Molecular detection of Middle East Respiratory Syndrome *Coronavirus* (MERS-CoV) in Tunisian dromedary camels (*Camelus dromedarius*)

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Abstract

Middle East Respiratory Syndrome *Coronavirus* (MERS-CoV) was identified in humans in 2012. Dromedary camels were considered as the major reservoir of the virus throw several studies. Tunisian dromedary camels were implicated in a serological survey and a seropositivity reaching 100% was observed among some of them. Therefore, more investigations are necessary to look for the current infection situation. In this study, we aimed to detect the MERS-CoV virus in our camels by molecular technics. Blood and nasal swabs samples from 64 dromedary camels from 4 Tunisian provinces were collected during June 2014 and April 2015. All the animals tested negative by real time reverse transcriptase–polymerase chain reaction assay (RT-PCR). These findings could not confirm the absence of MERS-CoV active shading among the Tunisian camel population and more researches should be carried on to survey dromedary camels in Tunisia and its neighboring countries.

Keywords: Middle East respiratory syndrome (MERS), Camels, Tunisia, RT-PCR, animal surveillance, *Coronavirus* (CoVs), molecular detection

Détection moléculaire du *Coronavirus* du Syndrome Respiratoire du Moyen-Orient (MERS-CoV) chez les dromadaires tunisiens (*Camelus dromedarius*)

Résumé

Le *Coronavirus* du syndrome respiratoire du Moyen-Orient (MERS-CoV) a été identifié chez l'Homme pour la première fois en 2012. A travers plusieurs études, les dromadaires ont été alors considérés comme étant le réservoir majeur du virus. Une enquête sérologique chez les dromadaires en Tunisie a permis de démontrer une séropositivité allant jusqu'à 100% chez certains sujets étudiés. Ainsi, des recherches supplémentaires semblent être nécessaires pour évaluer l'état d'infection actuel du pays. A travers cette étude, nous visons, par le biais de techniques moléculaires, la détection du virus (MERS-CoV) chez nos dromadaires tunisiens. Durant la période s'étalant entre juin 2014 et avril 2015, des échantillons de sang et des écouvillonnages nasaux ont été prélevés à partir de 64 individus prévenant de 4 gouvernorats. L'essai de la détection moléculaire, en utilisant la technique d'amplification génomique en chaîne de polymérase avec transcriptase inverse (RT-PCR), a démontré que tous les sujets étudiés ont donné un réponse négative. Cependant, ces résultats ne peuvent pas confirmer l'absence d'une excrétion active du virus chez la population cameline tunisienne, raison pour laquelle d'autres investigations doivent être enchaînées pour surveiller l'état d'infection cameline par le MERS-CoV non seulement en Tunisie mais également dans les pays voisins.

Mots-clés: Le Syndrome Respiratoire du Moyen-Orient (SRMO), dromadaires, Tunisie, RT-PCR, surveillance animale, *Coronavirus* (CoVs), détection moléculaire

INTRODUCTION

In 2012, a new *Coronavirus*, Middle East Respiratory Syndrome *Coronavirus* (MERS-CoV), was isolated from a Saudi Arabian patient (Zaki *et al.*, 2012). It causes severe lower respiratory tract infection in humans (Zaki *et al.*, 2012, WHO-MERS-CoV Research Group, 2013). As of 30 January 2017, 1.888 cases of laboratory-confirmed MERS have been reported to the World Health Organization (WHO) (WHO official web site, assessed: 31 January 2017). Although bats are shown to harbor CoVs phylogenetically related to MERS-CoV (Chan *et al.,* 2015), the discovery of closely related viruses and virus-neutralizing antibodies in dromedary camels (*Camelus dromedarius*) has led to the hypothesis that they are the major reservoir of MERS-CoV and are implicated in direct or indirect transmission to human beings (Zumla *et al.,* 2015, Crameri *et al.,* 2015).

In 2014, a serological survey has invested geographic distribution of MERS-CoV among dromedary camels in Africa including sera from dromedary camels from Tunisia (Reusken *et al.,* 2014). Surprisingly, a seropositivity ranging from 36% in Sidi Bouzid province to as much as 100% in Kebili province was observed among Tunisian dromedary camels, which confirmed that they must have had previous infection with MERS-CoV. These evidences have raised public health concerns and further investigations seemed to be needed for management and control recommendations.

