

Detection of pancoronavirus using PCR in *Camelus dromedarius* in Iran (first report)

Heydar Khalili Bagaloy¹ · Ehsanollah Sakhaee¹ · Mohammad Khalili²

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Abstract *Coronaviride* is a colossal family of viruses that cause a variety of diseases in humans and other animals. As of late, a novel coronavirus, not anterior-optically discerned in humans, has been identified in a denizen of the Middle East. There is growing evidence that the *Camelus dromedarius* is host species for the virus and plays an important role of a source of human infection. Along these lines, the authors decided to detect coronaviruses in dromedary camels in two high-risk areas of Iran by employing an reverse transcription polymerase chain reaction (RT-PCR) assay. In the present study, nasal swab specimens were collected from 98 camels (*C. dromedarius*) traditionally reared in southeast and northwest of Iran. The detection of pancoronavirus was carried out, using RT-PCR. Pancoronavirus RNA was observed in seven cases among 98 nasal swab samples. Among these, 4 positive samples belonged to Azerbaijan province located in northwest of Iran and 3 positive samples were taken from southeast of Iran. The results of this study contribute to raising the hypothesis to the extent of transmission and risk factors for human infection and public health in Iran.

Keywords Pancoronavirus · RT-PCR · Camels · Iran

Introduction

Coronaviride is an astronomically immense family of viruses that induce a diversity of diseases in humans, from a prevalent cold to severe acute respiratory syndrome (SARS). Additionally, these viruses cause disease in a wide assortment of animal species (Drosten et al. 2003; Kuiken et al. 2003; Perlman and Netland 2009; Vabret et al. 2006; Vabret et al. 2008; WHO 2013). In late 2012, a novel coronavirus, not already found in humans, was distinguished in an inhabitant of the Middle East (Corman et al. 2011; Zaki et al. 2012). Thus far, the coronavirus has been kenneed as the Middle East respiratory syndrome coronavirus (MERS-CoV) (Bermingham et al. 2012; Buchholz 2013; Van Boheemen et al. 2012; Zaki et al. 2012). As per the World Health Organization (WHO), worldwide cases meaning the Middle East respiratory syndrome (MERS) were 1599 cases affirmed by research facilities until October 2015. These cases included at least 574 deaths (case fatality rate of 36%) since the first cases were reported in September 2012 (WHO 2015).

There is confirmation that the dromedary camels are host species for the infection and they assume a part as a wellspring of human disease (WHO 2013). An investigation in Egypt, utilizing reverse transcription polymerase chain reaction (RT-PCR), detected MERS-CoV in 3.6% (4 out of 110) of ostensibly salubrious dromedary camels in an abattoir. The genetic sequence of the viruses demonstrates the lowest contrasts from a reference strain previously taken from a human case (Chu et al. 2014).

Viral culture is a standard test for diagnosis of respiratory infections (van Elden et al. 2002). However, as it is difficult to grow coronaviruses in cell culture (Siddell et al. 1983), RT-PCR has been produced to acquire more affectable and fast indicative results. This pancoronavirus assay is a utilizable implement for screening sample amassments for the presence

✉ Heydar Khalili Bagaloy
dr.khalilihaeydar@yahoo.com

¹ Department of Clinical Sciences, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

² Department of Pathobiology, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

of all known coronaviruses (Moës 2005; Zaki et al. 2012). As Iran recently reported its sixth human MERS-CoV case and the fifth death from the disease, it is vital to evaluate the situation of the infection in camels as a source of the virus. Therefore, the authors decided to detect coronaviruses in dromedary camels in two high-risk areas of Iran by applying an RT-PCR assay.

Materials and methods

Sample collection

After a 4-month period (from January to May 2014), the nasal swab samples were gathered aseptically with sterile absorbent swabs soaked in phosphate buffer solution (PBS) from 98 apparently healthy camels (*C. dromedarius*) which were reared traditionally in Kerman and West Azerbaijan provinces, Iran.

Sample processing

At first, the swab tips were squeezed, and vortexes and 140 μ l of the solution obtained were taken for RNA extraction. Subsequently, 60 μ l of RNA concentrate, which was received for the most part as portrayed by the producer's aide (Qiagen, Germany), was stored at -20 °C.

PCR process

The coronavirus RNA was recognized by amplifying a 251-bp section of the coronavirus polymerase gene utilizing the accompanying preliminary set: Cor-FW (5'-TGGG GAGTAATGAACCCGGTA-3') and Cor-RV (5'-ACAT GTAAAAGAGCTAATAACAC-3') (Copenhagen, Denmark). PCR amplifications were performed in 25- μ l reaction volumes containing 5 μ l of the extract and 20 μ l of the master mix (containing 10 μ l buffer, 2 μ l dNTP mix, 1.8 μ l enzyme Mix (a combination of reverse transcriptase and Hot Star Taq DNA polymerase), 4 μ M of forward and reverse primer, and RNase-free water). The reverse transcriptase reaction was launched during 30 min at 50 °C; it underwent PCR initiation at 95 °C for 15 min, 50 cycles of amplification (30-s denaturation at 94 °C and 1-min extension at 72 °C), and finally the last augmentation by hatching at 72 °C for 10 min. Then, 5 μ l of the product mixed with 1 μ l of the loading buffer was loaded on agarose gel (1.2%) and run at 90 V for 30 min. The coronavirus-positive samples will be subject to a sequence.

