marsa Matrouh sahara, 5) and examined serologically by microneutralization test . The results revealed that the overall prevalence was 59.3% (105 out of 177). The respective percentages of mentioned abattoirs and localities were 30, 44.4, 83.3, 65, 40, 57.1, 61.5, 86.7, 55, 35and 79.1. Meantime a total of 177 samples (155 nasal, 20 rectal, 1 oral and 1 ocular) were taken from the same camels and were undergo to Real time polymerase chain reaction (RT-PCR) to detect RNA of MERS-CoV .The results showed that the overall prevalence of MERS-CoV by RT-PCR was 11.9 (21 out of 177). It was found that 21 out of 155 (13.5%) of nasal swab were positive, while all rectal, ocular and oral swabs were negative RT-PCR. It was concluded that there was a detectable level of anti MERS-CoV antibodies in sera of investigated camels in the examined area of Egypt; moreover, viral RNA of MERS-CoV was also detected with varying percentages of camel nasal samples. This indicated the risk hazards of examined camels in Egypt as a potential reservoir for MERS-CoV infection in camels and humans, so the fully precautions and sanitary measures must be taken to avoid infection.

INTRODUCTION

Corona viruses infect many animals including camels, pigs, domestic and wild birds, bats, rodents, dogs, cats, and cattle. Sever Acute Respiratory Syndrome that affected humans in 2003 world-wide and MERS-CoV currently affecting people in the Middle East and Europe are said to have an animal origin. Since, Middle East respiratory syndrome *corona virus* (MERS-CoV) was first detected in 2012, approximately 1,000 human infections have been reported to the World Health Organization, all linked to residence in or travel to countries on the Arabian Peninsula (**WHO, 2015).** Dromedaries (*Camelus dromedarius*) are thought to play a central role in MERS epidemiology because widespread evidence of MERS-CoV–specific antibodies and virus shedding in camels was found (**Mackay and Arden, 2015).** Urgent epidemiological investigations are required to better understand the transmission patterns of MERS-CoV. Collaboration between human and animal health sectors in the affected countries is essential to understand the risk of

transmission of MERS-CoV between animals and humans, whether there is any seasonal variation in the circulation of the virus in animals, and the natural reservoir(s) of MERS-CoV **(WHO, 2105).** Recently, In Egypt Real-time PCR combined micro – neutralization assay was used for detection of MERS-CoV in camels in Egypt and revealed that Real-time PCR is more sensitive and specific technique (**Ali et al.2017).** From The public health and zoonotic significance of MERS-CoV as emerging zoonoes, this work was performed to investigate the seroprevalence and molecular detection of MERS-CoV in camels in different abattoirs and localities in Egypt.

MATERIALS AND METHODS

A cross sectional study was conducted to determine the prevalence of MERS-CoV serologically by using Microneutralisation test and molecularly by using RT-PCR. For this purpose, a total of 177 blood samples were collected from camels at different abattoirs and localities in Egypt (Nahya, 20,Alwarraque, 27, Belbais, 12, Kerdasa, 20, Kerdasa camel houe hold, 6, Banha, 7, camel studies and developing center farm in Marsa Matrouh sahara, 5) and examined serologically by microneutralization test .

Micro – neutralization test

A total of 177 collected camel serum samples were tested by micro – neutralization test according to **Perera et al. (2013).** MERS-CoV virus was obtained from Dr R Fouchier (Erasmus University Medical Centre, Rotterdam, and the Netherlands). SARS-CoV (strain HKU-39849) was taken from the virus repository at Hong Kong University. Virus stock for MERS-CoV was prepared in Vero cell culture (ATCC CCL-81) in minimal essential medium containing 2% fetal bovine serum, 100 units/mL penicillin and 100 µg/mL streptomycin. Virus aliquots were stored at -80 °C. Virus was titrated in serial half-log10 dilutions (from 0.5 log to 7 log) to obtain 50% tissue culture infectious dose (TCID50) on 96-well tissue culture plates of Vero cells (African green monkey cells). The plates were observed in a phase contrast microscope for cytopathic effect (CPE) daily for three days. The endpoint of viral dilution leading to CPE in 50% of inoculated wells was estimated by using the Reed Muench method and designated as one TCID50. SARS-CoV was grown and titrated in the same manner with the exception that Vero E6 cells (ATCC CRL-1586) were used.

