International Journal of Infectious Diseases 96 (2020) 323–326

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

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

Case Report

First molecular analysis of rabies virus in Qatar and clinical cases imported into Qatar, a case report

Bas B. Oude Munnink[a,1,*], Elmoubashar Abu Baker Abd Farag[b,1], Corine GeurtsvanKessel[a], Claudia Schapendonk[a], Anne van der Linden[a], Robert Kohl[a], Georgina Arron[a], Hisham Ziglam[c], Wael Goravey Mhjoop Goravey[c], Peter V. Coyle[c], Imad Ibrahim[c], Khaled A. Mohran[d,e], Muneera Mohammed Saleh Alrajhi[d], Md Mazharul Islam[d], Randa Abdeen[d], A. Aziz Mahmoud A.H. Al-Zeyara[d], Nidal Mahmoud Younis[d], Hamad Eid Al-Romaihi[b], Mohammad Hamad J. Al Thani[b], Richard Molenkamp[a], Reina S. Sikkema[a], Marion Koopmans[a]

[a] Erasmus MC, Department of Viroscience, WHO collaborating centre for arbovirus and viral hemorrhagic fever Reference and Research, Rotterdam, the Netherlands
[b] Ministry of Public of Health, Doha, Qatar
[c] Hamad Medical Corporation, Doha, Qatar
[d] Department of Animal Resources, Ministry of Municipals and Environment, Doha, Qatar
[e] Animal Health Research Institute, Biotechnology Departments ERC, Dokki, Egypt

ARTICLE INFO

Article history:
Received 19 November 2019
Received in revised form 23 April 2020
Accepted 25 April 2020

Keywords:
Nanopore sequencing
metagenomic sequencing
rabies virus

ABSTRACT

Identifying the origin of the rabies virus (RABV) infection may have significant implications for control measures. Here, we identified the source of a RABV infection of two Nepalese migrants in Qatar by comparing their RABV genomes with RABV genomes isolated from the brains of a RABV infected camel and fox from Qatar.

1. Introduction

Rabies virus (RABV) is a single-stranded RNA virus with a genomic length of around 12 kb and is part of the *Rhabdoviridae* family (Amarasinghe et al., 2017). Human infection may occur when exposed to infected animals, mainly carnivores but also bats, and is almost always fatal, ranking rabies among the most lethal diseases (Liu et al., 2019). RABV infection can be prevented through vaccination of humans and wild and domestic carnivores, which has successfully eliminated RABV from Western Europe (Muller and Freuling, 2018). However, RABV continues to be enzootic in large parts of the world, such as in Asia and Africa, where it causes an estimated 35,172 cases and 21,476 human deaths yearly (WHO). Dogs are the main reservoir for human rabies (Hampson et al., 2015).

RABV is commonly subdivided into six phylogenetic clades, namely the Africa 2, Africa 3, Arctic-related, Asian, Cosmopolitan, and Indian subcontinent clades. In Nepal, both the Arctic-related as well as the Indian subcontinent RABV clade are present (Pant et al., 2013), while the Cosmopolitan RABV clade currently circulates in the Arabic Peninsula (Horton et al., 2015; Troupin et al., 2016).

Recently, two RABV infections were diagnosed in Qatar in Nepalese migrant workers. In this study, we sought to unravel the source of these RABV infections. Therefore, whole genome sequences were generated from brain tissues of two human patients as well as from brain tissues of a rabid camel and a rabid fox from Qatar to determine their potential genetic relationship.

2. The study

On July 18th, 2018, the first patient, a 33-year-old Nepali was admitted to the Hamad General Hospital. The patient had arrived in Qatar one month earlier and reported an animal bite by an unknown animal three months ago in Nepal. On July 27th, a RABV infection was confirmed using two different real-time PCR assays

* Corresponding author.
[1] These authors contributed equally to this manuscript.

https://doi.org/10.1016/j.ijid.2020.04.070

on saliva samples (Wakeley et al., 2005; Wadhwa et al., 2017). The patient had no history of RABV vaccination and died on August 6th, 2018. On February 24th, 2019, a second patient, a 25-year-old Nepali from the Nuwakot district, visited the Qatar Red Crescent worker's health center in Mesaimeer after which he was transferred to the Hamad General Hospital. According to his relatives, he had been bitten by a raccoon (*Procyon lotor*) in early October 2018 in Nepal. RABV infection was confirmed on the 18th of April using the RT-PCRs described above. The patient died on March 19th, 2019. There were no RABV neutralizing antibodies detectable in serum by fluorescent antibody virus neutralization in both patients. Post-mortem tissue samples were taken after obtaining consent from the relatives.