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The purpose of this study is to investigate a current shading of MERS-CoV among camels from Tunisia by a molecular detection technics in order to implement adequate sanitary and control measures.

MATERIALS AND METHODS

Samples Collection

Blood and nasal swabs samples were collected from 64 apparently healthy dromedary camels. Since the infection epidemiology was not well understood, animal selection was hazardous and selected animals were of different age, breed and sex and from four Tunisian provinces (Figure 1). However, we intend to take samples from the same farms in two provinces (Sousse and Kebili) that showed positive results in the previous serological survey (Reusken *et al*, 2014). Samples were collected on the 3rd June 2014, 10th October 2014, 3rd November 2014 and 22nd April 2015, respectively (Table 1). The ages of the animals ranged from 7 months to 14 years (26 juvenile < 2 years of age; 38 adults > 2 years of age). Blood samples were taken by jugular vein puncture according to local laws. Nasal swabs were taken from the two nasal cavities of each animal and put in tubes containing viral transport medium. After sampling, all the samples were stored at 4°C and shipped to the microbiological laboratory of the Charles Nicolle Hospital in Tunis, Tunisia in less than 24 hours.

Molecular detection

RNAs were extracted from the nasal swabs and the plasma's samples with the use of the QIAamp Viral RNA Mini Kit (Qiagen), according to the manufacturer's instructions. Eluted RNAs were screened for the MERS-CoV upstream region of the E gene (upE region) with the use of real-time RT-PCR, as described previously (Haagmans *et al.,* 2014; Corman *et al.,* 2012).

RESULTS

Absence of RNA MERS-CoV in Tunisian dromedary camels

Real time RT-PCR Up-E assay was used to detect MERS-CoV RNA in Tunisian camels. Among the 64 studied dromedary camels, we note that 40.6 % of the individuals are juveniles (<2 years old) while 59.4 % are adults (> 2 years old).

As all the analyzed animals showed a negative result for MERS-CoV RNA detection, including animals that were sampled from Kebili and Sousse provinces where a seropositivity of 40% and 100% was previously reported (Reusken *et al.,* 2014), only results from selected plasma and nasal swabs samples (at least 5 individuals from each region except Ben Guerdene province) are given in table 2 to permit a clearer presentation of the findings (Figure 2).

As we noticed, only positive control sample showed a positive result.

Figure 1: ***The 4 regions sampled in this study***
(Map adapted from http://d-maps.com/index.php)

DISCUSSION

This study aimed to find out whether MERS-CoV is actually circulating among camels in Tunisia, after finding specific antibodies against MERS-CoV in some of these animals, as previously reported (Reusken *et al.,* 2014). Our work was based only on molecular technics that allow a direct detection of the virus and so the detection of an eventual recent infection, whereas, serological methods as used in the previous survey (Reusken *et al.*, 2014) do not offer this precision.

MERS-CoV transmission and ecology are not yet well know and different studies are actually focusing on these properties in order to understand its complex epidemiology (Funk *et al.,* 2016). During our work animal sampling was hazardous and we have especially focused on apparently healthy dromedary camels. However, different considerations related to the virus properties were missed. First of all, camels infected with MERS-CoV can develop rhinitis or show no signs of infection (Zumla *et al.,* 2015). Besides, despite that both adults and calves can be infected (Hemida *et al.,* 2014); juvenile dromedary camels might at higher risk of contracting the virus than adults due to their naïve status (Mohd *et al.,* 2016). In fact, in 2017 in Jordan;

van Doremalen *et al., (2017)* detected a high prevalence of MERS-CoV in dromedary camels younger than 3 years. In addition to that, calving season is believed as a period of higher risk for virus transmission (Funk *et al.,* 2016). These elements indicate that same camels with active virus shading and that capable of showing a positive result might be missed while sampling. Actually, the probability of finding dromedary camels with virus excretion by the time of sampling would be higher if we have concentrated on juvenile individuals rather than adults, animals expressing respiratory disease rather than apparently healthy camels and if we have chosen calving season as the best period of collecting samples. Moreover, it should be mentioned that by gathering these factors to increase the probabil-

Table 1: Camel samples collected in this study

Animal number	Date of collection	Animal originated from
23	03-06-2014	Sousse (central-east of Tunisia)
10	10-10-2014	Gafsa (southwest Tunisia)
2	03-11-2014	Ben Gardane (south eastern Tunisia)
29	22-04-2015	Kebili (south-western Tunisia)