Microneutralisation procedures:

Serial two-fold dilutions of heat-inactivated sera (56 ºC for 30 minutes) were made, starting with a dilution of 1:10. The serum dilutions were mixed with equal volumes of 200 TCID50 of MERS-CoV or SARS-CoV as indicated. After one h of incubation at 37 ºC, 35 µL of the virus–serum mixture was added in quadruplicate to Vero or Vero-E6 cell monolayers for MERS-CoV and SARS-CoV, respectively, in 96-well microtiter plates. After 1 h of adsorption, an additional 150 µL of culture medium were added to each well and the plates incubated for three more days at 37 ºC in 5% CO2 in a humidified incubator. A virus back-titration was performed without immune serum to assess input virus dose. Cyto Pathogenic Effect (CPE) was read at 3 days post infection. The highest serum dilution that completely protected the cells from CPE in half of the wells was taken as the neutralising antibody titre and was estimated using the Reed-Muench method. Positive and negative control sera were included to validate the assay **(Perera et al., 2013).**

Real-time PCR

a total of 177 samples (155 nasal, 20 rectal, 1 oral and 1 ocular) were taken from the same camels and were undergo to Real time polymerase chain reaction (RT-PCR) to detect RNA of MERS - CoV .

RNA was extracted from the samples as described by **Drosten et al. (2003**) using a viral RNA mini kit (Qiagen). samples were pretreated with 2× mucus lyses buffer (10 g of N acetylcysteine/litre, 0.9% sodium chloride) for 30 minutes in a shaking incubator. Swabs were immersed in lyses buffer. Real-time reverse-transcription polymerase chain reaction screening assay upstream of E gene (upE assay(A 25-µl reaction was set up containing 5 µl of RNA,12.5 µl of 2 X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each dNTP and 3.2 mM Magnesium sulfate), 1 µl of reverse transcriptase/Taq mixture from the kit, 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen – not provided with the kit), 1 µg of non-acetylated bovine serum albumin (Sigma), 400 nM concentrations of primer upE-Fwd (GCAACGCGCGATTCAGTT) and primer upE-Rev (GCCTCTACACGGGACCCATA), as well as 200 nM of probe up E-Prb (6)carboxyfluorescein[FAM])CTCTTCACAT AATCGCCCCGAGCTCG-6-carboxy N,N,N,N′- tetramethylrhodamine [TAMRA]). All oligonucleotides were synthesized and provided by Tib-Molbiol, Berlin. Thermal cycling involved 55°C for 20 min, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s, 58°C for 30 s. **(Corman et. al., 2012)**.

Table (1): Seroprevalence of MERS-CoV in camel serum samples collected from different Localities in Egypt by microneutralization test.

Places	Number of examined samples.	No. of positive	Prevalence rate.
Nahya Abattoir	20	6	30%
Al waraque Abattoir	27	12	44.4%
Belbais abattoir	12	10	83.3%
Kerdasa abattoir	20	13	65%
Kerdasa camel house hold	6	2	40%
Banha abattoir	7	4	57.1%
Camel studies and developing center farm in Marsa Matrouh .	13	8	61.5%
Marsa Matrouh sahara.	15	13	86.7%
Berkash market	20	11	55%
Kerdasa farm	13	7	35%
Basaten abattoir	24	19	79.1%
Total	177	105	59.3%

Table (2): MERS-CoV prevalence in camel nasal swap samples as detected by RT- PCR.

Places	Number	RNA + ve by PCR	Prevalence
Nahya Abattoir	18	0	0%
Al waraque Abattoir	17	1	5.9 %
Belbais abattoir	6	3	50 %
Kerdasa abattoir	19	3	15.8%
Kerdasa camel house hold	5	0	0
Banha abattoir	7	0	0%
Camel studies and developing center farm in Marsa Matrouh .	13	0	0%
MarsaMatrouh sahara.	15	2	13.3%
Berkash market	20	7	35%
Kerdasa farm	11	1	9%
Basaten abattoir	24	4	16.7%
Total	I55	21	13.5%

Table (3): MERS-CoV prevalence in camel rectal swab samples as detected by RT- PCR.

places	Number	RNA + ve by PCR	Prevalence
NahyaAbattoir	2	0	0%
Al waraque Abattoir	10	0	0%
Belbais abattoir	5	0	0%
Kerdasa abattoir	1	0	0%
Kerdasa camel house hold	1	0	0%
Banha abattoir	0	0	0%
Camel studies and developing center farm in Marsa Matrouh.	0	0	0%
Marsa Matrouh sahara.	0	0	0%
Berkash market	0	0	0%
Kerdasa farm	1	0	0%
Basaten abattoir	0	0	0%
Total	20	0	0%

Table (4): MERS-CoV prevalence in camel ocular and oral swabs samples by RT- PCR.

