Transboundary and Emerging Diseases

RAPID COMMUNICATION

Middle East Respiratory Syndrome Coronavirus Antibody Reactors Among Camels in Dubai, United Arab Emirates, in 2005

S. Alexandersen[1], G. P. Kobinger[2], G. Soule[2] and U. Wernery[3]

[1] National Centres for Animal Disease (NCAD), Canadian Food Inspection Agency, Canadian Science Centre for Human and Animal Health, Winnipeg, MB, Canada
[2] National Microbiology Laboratory (NML), Public Health Agency of Canada, Canadian Science Centre for Human and Animal Health, Winnipeg, MB, Canada
[3] Central Veterinary Research Laboratory (CVRL), Dubai, United Arab Emirates

Keywords:
Middle East respiratory syndrome coronavirus; coronavirus; antibodies; camels; dromedaries; Middle East

Correspondence:

S. Alexandersen. National Centres for Animal Disease (NCAD), Canadian Food Inspection Agency, Canadian Science Centre for Human and Animal Health, 1015 Arlington Street, Winnipeg MB R3E 3M4, Canada.
Tel.: +1 204 789 2102;
Fax: +1 204 789 2038;
E-mail: soren.alexandersen@inspection.gc.ca

Received for publication January 6, 2014

doi:10.1111/tbed.12212

Summary

We tested, using a low starting dilution, sequential serum samples from dromedary camels, sheep and horses collected in Dubai from February/April to October of 2005 and from dromedary camels for export/import testing between Canada and USA in 2000–2001. Using a standard Middle East respiratory syndrome coronavirus (MERS-CoV) neutralization test, serial sera from three sheep and three horses were all negative while sera from 9 of 11 dromedary camels from Dubai were positive for antibodies supported by similar results in a MERS-CoV recombinant partial spike protein antibody ELISA. The two negative Dubai camels were both dromedary calves and remained negative over the 5 months studied. The six dromedary samples from USA and Canada were negative in both tests. These results support the recent findings that infection with MERS-CoV or a closely related virus is not a new occurrence in camels in the Middle East. Therefore, interactions of MERS-CoV at the human–animal interface may have been ongoing for several, perhaps many, years and by inference, a widespread pandemic may be less likely unless significant evolution of the virus allow accelerated infection and spread potential in the human population.

The Middle East respiratory syndrome coronavirus (MERS-CoV) has been linked to severe human respiratory disease starting in September of 2012 (van Boheemen et al., 2012; Zaki et al., 2012). As of 27 December 2013, a total of 170 laboratory-confirmed human cases of infection with MERS-CoV, including 72 deaths, are reported by the World Health Organization (WHO) (WHO update 27 December 2013 accessed online). Most cases originated in the Middle East, including Jordan, Kuwait, Quatar, Saudi Arabia, Oman and United Arab Emirates, and due to the findings of antibodies reacting to this virus, as well as recent positive RT-PCR detection of fragments of the MERS-CoV RNA, in dromedary camels, it has been hypothesized that camels may be an original or intermediary host of the MERS-CoV (Butler, 2013; Haagmans et al., 2013; Hawkes, 2013; Hemida et al., 2013; Kupferschmidt, 2013; Perera et al., 2013; Reusken et al., 2013a,b; de Wit and Munster, 2013). Furthermore, a very recent online report has indicated that antibody reactors have been present in camels in Dubai, United Arab Emirates, going back to at least 2003 (Meyer et al., 2014). However, due to limited amounts of the sera available to those authors, they used a high starting dilution for their testing and that study could consequently only detect strong antibody reactors, and therefore, although older camels included in the study indeed were found to be positive, the few young and relatively young camels deemed as negative could indeed have been positive albeit at a lower titre.