Table 2: RT-PCR results of selected plasma and nasal swabs' samples

Camel's number	Region	Age*	Sexe**	RT-PC on plasma samples	RT-PCR on nasal swabs' samples
1	Sousse	A	M	Neg	Neg
2	Sousse	A	M	Neg	Neg
3	Sousse	A	F	Neg	Neg
4	Sousse	A	F	Neg	Neg
5	Sousse	A	F	Neg	Neg
24	Gafsa	J	M	Neg	Neg
25	Gafsa	J	M	Neg	Neg
26	Gafsa	J	M	Neg	Neg
27	Gafsa	A	F	Neg	Neg
28	Gafsa	A	F	Neg	Neg
34	Ben Guerdene	A	F	Neg	Neg
35	Ben Guerdene	J	M	Neg	Neg
60	Kebili	A	F	Neg	Neg
61	Kebili	A	F	Neg	Neg
62	Kebili	A	M	Neg	Neg
63	Kebili	A	M	Neg	Neg
64	Kebili	A	M	Neg	Neg

*Camel age range: J, juvenile ≤2 years of age; A, adult >2 years of age

**Camel sexes: M, Male; F, Female

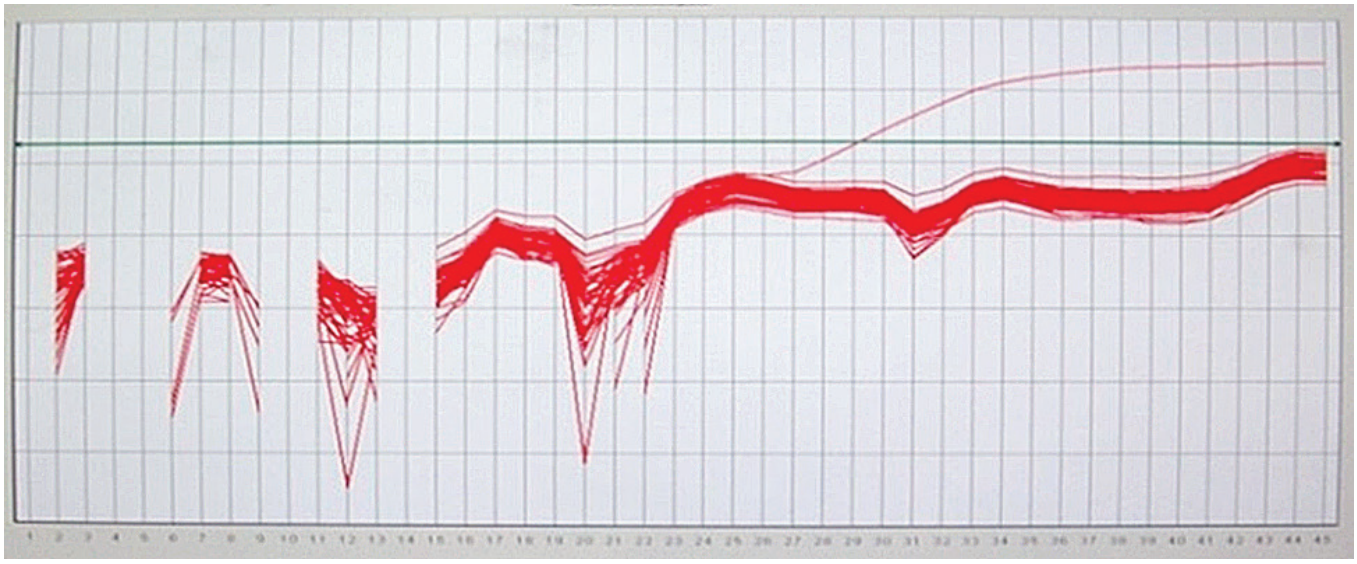

Figure 2: Real time RT-PCR results of dromedary camels from Kebili region

ity of discovering sick animal replicating the virus, the likelihood of falling ill during pre-treatment would also increase. Hence, we must respect necessary biosafety precautions while sampling.