Sample	Number	RNA + ve by PCR	Prevalence
Ocular swab (Kerdasa farm)	1	0	0%
Oral swab (Belbais abattoir)	1	0	0%

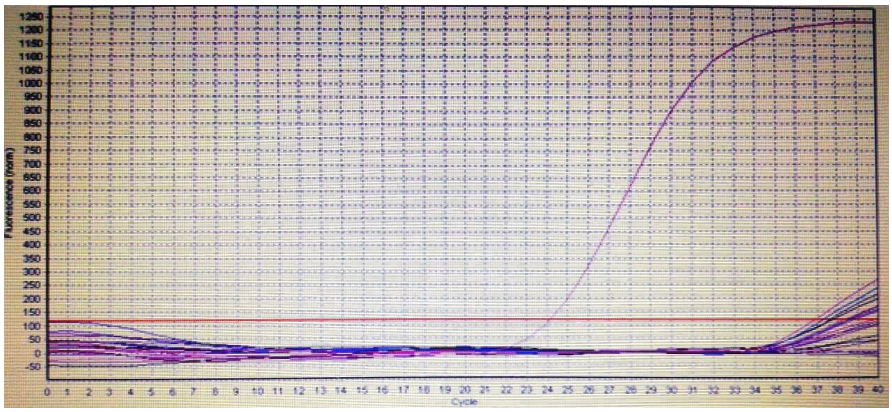

Fig (1): Represented figure to Real time PCR results of MERS-CoV.

RESULTS & DISCUSSION

In analogy to SARS-CoV, the novel MERS-CoV may share a putative origin in bats **(Amman et al., 2013).** In August 2013, the possible source of the MERS-CoV virus was traced to an Egyptian tomb bat found in a building in close proximity to the index-patient's home. The virus genome fragment isolated from the bat was a 100% genetic match to the virus isolated from the index-patient **(Jobs, 2013).**

Moreover, 100% of sera from examined dromedary Omani camels and 14% from Spanish camels had protein-specific neutralizing antibodies against MERS-CoV spike **(Reuskin et al., 2013).** Antibodies in the sera of dromedary "Arabian" camels from Oman and Spain that reacted or cross-reacted with a recombinant antigen of the MERS-CoV spike protein was described. While, whole or infections virus or viral RNA did not find **(Reuskin et al., 2013).** In further studies carried out in Saudi Arabia and Egypt on dromedary camels revealed the presence of high rates of MERS-CoV sero - prevalence of the evaluated camels **(Hemida, 2013 and Perera, 2013)**, suggesting that dromedary camels not only could be the main reservoir of MERS-CoV, Moreover, MERS-CoV was identified in 3 dromedary camels in a herd in Qatar in a barn, which was linked to two confirmed human cases who have since recovered **(Qatar Supreme Council of Health, 2013).**

For the utmost importance of MERS-CoV as an emerging zoonotic disease, this work was taken to investigate some aspects of MERS-CoV in Egypt by serological and molecular

investigation. From results of microneutralization test performed on 177serum samples of camels in Egypt it revealed that the overall prevalence was 11.9 (21 out of 177) by RT- PCR while the total serum + ve by microneutralization assay is 105 with seroprevalence 59.3 % (105 of 177) these seroprevalence results are less than that reported by **Ali et al. (2017)** who recorded 84.5% sero- prevalence, positive higher RT PCR results which were 3.8%. **Chu et al. (2014)** Carried out a study at four slaughterhouses in Egypt showed an overall RNA prevalence in nasal swabs of 3.6% among 110 camels.

Results in tables (1-4) and figure (1) revealed that MERS-CoV prevalence detected by RT- PCR. The overall prevalence of MERS-CoV was 13.5% (21 of 155 nasal swabs) percentages in Al waraque Abattoir, Belbais abattoir, Kerdasa abattoir, Kerdasa camel house hold, Banha abattoir, Camel studies and developing center farm in Marsa Matrouh, Marsa Matrouh sahara (free roaming flocks), Berkash market, Kerdasa farm and Basateen abattoir was sequentially , 3.7%,3.7%,3.7%,0%,0%,0%,13.3%,35%,7.7% and 16.7% .