To further study antibodies to the MERS-CoV, or to a closely related coronavirus, present in the dromedary camels in the Middle East for some time, we did serology on 47 sera from 11 dromedary camels, 20 sera from three sheep and 17 sera from 3 horses collected in Dubai in the period from February/April to October of 2005. The details of the studies from which these samples originated have been described previously (Frederiksen et al., 2006; Wernery et al., 2006; Alexandersen et al., 2008). The 11 dromedaries are all from the Dubai Emirate and included two dromedary calves born at the Central Veterinary Research Laboratory and without any contact to other camels. For comparison, we also tested sera from 6 dromedary camels collected for export/import testing between Canada and USA in 2000 and 2001; these camels most likely were imported into North America from Australia in the mid- to late nineties where such import was allowed. The Australian dromedary camel population has been geographically separated from the camel populations of the Middle East and Asia for close to one hundred years and consequently provide a good control for our studies described here. Before doing serology, we extracted RNA from an aliquot of all the sera and tested them in a standard MERS-CoV RT-PCR with negative results. The antibody detection tests used heat inactivated sera and included a virus neutralization test (VNT) starting at a serum dilution at 1 : 12 and using standard methods as described previously for the SARS coronavirus (Kobinger et al., 2007) on all the sera and for the camel sera only also a MERS-CoV indirect antibody ELISA starting at a serum dilution of 1:100 and using recombinant partial spike protein (S1 fragment, catalogue number 40069-V08H from Sino Biological Inc., Beijing, China, www.sinobiological.com) and a horseradish peroxidase-labelled goat anti-camel immunoglobulin detection antibody (product code V2023 obtained from Lillidale LTD., Dorset, UK) using standard ELISA conditions as described previously for detecting antibodies to, for example, ebola virus proteins (Wong et al., 2012). All the sera from sheep and horses were negative in VNT and the 6 North American dromedary camels were negative in both tests. In contrast, all 33 samples collected from 9 dromedaries from February to October 2005 had VNT titres of 1 : 24–1 : 384, and of these, 30 samples also had ELISA titres of 1 : 200–1 : 6400. A single sample from each of three individual camels was ELISA negative in February and October, respectively, but these animals had a titre of 1 : 200–1 : 800 in the other samples collected in October. All seven samples available from these three animals, 1 sample collected in February and the other six samples collected 2 weeks apart in October 2005, also had relatively low VNT titres (1 : 24–1 : 96), and the single negative ELISA results for each of these three dromedaries therefore correlated with a low level of antibodies close to the detection limit of the ELISA. The 14 samples collected from two dromedary calves from April to October 2005 were consistently negative for antibodies in both tests. Details of the results can be seen in Table 1.

Although our results do not provide proof for the presence of MERS-CoV in dromedary camels, they do support the finding (Meyer et al., 2014) that this virus, or a closely related coronavirus, is not a new infection in dromedary camels and likely has been ongoing for at least 10 years or more. From the data available, it appears that young dromedary calves, at least under the conditions they were kept in Dubai, are not antibody positive, while the older camels studied all had been exposed and had antibodies before the study started in February/April of 2005. None of the camels in this study seroconverted or changed significantly in regard to titre over the time period studied. Although limited in numbers, the dromedary calves were completely seronegative while the older animals were seropositive. Interestingly, the oldest camel in the study, a more than 20-year-old female dromedary born in the Dubai Emirate as an offspring of camels imported from Sudan (labelled number 10 Sudani Lady in Table 1) had low titres relative to other younger camels of between 4 and 11 years of age. As seroconversion was not observed, one can only speculate as to when, where and how the camels may have been exposed to MERS-CoV or a closely related coronavirus. The possibility exists that these camels, kept at the Central Veterinary Research Laboratory grounds in Dubai and all originating from the Dubai Emirate, may have been exposed elsewhere or that these exposures may have taken place at a time outside of the period studied (i.e. perhaps in the period from November to February/March which happens to be the relatively cooler and more humid period in Dubai) or even years earlier. From the data available here, it appears that these exposures occurred at an age that is between the age of the calves and the age of the young adults. Although not directly comparable from laboratory to laboratory, the VNT titres we observed in the seropositive dromedary camels sampled in Dubai in 2005 were in the same order as described in recent sero-survey studies in Saudi Arabia, Jordan and Egypt from 2010 to 2013 (Hemida et al., 2013; Perera et al., 2013; Reusken et al., 2013a), but much lower than described in an active outbreak investigation in Qatar from 2013 (Haagmans et al., 2013) and, interestingly, also lower than what has been reported in another study on camel sera collected in the Dubai Emirate in 2003 and 2013, which however, only tested at high serum dilutions (Meyer et al., 2014). Nevertheless, this may be caused by differences in the details of the assays used or potentially suggests that exposure/active infection of dromedary camels is not temporally homogeneous, but may occur or be more widespread in some settings or some years. If that is the case, the samples studied by Meyer et al.

