One Health 1 (2015) 76–82

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Absence of MERS-CoV antibodies in feral camels in Australia: Implications for the pathogen's origin and spread

Gary Crameri [a], Peter A. Durr [a,*], Jennifer Barr [a], Meng Yu [a], Kerryne Graham [a], Owen J. Williams [b], Ghazi Kayali [c], David Smith [d,e], Malik Peiris [f,g], John S. Mackenzie [h], Lin-Fa Wang [a,i,**]

[a] CSIRO Australian Animal Health Laboratory, Geelong, Australia
[b] Earlville Veterinary Surgery, Cairns, Australia
[c] Division of Environmental Research, National Research Centre, Giza, Egypt
[d] School of Pathology and Laboratory Medicine, University of WA, Nedlands, Australia
[e] PathWest Laboratory Medicine WA, Nedlands, Australia
[f] School of Public Health, the University of Hong Kong, Hong Kong, China
[g] HKU-Pasteur Pole, the University of Hong Kong, Hong Kong, China
[h] Faculty of Health Sciences, Curtin University, Perth, WA, Australia
[i] Program in Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore

ARTICLE INFO

Article history:
Received 22 July 2015
Received in revised form 23 October 2015
Accepted 26 October 2015
Available online 2 November 2015

Keywords:
Middle East respiratory syndrome (MERS)
Severe acute respiratory syndrome (SARS)
Coronavirus (CoV)
Camels
Bats
Serological surveillance

ABSTRACT

Middle East respiratory syndrome coronavirus (MERS-CoV) infections continue to be a serious emerging disease problem internationally with well over 1000 cases and a major outbreak outside of the Middle East region. While the hypothesis that dromedary camels are the likely major source of MERS-CoV infection in humans is gaining acceptance, conjecture continues over the original natural reservoir host(s) and specifically the role of bats in the emergence of the virus. Dromedary camels were imported to Australia, principally between 1880 and 1907 and have since become a large feral population inhabiting extensive parts of the continent. Here we report that during a focussed surveillance study, no serological evidence was found for the presence of MERS-CoV in the camels in the Australian population. This finding presents various hypotheses about the timing of the emergence and spread of MERS-CoV throughout populations of camels in Africa and Asia, which can be partially resolved by testing sera from camels from the original source region, which we have inferred was mainly north-western Pakistan. In addition, we identify bat species which overlap (or neighbour) the range of the Australian camel population with a higher likelihood of carrying CoVs of the same lineage as MERS-CoV. Both of these proposed follow-on studies are examples of "proactive surveillance", a concept that has particular relevance to a One Health approach to emerging zoonotic diseases with a complex epidemiology and aetiology.

Introduction

Since the first detection and isolation of the Middle East respiratory syndrome coronavirus (MERS-CoV) in September 2012 from a fatal human case in Saudi Arabia [1], there have been more than 1000 human cases reported with a mortality rate of approximately 40% [2]. Although the primary cases are limited to nations in the Arabian Peninsula, secondary cases have been reported in many countries outside of the region, with the latest outbreak in South Korea already claiming 36 lives with more than 185 confirmed infections [3].

In contrast to the severe acute respiratory syndrome coronavirus (SARS-CoV), which was introduced into the human population through a single or a limited number of spill-over event(s) [4,5], current epidemiological studies suggest that there have been multiple introductions of different MERS-CoV strains into human population from animal reservoir(s) [6]. For SARS-CoV, there is now increasing evidence indicating that it is a bat-borne virus transmitted to humans via intermediate hosts such as palm civets and raccoon dogs [7–11]. For MERS-CoV, the natural reservoir host(s) has not yet been determined, nor how different strains of the virus have been transmitted to human populations on multiple occasions since its first discovery in 2012 [6]. Although there are reports of MERS-like CoVs in different bats around the world, discovery of closely related viruses and virus-neutralising antibodies in dromedary camels has led to the hypothesis that they are likely to be the major reservoir of MERS-CoV and camel-to-human transmission is the main route of spill-over events [6]. However, it is

* Corresponding author.
** Corresponding author at: CSIRO Australian Animal Health Laboratory, Geelong, Australia.
E-mail addresses: peter.durr@csiro.au (P.A. Durr), linfa.wang@duke-nus.edu.sg (L.-F. Wang).

http://dx.doi.org/10.1016/j.onehlt.2015.10.003

presently not clear whether MERS-CoV was introduced into the camel populations recently or whether it has adapted to camels as a natural reservoir from ancient times. A retrospective search for MERS-CoV antibodies indicated that the virus was circulating among the camel populations in the Middle East and Africa as early as 1992 and 1983, respectively [12–15]. In a recent study, the detection of a MERS-CoV conspecific virus from an African bat suggests that the MERS-CoV may have originated from an African bat, followed by bat-to-camel transmission in Africa, then the introduction of MERS-CoV to the Middle East through camel exportation/importation [6,16].

In this context, it was hypothesized that examining the serological status of Australian camels may help elucidate when MERS-CoV entered the camel population. Dromedary camels (*Camelus dromedarius*) were introduced into Australia from the mid-19th century to assist with exploration and development of the arid centre of the continent [17]. Between 1840 and 1907 many thousands of camels were imported into Australia, into an area ranging from Central Australia to the De Grey River in Western Australia. As rail services extended north to Alice Springs in Central Australia in 1929, and with the subsequent growth of motor transport, many working camels were turned loose and their feral progeny were able to survive and breed in the desert.