The highest prevalence was 35% which detected in Berkash market, where, all local, imported types, categories and ages of camels are being in one place which increases risk of infection. These results support the hypotheses supposed that and the possibility that camels could spread MERS-CoV infection with pandemic risk to other countries and regions unaffected by this virus **(Karagöz et al .,2014).**

In Egypt **Chu et al. (2014)** carried out a study at four slaughterhouses in Egypt and showed an overall RNA prevalence in nasal swabs of 3.6% among 110 camels. Moreover **Ali et al. (2017)** reported a percentage of 3.8% by RT- PCR.

The results shown in tables (2 - 4) and figure (1) clarified that all positive samples detected in nasal swaps, however MERS-CoV RNA were not detected by RT- PCR in neither ocular nor rectal swaps. These results support the hypotheses suggested that the high viral load of MERS-CoV detected in nasal swabs of camels may facilitate the zoonotic transmission through the respiratory route **Memish et al. (2014)**. It was concluded that in the results achieved in tables (1-4) and figure (1) there were detectable levels of anti MERS-CoV antibodies in sera of investigated camels in the examined area of Egypt, moreover, viral RNA of MERS-CoV was also detected in varying percentages of camel's samples. This indicated the risk hazards of examined camels in Egypt as a potential reservoir for MERS-CoV infection in camels and humans, so the following precautions must be taken to avoid infection, heat treatment of camel meat and efficient milk pasteurization with public health education to prevent drinking of raw milk or urine of camels, butchers, veterinarians should apply hygienic measures during handling, slaughtering and examining of camels .periodical serological and molecular detection of MERS-CoV in camels populations in Egypt must be carried out in future to obtain clear picture about MERS-CoV infection.

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الملخص العربي

مدي الانتشار السيرولوجى والكشف الجزيئى لمتلازمة الشرق الاوسط التنفسية المسببة بفيروس الكورونا في بعض الجمال في مصر

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اجريت هذه الدراسة للبحث عن معدل الحدوث السيرولوجي والكشف الجزيئى لمتلازمة الشرق الاوسط التنفسية المسببه بفيروس الكورونا في بعض الابل في مصر ولهذا الغرض تم تجميع ١٧٧ عينة دم من مجازر واماكن مختلفة (ناهيا ،٢٠ ، الوراق، ٢٧، بلبيس ١٢، كرداسة٢٠، التربية المنزلية بكرداسة ٦، بنها ٧، مزرعة مركز دراسة وتطوير الابل بمرسى مطروح ١٣، صحارى مرسى مطروح ٥) وتم اختبارها سيرولوجيا باختبار وأشارت النتائج الي ان مدي حدوث الانتشار السيرولوجي الكلي كان ٣, ٥٩% (١٠٥ من ١٧٧) وكانت النسب بالتوالي فى تلك الاماكن كالتالى ٣٠ ،٤٤,٤ ،٨٣,٣ ،٦٥,٤ ،٥٧,١ ،٦١,٥ ،٨٦,٧ ،٥٥,٣٥ ،٧٩,١ في نفس الوقت تم تجميع ٧٧عينه (١٥٥ مسحات انفيه ،٢٠مسحات شرجيه ،١مسحه فميه ،١مسحه من العين) من الابل ومن نفس المجازر واماكن مختلفه في مصر وأخضعت لاختبار RT- PCR للكشف عن RNA (الحمض النووي) لفيروس الكورونا المسبب لمتلازمه الشرق الاوسط التنفسيه .

أظهرت نتائج الدراسة ان معدل الحدوث الكلي باختبار كان ١١,٩% (٢١من١٧٧) ووجدت ان ٢١ من ١٥٥ بنسبه حدوث ١٣,٥% من المساحات الانفيه أحرزت نتائج ايجابيه بينما كانت المساحات العينيه والفميه سلبيه باختبار RT- PCR ويستنتج من ذلك عند الكشف عن الاجسام المناعية ضد متلازمة الشرق الأوسط التنفسية المسببه بفيروس الكورونا بمستويات مختلفة وبنسب مختلفة في عينات امصال الابل في المناطق تحت الدراسة بمصر. علاوة علي ذلك تم الكشف عن الحمض النووي لفيروس الكورونا بنسب مختلفة فى عينات الانف للابل في مناطق الدراسة . مما يؤكد وجود الفيروس في الجمال مسببا خطورة كمستودع ومخزن للعدوي للجمال الاخرى للانسان. ولذلك قد تم مناقشة الإجراءات الصحية اللازمة لمنع العدوي.