In September 2018, the Animal Health Department in Qatar was notified of a fox (*Vulpes vulpes*) attack at a camel (*Camelus dromedarius*) farm in the Alkharsah area. Approximately two weeks after the fox bite, a camel started to develop neurological symptoms and died. The fox was captured, and brain tissue from both the fox and the camel tested positive for RABV by RT-PCR (Hoffmann et al., 2010) at the Animal Health Department in Qatar

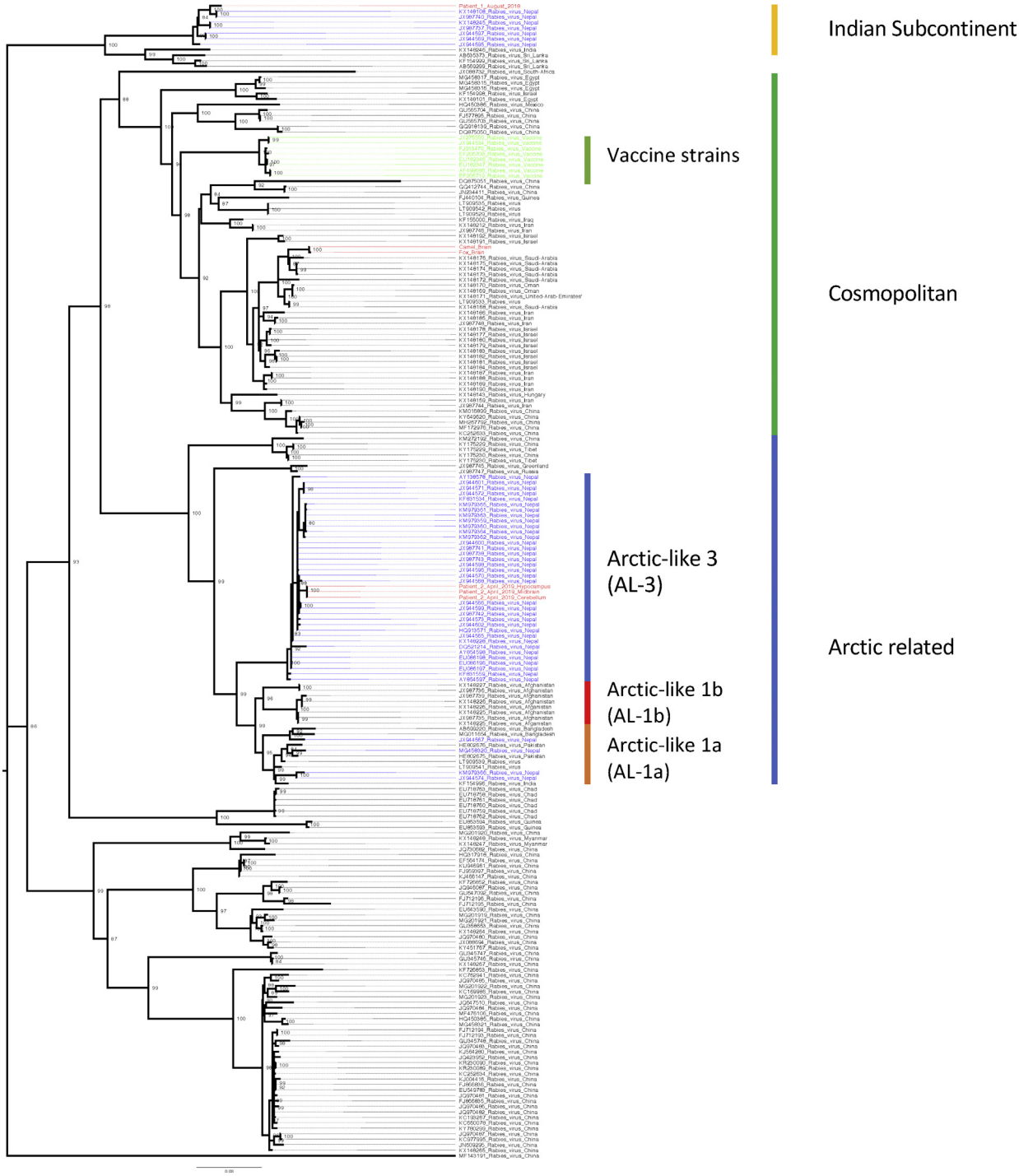

Figure 1. Phylogenetic analysis of the N-gene of the rabies virus genomes sequenced in this study. Green indicates the vaccine strains, red indicates the newly sequenced samples, and blue indicates the viruses from Nepal. A maximum-likelihood tree was constructed under the GTR + F + I + G4 model as the best-predicted model using the best model prediction function, ultrafast bootstrapping, and 1000 replicates. The tree is midpoint rooted, and the scale bar represents the number of substitutions per site.