Results

Pancoronavirus RNA was identified in seven cases among 98 nasal swab samples. Four positive samples belonged to West Azerbaijan province, northwest of Iran, and the remaining three were taken from Kerman province, southeast of Iran (Tables 1 and 2).

Discussion

The late dangerous human contaminations made by the MERS-CoV realized an astonishing interest in the revelation of coronaviruses in individuals and farm animals. The first studies were carried out to investigate whether or not a camel is a possible source and reservoir of the virus (Haagmans et al. 2014a; Memish et al. 2014).

The positive serological results from 50 serum tests taken from dromedary camels in Oman exhibited a high titer of neutralizing antibodies against MERS-CoV (Reusken et al. 2013b). Camels infected with MERS-CoV may not show any clinical signs (Assiri et al. 2013; Azhar et al. 2014; WHO 2014a, b).

Thus, it is not possible to know whether or not an animal in a farm, market, race track, or slaughterhouse is excreting the virus and is able to infect humans through their nasal discharges and feces and potentially in their milk and urine (Adney et al. 2014). The virus may also be found in the organs and meat of the infected animals (WHO 2014). *C. dromedarius* is increasingly apperceived as an indigenous reservoir for human coronaviruses. A pancoronavirus RT-PCR examination is an apt strategy to distinguish the majority of the coronaviruses in clinical specimens. Other than speedy screening for a few pathogens in one test, it supplies the likelihood to distinguish aforesaid obscure coronaviruses.

In recent studies, scientists have tested the sera of sheep, dairy cattle, goats, and camels from the Middle East and some different districts for the nearness of antibodies against the MERS-CoV. Interestingly, the results showed that all samples taken from the camels were positive against MERS-CoV (Hemida et al. 2013; Meyer et al. 2014; Perera et al. 2013; Reusken et al. 2013a). However, no anti-MERS-CoV

Table 1 Number and frequency (%) of positive and negative cases among 98 apparently healthy camels from Kerman and West Azerbaijan provinces, Iran

Province	Positive	Negative	Total
West Azerbaijan	4 (4.08%)	66 (67.33%)	70 (71.4%)
Kerman	3 (3.06%)	25 (25.51%)	28 (28.6%)
Total	7 (7.14%)	91 (92.85%)	98 (100%)

Table 2 Number and frequency (%) of positive and negative cases in each province

Province	Positive	Negative
West Azerbaijan	4 (5.7%)	66 (94.3%)
Kerman	3 (10.7%)	25 (89.3%)

antibodies were found in any of the other tested animals (Reusken et al. 2013b).

The results of previous studies also indicate that humans who had had interaction with camels developed a higher seroprevalence than those without contact (Reusken et al. 2013a; Reusken et al. 2013b). Be that as it may, camels do not essentially need to build up the sickness; however, they are liable to transmit the pathogen (Müller et al. 2015). A recent study has demonstrated that indistinguishable MERS-CoV RNA sections were identified in an air test gathered from the dwelling place of the camel where there was a common comparable MERS-CoV with a contaminated individual (Azhar et al. 2014).

On 11 June 2014, the World Health Organization (WHO) reported that Kuwait found the virus in five camels. They also confirmed the first two MERS-CoV cases in Iran (WHO 2014b). According to the WHO recent report, all the patients who died from MERS-CoV were inhabitants of Kerman province, Iran (WHO 2014a; Yousefi et al. 2016). Therefore, our research group designed a study to focus on the detection of coronaviruses in camels in Kerman province, Iran.

However, the Ministry of Health and Medical Education of Iran has reported that the above-mentioned patients had no contact with animals or did not consume raw camel products before their illness; however, they had close contact with Umrah pilgrims who had an influenza-like illness. Previous studies affirm a remarkable similarity between MERS-CoVs conveyed by humans and camels (Abdulaziz et al. 2014; Haagmans et al. 2014b) and bolster the theory that human MERS-CoV contamination might be procured straightforwardly from camels.

The first reported case of coronavirus infection in *C. dromedarius* in Iran raises the possibility that a significant proportion of cases might be infected or will be infected in the future, thus improving the hypothesis to the extent of transmission and risk factors for humans.

Conclusion

In conclusion, the implementation of a perpetual veterinary surveillance program for detecting coronaviruses in dromedary camels may avail our control of the transit method of coronavirus infections.

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Compliance with ethical standard

Ethical approval All ethical considerations utilizing animals were considered conscientiously, and the experimental protocol was affirmed by the Ethics Committee of KUMS, Kerman, Iran.

Conflict of interest The authors declare that they have no conflict of interest.

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