In the other hand, the absence of RNA MERS-CoV detection and high seropositivity reported in the previous study (Reusken *et al.*, 2014) may indicate only a past contact of Tunisian camels with the virus. In fact, seropositivity was noted in camels in Somalia and Sudan using archived serum samples accumulated during the past 30 years (Müller *et al.,* 2014). The presence of antibodies in archived samples indicates that this infection is not recent in Africa. In addition to that, serological surveys investing dromedaries of the Canary Islands revealed a decrease in specific seropositivity between 2013 and 2015. While throw the first study a seropositivity rate of 14% was recorded (Reusken *et al.,* 2013), the second study showed a lower rate (4.1%) (Gutiérrez *et al.,* 2015). These findings indicate that these dromedaries had contacted the virus at same point of their lives; however, a current infection doesn't exist. So, basing on these facts that confirm that MERS-CoV is a very immunogenic virus, additional serological studies should be done on different dromedary camels, especially those expressing clinical respiratory disease, in order to eventually demonstrate a recent infection.

The hypothesis that MERS-CoV infection in dromedaries in Tunisia is a very rare event is a probable idea that should be considered. Although Corman *et al.*, (2014) indicated that MERS-CoV originated from sub-Saharan African bats, the MERS epidemic has started in the Middle East, where almost all primary cases of MERS-CoV have been registered. This reminds us of a particularity related to the dromedary farming in the Middle East. Indeed, since the 1960s, throw the intensification and the concentration of the production around cities, there have been radical changes in dromedary farming practices in the Arabian Peninsula (Gossner *et al.,* 2016). These modifications in the overall dynamics of dromedary populations as well as the recent intensification of their breeding might increase the number of viral spawners and the attack rate in dromedary herds. As a consequence, there have been an increased spread of the virus among camels and so the augmentation of zoonotic infections from dromedaries to humans (Gossner *et al.,* 2016). The circumstances of MERS-CoV emergence in the Middle East remind us then of those of the SARS-CoV in China in 2003, as particular conditions have also favored the transmission of the virus from bats to humans through encaged civets.

CONCLUSION

Additional researches are needed to confirm or invalidate a current infection of camel herds in Tunisia and its neighboring countries. Samples collection should be more selective basing on age, sex, symptoms and the season. Vigilance and specific concerned authorities' measures should be kept to avoid a potential epidemic in North Africa.

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REFERENCES

Chan J.F., Lau S.K., To K.K., Cheng V.C., Woo P.C., Yuen K.Y. (2015). Middle East respiratory syndrome *Coronavirus*: another zoonotic *betacoronavirus* causing SARS-like disease. *Clin. Microbiol. Rev*., 28: 465-522.

Corman V.M., Ithete N.L., Richards L.R., Schoeman M.C., Preiser W., Drosten C., Drexler J.F.. (2014). Rooting the phylogenetic tree of Middle East Respiratory Syndrome *Coronavirus* by characterization of a conspecifivirus from an African bat. *J. Virol*., 88:11297-11303.

Corman V.M., Eckerle I., Bleicker T., Zaki A., Landt O., Eschbach-Bludau M., Van Boheemen S., Gopal R., Ballhause M., Bestebroer T.M., Muth D., Müller M.A., Drexler J.-F. , Zambon M.C., Osterhaus A.D.M.E., Fouchier R.A.M., Drosten C. (2012). Detection of a novel human *Coronavirus* by realtime reverse-transcription polymerase chain reaction. *EuroSurveill*., 17: pii/20285

Crameri G., Durr P.A., Barr J., Yu M., Graham K., Williams O.J., Kayali G., Smith D., Peiris M., Mackenzie J.S., Wang L.F. (2015). Absence of MERS-CoV antibodies in feral camels in Australia: implications for the pathogen's origin and spread. *One Health.,* 1: 76-82.

Funk A.L., Goutard F.L., Miguel E., Bourgarel M., Chevalier V., Faye B., Peiris J.S., Van Kerkhove M.D., Roger F.L. (2016). MERS-CoV at the Animal–Human Interface: Inputs on Exposure Pathways from an Expert-Opinion Elicitation. *Front. Vet. Sci.,* 3: 88.

Gossner C., Danielson N., Gervelmeyer A., Berthe F., Faye B., Kaasik Aaslav K., Adlhoch C., Zeller H., Penttinen P., Coulombier D. (2016). Human–Dromedary Camel Interactions and the Risk of Acquiring Zoonotic Middle East Respiratory Syndrome *Coronavirus* Infection. *Zoonoses and Public Health*, 63: 1-9.