in September 2018 and November 2018, respectively. Even though the Nepali had no direct animal contact in Qatar, these animal samples were included as there is no RABV genomic sequence data from Qatar available for comparison and source tracking. Brain material from both animals was collected, stored in Virus Transport Medium (VTM), and shipped to the Erasmus MC together with the samples from both human patients for virus isolation and sequencing.

Human and animal brain samples were suspended in supplemented DMEM (Thermofisher). From patient 1 material from the left brain was cultured while from patient 2 material from the hippocampus, the midbrain and the cerebellum were cultured. Cerebellum samples were taken from the animals. Mouse neuroblastoma astrocytes cells were seeded in supplemented DMEM in a 24-well plate. Upon 80% confluency, 200 μl of brain suspension was inoculated on the cells, followed by 15 minutes centrifugation at 3500*xg*. The plate was centrifuged in a plate centrifuge for 15 minutes at 3500xg, and the culture medium was refreshed. Cultures were placed at 36,5 °C in a 5% CO2 humidified incubator and checked for cytopathic effect daily. Once CPE was visible, the supernatant of the culture was collected, centrifuged for five minutes at 5000xg**,** and filtered using a 0.45 μm filter. Nucleic Acid was extracted, and cDNA was made using superscript IV (ThermoFisher) and random primers (ThermoFisher). dsDNA was made using Klenow (NEB) and used as input for a multiplexed metagenomic Nanopore sequencing using the SQK-PBK004 kit (Nanopore) on an R9 flowcell.

Sequences were demultiplexed and mapped to a randomly derived Nepalese RABV genome from GenBank (KX148228) using minimap2 (Li, 2018). The consensus sequences were compared to the non-redundant database using BLASTn, and the reference-based alignment was repeated with the closest reference sequence, using a 100x read coverage cut-off (Oude Munnink et al., 2019). Phylogenetic analysis was performed on the 10 closest BLAST hits for the different viruses, and also all complete RABV genomes from the Arabian Peninsula, Nepal, and neighboring countries were included (GenBank, 27-04-2019). Phylogenetic analysis was also performed on all full-length N-gene sequences from Nepal, as this has been studied previously in Nepal (Pant et al., 2013). Sequences were aligned using MUSCLE, after which the alignment was manually inspected. Phylogenetic analysis was performed in IQ-TREE (Nguyen et al., 2015) under the GTR + F + I + G4 model as the best-predicted model using the best model prediction function, ultrafast bootstrapping, and 1000 replicates. Phylogenetic analysis based on the full-length N-gene and on full-length sequences revealed that the RABV from patient 1 clustered with sequences from the Indian subcontinent clades while the RABV sequence from patient 2 clustered with the Arctic-like 3 clades (Figure 1 and **Supplementary Figure 1**). Both human patient viruses clustered most closely with viruses previously detected in Nepal. The RABV sequences from the fox and camel cluster within the Cosmopolitan clades, with viruses from the Arabic Peninsula.

3. Conclusions

Here, we describe the first two complete animal RABV sequences from Qatar and two RABV sequences from Nepalese patients diagnosed with RABV in Qatar. We show that the human RABV strains cluster with RABV sequences previously identified in Nepal. In contrast, the animal RABV sequences from Qatar are part of another clade and cluster with sequences from the Arabic Peninsula, which is in line with epidemiological information and exposure history. Therefore, we conclude that these human RABV infections were acquired in Nepal and not in Qatar. However, given the observed diversity of RABV, more information about the diversity of RABV circulating in Qatar is needed for more robust conclusions. The identification of two imported cases of RABV demonstrates that Qatar is at risk of introducing RABV, especially in migrant workers.