Table 1. Detection of antibodies to Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in Dubai dromedary camel sera from 2005 using virus neutralization test (VNT) and S1 ELISA

	MERS-CoV VNT titres								S1 ELISA titres							
	10-02-2005	05-04-2005	19-04-2005	10-05-2005	02-08-2005	06-09-2005	04-10-2005	21-10-2005	10-02-2005	05-04-2005	19-04-2005	10-05-2005	02-08-2005	06-09-2005	04-10-2005	21-10-2005
Camel 19 female		384	192	96	96	96	96	48		400	800	1600	800	1600	400	400
Camel 47 female		192	192	192	96	192	192	192		400	1600	1600	800	6400	800	800
Camel 49 female		384	384	192	384	192	192			200	800	400	800	1600	800	
Camel 40 male calf		Neg	Neg	Neg	Neg	Neg	Neg	Neg		Neg	Neg	Neg	Neg	Neg	Neg	Neg
Camel 45 male calf		Neg	Neg	Neg	Neg	Neg	Neg	Neg		Neg	Neg	Neg	Neg	Neg	Neg	Neg
Camel 50 (30) male							96	24							200	Neg
Camel 9 (33) male							192	192							1600	800
Camel 34 (6) pregnant female							96	48							1600	800
Camel 45 (32) female							48	24							1600	800
Camel 35 (20) female							24	48							Neg	400
Camel 10 (Sudani Lady) female	96						96	24	Neg						800	800

Titres given as reciprocal dilution testing positive in the test. Negative (Neg) means negative at 1 : 12 dilution for the VNT, and negative for the S1 ELISA means negative at 1 : 100 dilution. All sheep and horse sera tested were negative in VNT, and the North American dromedary sera tested were negative in both tests.

(2014) and collected in the Dubai Emirate in 2003 and 2013, as well as the outbreak investigation in Qatar in 2013 (Haagmans et al., 2013), may have included recently infected dromedaries (with a high antibody titre) while our Dubai camels may have been exposed some time before 2005, perhaps 2003 or even earlier, and not be a recent event. If indeed widespread infection/exposure of dromedary camels only occurs in certain years or in special, as yet undetermined, settings or conditions, that may explain why the infection, if either transferred to humans from dromedaries or under conditions similar to what may cause widespread camel exposure perhaps by vectors, may have remained undetected for this long. It is worth noting that 6 North American dromedaries bled in 2000/2001 were completely seronegative which in turn, as these animals most likely originated in Australia, may suggest that the infection is not established in the Australian/North American dromedary populations that have been separated from dromedaries in the Middle East and Asia for almost one hundred years or alternatively, but considered less likely if not unlikely, have got established in the camel population sometime between year 2000 and 2005/2003.

This study detected the presence of antibodies to MERS-CoV, or a closely related coronavirus, in dromedary camels in the Middle East dating back to 2005 supporting and extending findings reported by Meyers et al. online (Meyer et al., 2014). Taken together, the data indicate that MERS-CoV or a MERS-CoV-like virus did not emerge in camels as recently as 2012 but rather is an occurrence that preceded 2005/2003. Consequently, this suggests that limited spillovers to humans may have been occurring sporadically, albeit undetected, for a longer period of time than currently suspected. Provided that the virus has not recently acquired additional potential for human infection and transmission, these results may ease fears of an immediate widespread pandemic in humans.

Acknowledgements

We acknowledge the staff at the Danish Veterinary Institute, Lindholm, Denmark, for storing the Dubai sera from when they were collected in 2005 and for sharing them for use in this study. We also acknowledge Kathleen Hooper-McGrevy and Tom Hunt for sharing North American sera and thank Kate Hole, Tim Salo and Greg Smith for technical assistance.

References

Alexandersen, S., U. Wernery, P. Nagy, T. Frederiksen, and P. Normann, 2008: Dromedaries (*Camelus dromedarius*) are of low susceptibility to inoculation with foot-and-mouth disease virus serotype O. *J. Comp. Pathol.* 139, 187–193.

van Boheemen, S., M. de Graaf, C. Lauber, T.M. Bestebroer, V.S. Raj, A.M. Zaki, A.D. Osterhaus, B.L. Haagmans, A.E. Gorbalenya, E.J. Snijder, and R.A. Fouchier, 2012: Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *MBio.* 3, e00473–12.

Butler, D., 2013: Progress stalled on coronavirus. *Nature* 501, 294–295.

Frederiksen, T., J. Borch, J. Christensen, K. Tjornehoj, U. Wernery, and S. Alexandersen, 2006: Comparison of FMDV Type O, A and Asia 1 Antibody Levels After Vaccination of Cattle, Sheep, Dromedary Camels and Horses. Session of the Research Group of the Standing Technical Committee of EuFMD, Paphos, Cyprus, 17-20 October 2006 Appendix 36, pp. 243–249. Available at: http://www.fao.org/ag/againfo/commissions/docs/research_group/paphos/App36.pdf (accessed December 28, 2013).