Gutiérrez C., Tejedor-Junco M.T., González M., Lattwein E., Renneker S. (2015). Presence of antibodies but no evidence for circulation of MERS-CoV in dromedaries on the Canary Islands, 2015. *Euro Surveill.* 20(37):pii=30019. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2015.20.37.30019

Haagmans B.L., Al Dhahiry S.H., Reusken C.B., Raj V.S., Galiano M., Myers R., Godeke G.J., Jonges M., Farag E., Diab A., Ghobashy H., Alhajri F., Al-Thani M., Al-Marri S.A., Al Romaihi H.E., Al Khal A., Bermingham A., Osterhaus A.D. M. E., AlHajri M.M., Koopmans M.P.G. (2014). Middle East respiratory syndrome *coronavirus* in dromedary camels: an outbreak investigation. *Lancet Infect. Dis.,* 14:140-5.

Hemida M.G., Chu D.K., Poon L.L., Perera R.A., Alhammadi M.A., Hoi-yee Ng, Siu L.Y., Guan Y., Alnaeem A., Peiris M. (2014). MERS *Coronavirus* in Dromedary Camel Herd, Saudi Arabia. *Emerging Infectious Diseases,* 20: 1231-1234.

Mohd H.A., Al-Tawfiq J.A., Memish Z.A. (2016). Middle East Respiratory Syndrome *Coronavirus* (MERS-CoV) origin and animal reservoir. *Virology Journal*, 3: 13: 87.

Müller M.A., Corman V.M., Jores J., Meyer B., Younan M., Liljander A., Bosch B.J., Lattwein E., Hilali M., Musa B.E., Bornstein S., Drosten C. (2014) MERS *Coronavirus* neutralizing antibodies in camels, Eastern Africa, 1983-1997. *Emerging Infectious Diseases*, 20: 2093-2095.

Reusken C.B., Messadi L., Feyisa A., Ularamu H., Godeke G.J., Danmarwa A., Dawo F. , Jemli M. , Melaku S. , Shamaki D. , Woma Y. , Wungak Y. , Gebremedhin E. Z. , Zutt I. , Bosch B.J. , Haagmans B.L. , Koopmans M.P.G. (2014). Geographic distribution of MERS *Coronavirus* among dromedary camels, Africa. *Emerg. Infect. Dis. J.,* 20: 1370-1374.

Reusken C.B., Haagmans B.L., Müller M.A., Gutierrez C., Godeke G.J., Meyer B., Muth D., Raj V.S., Smits-De Vries L., Corman V.M., Drexler J.F., Smits S.L., El Tahir Y.E., De Sousa R., van Beek J., Nowotny N., van Maanen K., Hidalgo-Hermoso E., Bosch B.J., Rottier P., Osterhaus A., Gortázar-Schmidt C., Drosten C., Koopmans M P G. (2013). Middle East respiratory syndrome *Coronavirus* neutralizing serum antibodies in dromedary camels: a comparative serological study. *Lancet. Infect. Dis*., 13: 859-866.

Van Doremalen N., Hijazeen Z.S.K., Holloway P., Al Omari B., McDowell C., Adney D., Talafha H.A., Guitian J., Steel J., Amarin N., Tibbo M., Abu-Basha E., Al-Majali A., Munster V.J., Juergen A. Richt J.A. (2017). High Prevalence of Middle East Respiratory *Coronavirus* in Young Dromedary Camels in Jordan. *Vector-borne and zoonotic diseases*, 17: 155-159.

World Health Organization, MERS-CoV Research Group (2013). State of knowledge and data gaps of Middle East respiratory syndrome *Coronavirus* (MERS-CoV) in humans. *PLoS Curr*. 5:pii: ecurrents. outbreaks.0bf 719e352e7478f8ad85fa30127ddb8

World Health Organization (WHO) (2017). Middle East respiratory syndrome *Coronavirus* (MERS-CoV). Geneva: WHO. [Accessed: 31 January 2017]. Available from: http://www.who.int/emergencies/mers-cov/en/

Zaki A.M., van Boheemen S., Bestebroer T.M., Osterhaus A.D., Fouchier R.A. (2012). Isolation of a novel *Coronavirus* from a man with pneumonia in Saudi Arabia. *N. Engl. J. Med*., 367: 1814-1820.

Zumla A., Hui D.S., Perlman S. (2015). Middle East Respiratory Syndrome. *The Lancet*, 386: 995-1007.