The last report of an animal RABV infection in Qatar dates from 2009, according to the World Organisation of Animal Health (OIE World Animal Health Information System, 2019). Our report demonstrates that RABV is still present in Qatar. This shows the need for further epidemiological research and control measures on the human-animal interface. If RABV is detected in regions with stray dogs and foxes, wild carnivore vaccination is an option to prevent economic damage to camels. Also, in areas where RABV is identified, human and animal health care workers should be informed and alerted to the presence of RABV and veterinary surveillance should be put in place, as well as careful assessment and possible treatment of humans after animal bites.

Ethical approval

Ethical approval for all activities described in this manuscript was obtained by the Health Research Governance Department at the Ministry of Public Health in Qatar under the protocol ID MRC-04-19-387.

Conflict of interest

The authors have no reported conflict of interest.

Acknowledgments

This work has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 643476 (COMPARE). We would like to thank Saleh Jaralla Almarri, Abdulla Mohd ZNAL-Marri, Tarek MAM Elsherbini, Ahmed Alatafi Almutawali Aldasuki and Ibrahim Mahmoud Ibrahim Ali for the sample collections and Tony Vincent Chawla and Jalaluddin Bhuiyan for facilitating the shipment. We would like to thanks Annemiek van der Eijk for clinical support.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.ijid.2020.04.070.

References

Amarasinghe GK, Bào Y, Basler CF, Bavari S, Beer M, Bejerman N, et al. Taxonomy of the order Mononegavirales: update 2017. Arch Virol. 2017;162(8):2493–504.
Hampson K, Coudeville L, Lembo T, Sambo M, Kieffer A, Attlan M, et al. Estimating the Global Burden of Endemic Canine Rabies. PLoS Negl Trop Dis. 2015;9(4).
Hoffmann B, Freuling CM, Wakeley PR, Rasmussen TB, Leech S, Fooks AR, et al. Improved safety for molecular diagnosis of classical rabies viruses by use of a TaqMan real-time reverse transcription-PCR "double check" strategy. J Clin Microbiol. 2010;48(11):3970–8.
Horton DL, McElhinney LM, Freuling CM, Marston DA, Banyard AC, Goharrriz H, et al. Complex Epidemiology of a Zoonotic Disease in a Culturally Diverse Region: Phylogeography of Rabies Virus in the Middle East. Rupprecht CE, editor. PLoS Negl Trop Dis 2015;9(3):e0003569.
Li H. Minimap2: pairwise alignment for nucleotide sequences. Birol I, editor. Bioinformatics. 2018;34(18):3094–100.
Liu JJ, Duo L, Tao XY, Zhu WY. [Epidemiological characteristics of human rabies in China, 2017]. Zhonghua Liu Xing Bing Xue Za Zhi. 2019;40(5):526–30.
Muller FT, Freuling CM. Rabies control in Europe: an overview of past, current and future strategies. Rev Sci Tech l'OIE. Aug 1 2018;37(2):409–19.
Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2015;32(1):268–74.
OIE World Animal Health Information System. OIE World Animal Health Information System [Internet]. 2009.. 2019 [cited 2019 Jul 29]. Available from: http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Animalsituation.
Oude Munnink BB, Kik M, de Bruijn ND, Kohl R, van der Linden A, Reusken CBEM, et al. Towards high quality real-time whole genome sequencing during outbreaks using Usutu virus as example. Infect Genet Evol. Sep 1 2019;73:49–54.

Pant GR, Lavenir R, Wong FYK, Certoma A, Larrous F, Bhatta DR, et al. Recent Emergence and Spread of an Arctic-Related Phylogenetic Lineage of Rabies Virus in Nepal. Munoz-Zanzi C, editor. PLoS Negl Trop Dis 2013;7(11):e2560.

Troupin C, Dacheux L, Tanguy M, Sabeta C, Blanc H, Bouchier C, et al. Large-Scale Phylogenomic Analysis Reveals the Complex Evolutionary History of Rabies Virus in Multiple Carnivore Hosts. Parrish C, editor. PLOS Pathog 2016;12(12):e1006041.

Wadhwa A, Wilkins K, Gao J, Condori Condori RE, Gigante CM, Zhao H, et al. A Pan-Lyssavirus Taqman Real-Time RT-PCR Assay for the Detection of Highly Variable Rabies virus and Other Lyssaviruses. PLoS Negl Trop Dis. Jan 12 2017;11(1).

Wakeley PR, Johnson N, McElhinney LM, Marston D, Sawyer J, Fooks AR. Development of a real-time, TaqMan reverse transcription-PCR assay for detection and differentiation of lyssavirus genotypes 1, 5, and 6. J Clin Microbiol. 2005;43(6):2786–92.