Haagmans, B.L., S.H. Al Dhahiry, C.B. Reusken, V.S. Raj, M. Galiano, R. Myers, G.J. Godeke, M. Jonges, E. Farag, A. Diab, H. Ghobashy, F. Alhajri, M. Al-Thani, S.A. Al-Marri, H.E. Al Romaihi, A. Al Khal, A. Bermingham, A.D. Osterhaus, M.M. Alhajri, and M.P. Koopmans, 2013: Middle East respiratory syndrome coronavirus in dromedary camels: an outbreak investigation. *Lancet Infect. Dis.* 12, pii: S1473–3099.

Hawkes, N., 2013: Camels could be the source of MERS coronavirus, research finds. *BMJ* 347, f5052.

Hemida, M., R. Perera, P. Wang, M. Alhammadi, L. Siu, M. Li, L. Poon, L. Saif, A. Alnaeem, and M. Peiris, 2013: Middle East Respiratory Syndrome (MERS) coronavirus seroprevalence in domestic livestock in Saudi Arabia, 2010 to 2013. *Euro. Surveill.* 18, pii: 20659.

Kobinger, G.P., J.M. Figueredo, T. Rowe, Y. Zhi, G. Gao, J.C. Sanmiguel, P. Bell, N.A. Wivel, L.A. Zitzow, D.B. Flieder, R.J. Hogan, and J.M. Wilson, 2007: Adenovirus-based vaccine prevents pneumonia in ferrets challenged with the SARS coronavirus and stimulates robust immune responses in macaques. *Vaccine* 25, 5220–5231.

Kupferschmidt, K., 2013: Emerging diseases. Researchers scramble to understand camel connection to MERS. *Science* 341, 702.

Meyer, B., M.A. Müller, V.M. Corman, C.B.E.M. Reusken, D. Ritz, G.-J. Godeke, E. Lattwein, S. Kallies, A. Siemens, J. van Beek, J.F. Drexler, D. Muth, B.-J. Bosch, U. Wernery, M.P.G. Koopmans, R. Wernery, and C. Drosten, 2014: Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. *Emerg. Infect. Dis.* [Internet]. Available at: http://dx.doi.org/10.3201/eid2004.131746 (accessed January 2, 2014).

Perera, R.A., P. Wang, M.R. Gomaa, R. El-Shesheny, A. Kandeil, O. Bagato, L.Y. Siu, M.M. Shehata, A.S. Kayed, Y. Moatasim, M. Li, L.L. Poon, Y. Guan, R.J. Webby, M.A. Ali, J.S. Peiris, and G. Kayali, 2013: Seroepidemiology for MERS coronavirus using microneutralisation and pseudoparticle virus neutralisation assays reveal a high prevalence of antibody in dromedary camels in Egypt. *Euro. Surveill.* 18, pii: 20574.

Reusken, C., M. Ababneh, V. Raj, B. Meyer, A. Eljarah, S. Abutarbush, G. Godeke, T. Bestebroer, I. Zutt, M. Muller, B. Bosch, P. Rottier, A. Osterhaus, C. Drosten, B. Haagmans, and M. Koopmans, 2013a: Middle East Respiratory Syndrome coronavirus (MERS-CoV) serology in major livestock species in an affected region in Jordan, June to September 2013a. *Euro. Surveill.* 18, pii: 20662.

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

Wernery, U., P. Nagy, C.M. Amaral-Doel, Z. Zhang, and S. Alexandersen, 2006: Lack of susceptibility of the dromedary camel (*Camelus dromedarius*) to foot-and-mouth disease virus serotype O. *Vet. Rec.* 158, 201–203.

WHO 2013: WHO Global Alert and Response (GAR): Middle East respiratory coronavirus – update 27 December 2013. http://www.who.int/csr/don/2013_12_27/en/index.html (accessed December 27, 2013).

de Wit, E., and V.J. Munster, 2013: MERS-CoV: the intermediate host identified?. *Lancet Infect Dis.* 13, 827–828.

Wong, G., J.S. Richardson, S. Pillet, A. Patel, X. Qiu, J. Alimonti, J. Hogan, Y. Zhang, A. Takada, H. Feldmann, and G.P. Kobinger, 2012: Immune parameters correlate with protection against ebola virus infection in rodents and nonhuman primates. *Sci. Transl. Med.* 4, 158ra146.

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