Since this time, the feral camel population in central Australia has undergone an exponential increase. In 1966, the population was estimated to be 15,000–20,000, and by the mid-1980s the estimate increased to a minimum population of 43,000 [18]. By 2008, the minimum population was re-estimated to be about 1 million animals [19], and there was increasing concern of the economic, social and environmental damage the uncontrolled population was causing [20]. In response, plans have been adopted for population control, including a large culling operation between 2010 and 2014, when over 150,000 camels were killed [21]. Currently Australia has the largest herd of camels anywhere in the world, and the only population of wild camels. Long-term sustainable control measures to permanently maintain a lower population density are focused on developing a viable commercialisation, particularly based on mustering to process meat for human consumption for the export market.

As a first step toward a risk assessment of potential bat-to-camel transmission of MERS-CoV like pathogens in Australia, we examined the current camel distribution in Australia and its overlap with the habitat of native bat species related to those from which MERS-CoV-like viruses have been detected. Furthermore, we conducted a MERS-CoV sero-prevalence study on more than 300 camel serum samples collected from three different locations at four time points from December 2013 to August 2014.

Materials and methods

Camel serum sampling

Blood or serum samples were collected from two locations, an abattoir that processes wild caught and farmed camels mainly for export, and from an area nearby Alice Springs, Northern Territory during a muster for the Department of Agriculture. Samples were collected on the 16th December 2013, 22nd January 2014, 17th April 2014 and 6th August 2014, respectively (see Table 1 for detail). This study was approved by both the Federal and State Agricultural Departments via the Animal Health Committee and by the Federal Department of Health in Australia.

Table 1
Camel serum samples collected in this study.

Sample#	Provider of samples	Date of collection	Animal originated from
1–31	Abattoir	16-12-2013	Central Australia
32–131	Abattoir	22-01-2014	Central Australia
132–231	Abattoir	17-04-2014	Central Australia
232–307	Camel muster	06-08-2014	Central Australia

Luminex antibody test

A Luminex-based assay was developed using recombinant nucleocapsid (N) proteins of MERS-CoV and SARS-CoV using methodology established in our group for henipaviruses [22]. Recombinant CoV N proteins were produced in *E. coli* and purified directly from SDS-PAGE gels as previously described [23]. For coating onto Luminex beads, a total of 100 μg each of the two N proteins were coupled onto 100 μl of bead set 28 (SARS-CoV) and bead set 34 (MERS-CoV), respectively. Briefly, coupled microsphere sets were vortexed and sonicated prior to dilution in PBS-T containing 2% skim milk and transferred to 96-well plate. The diluent was removed using an automated magnetic vacuum manifold followed by the addition of 100 μl of camel sera diluted 1:100 in PBS-T and incubated, shaking for 30 min at room temperature. Positive control camel sera used in this assay were derived from the natural infection of dromedary camels in Egypt during 2013 as part of a seroepidemiology study [24]. The serum was removed and the plate was washed twice with PBS-T followed by addition of Biotinylated Protein A (Pierce, Rockford, USA) and Protein G (Pierce, Rockford, USA) conjugates and incubated as described above. The conjugate was removed and the beads washed twice with PBS-T followed by addition of Streptavidin–phycoerythrin (Qiagen Pty Ltd, Australia) and a final incubation as described above. Assays were performed on a Bio-Plex Protein Array System integrated with Bio-Plex Manager Software (v 6.0) (Bio-Rad Laboratories, Inc., CA, USA). Results were recorded as median florescent intensity (MFI).

Virus neutralisation test (VNT)

VNT was conducted as previously described for SARS-CoV [10,25]. Briefly, each camel serum was tested in duplicate by doubling dilution in EMEM starting at 1:10 out to 1:1280. To 50 μl of sera an equal volume of EMEM containing 200 TCID50 of a Dromedary camel isolate of MERS virus [24] was added and incubated for 30 min at 37 °C. Vero cells were then added to each well and the plates incubated at 37 °C and subsequently read for the presence of cytopathic effect (CPE) after 4 days. Neutralising titres were recorded at the dilution at which at least one duplicate well was negative for CPE. The same positive control camel sera were used in this assay.

Analysis of the distributions of the Australian feral camels and potential MERS-CoV reservoir bat species

MERS-CoV belongs to a distinct lineage ("C") of the beta-coronavirus genus [26]. This lineage was initially defined from isolates from sampling in Hong Kong where two species of lineage C β-CoV were described: "Ty-Bat CoV HKU4" and "Pi-Bat CoV HKU5" [6].

On the assumption that lineage C β-CoVs are more likely to be found in Australian bats of the same genus as those from which they have been isolated overseas, we surveyed the peer-reviewed literature and the GenBank sequence repository for lineage C β-CoV - bat genera associations. Consequently, we identified seven Australian microbat species belonging to the families Vespertilionidae and Emballonuridae that satisfied this criterion.

There is little published literature on these seven species, and to determine potential overlap of their distribution with those of camels, we undertook habitat modelling distribution using *Maxent* version 3.3 [27]. As input for the modelling, we used the locational data stored within the Atlas of Living Australia (ALA) online database, which collates data on museum collections and sightings for most bat species of Australia (http://www.ala.org.au/). Predictor variables used were all the BioClim bioclimatic variables, Australian Land Use and Management Classification Version 7 and the NVIS Major Vegetation Subgroups (Version 4.1).

All modelling was undertaken at the resolution of 0.008° (30 s) using the WGS84 projection.