DOI : 10.5958/2277-8934.2014.00001.0 *Vol 21 No 1, p 1-4*

SOME EPIDEMIOLOGICAL STUDIES ON MERS CORONAVIRUS IN DROMEDARIES IN THE UNITED ARAB EMIRATES- A SHORT COMMUNICATION

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ABSTRACT

The Middle East Respiratory Syndrome (MERS) caused by a coronavirus emerged in the Middle East in 2012, and has killed so far more than 300 people most of them in Saudi Arabia.

MERS is a zoonotic disease and transmission from the dromedary camel to humans has been documented. However, most cases occur between humans. The low incidence of transmission from camel to human has several reasons. The virus is excreted only for 8 days and mainly young dromedaries are infected which have very little or no contact to their caretakers. It has yet not been proven how and from where the calves get there infection. Over 90% of adult dromedaries possess specific MERS- CoV antibodies and do not shed the virus.

Thirty dromedaries (15 dams and 15 calves) were tested at the Saudi Arabian border to the UAE for MERS-CoV infection. All dams had seroconverted, but were PCR and virus negative. However, 13 of their offsprings had antibodies to MERS-CoV, 11 (73%) were positive in PCR and from 5 (33%) MERS-CoV was isolated. A visit 8 days later showed that all had seroconverted, 4 (27%) remained PCR positive but none exhibited virus in their nasal cavities.

Key words: Dromedary, epidemiology, MERS-CoV

In 2012 a formerly unknown coronavirus infection emerged in the Middle East to which several hundred people succumbed so far. Most human cases were reported from Saudi Arabia but also other countries of the Arabian Peninsula observed human fatalities.

Through intensive serological investigations it was shown that over 90% of dromedaries on the Arabian Peninsula had antibodies against the Middle East Respiratory Syndrome (MERS) corona virus (Co), some dating back as far as 10 to 20 years (Meyer *et al,* 2014; Alexandersen *et al,* 2014).

In contrary, no MERS-CoV antibodies were found in camelids from North America, Europe or Australia.

Recently it was shown that direct contact to a dromedary in Saudi Arabia by its owner resulted in a MERS-CoV infection with fatal consequence. The full genome sequences of both, the dromedary and human isolates were identical (Azhar *et al,* 2014). However, most of the MERS-CoV infections were reported to occur between humans.

New scientific evidence has arisen of MERS-CoV excretion after 3 dromedaries were artificially infected in the USA (Adney *et al,* in press). An epidemiological study on a small herd of 15 dromedary dams including 15 calves was conducted, the results of which are reported here.

Materials and Methods

Fifteen dromedary mothers and their 3 to 5 month- old calves from the Emirate of Dubai which had spent several months in the desert of Saudi Arabia for grazing were quarantined at the UAE-Saudi Arabian border in Gheweifat near Qatar. Samples were taken twice within 8 days of each other. All camel handlers and people who took samples wore protection equipment including face masks and surgical gloves.

From all 30 dromedaries (15 dams and 15 calves) blood was taken from the jugular vein as well as nasal swabs. One nasal swab was taken from both nostrils by inserting the swab deep into the nose cavity and turning it 10 to 20 times in the nose. The swab was then inserted into RNAlater to preserve the RNA. The second swab was taken the same way and inserted into viral transport medium which included antibiotics. All samples were stored in a cool box and

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brought by helicopter to CVRL where the blood was separated and serum frozen at -20°C as were the virus transport medium samples. RNAlater samples were kept at 4°C until testing.

Serum samples were examined for MERS-CoV antibodies using the commercially available indirect antibody ELISA (Euroimmune, Germany). The samples for virus isolation were tested at the high security Laboratory (BSL-3 Lab) at CVRL.

All 30 virus transport medium samples were subjected to bacterial filtration (0.4µm filter) and then pipetted onto Vero cells. The cells were adspected under an inverted microscope every day for 4 days for the appearance of a cytopathic effect (CPE). PCR was performed using primers as described by Haagmans *et al* (2014). Samples from the 15 dams were taken only on the first visit to the quarantine.

Results

Table 1 shows the PCR, virus isolation and serology results of 15 dromedary mothers and their offsprings returning to the UAE from Saudi Arabia. Samples were taken twice from the 15 dromedary calves but not from their mothers.

All 15 dams possessed MERS-CoV antibodies but no virus was isolated from the nasal swabs and also the PCR was negative.

Eleven out of 15 dromedary calves (73%) were positive in PCR on the first visit and 8 days later only 4 calves (27%) remained PCR positive. Out of the 15 calves MERS CoV was isolated from 5 calves (33%) on the first trip and none 8 days later. From 11 PCR positive cases only 5 were also virus positive (45%). The virus grew well on Vero cells and a CPE was detected 48h after incubation (Fig 1).

Serum samples withdrawn from calves during the first trip revealed 11 positive, 2 dubious and 2 negative samples, where as all 15 camel calves were positive 8 days later in the indirect ELISA. Only 1 camel (No 3) displayed clear nasal discharge from both nostrils, but no virus was isolated. However, it was antibody positive on both days and PCR positive on the first trip, but negative when retested 8 days later. All dams did not excrete any nasal fluid when swabbed.

Discussion

Coronavirus infection have been reported in camelids (Wernery *et al,* 2014). The disease is caused by the bovine coronavirus (BCoV). Group 1 was isolated from New World camels (NWC) with respiratory disease and group 2 from NWCs and dromedaries with diarrhoea. It is mainly a disease of young animals. A novel dromedary Betacoronavirus (Dc CoV UAE-HKN23) was recently isolated from faecal samples of 3 adult dromedaries with no clinical signs (Woo *et al,* 2014). MERS-CoV infection in dromedaries were reported from Oman (Nowotny

Table 1. Serological, viral and PCR results for MERS-CoV of 15 dromedary mothers and their calves.

ID	ELIS Ab	Virus Isolation	PCR	ID	Ab ELISA		Virus Isolation		PCR	
					Day 0	Day 8	Day 0	Day 8	Day 0	Day 8
M1	+	-	-	C1	±	+	+	-	+	-
M2	+	-	-	C2	+	+	-	-	-	-
M3	+	-	-	C3	+	+	-	-	+	-
M4	+	-	-	C4	-	+	-	-	+	+
M5	+	-	-	C5	±	+	-	-	+	-
M6	+	-	-	C6	-	+	-	-	+	-
M7	+	-	-	C7	+	+	-	-	-	-
M8	+	-	-	C8	+	+	-	-	-	-
M9	+	-	-	C9	+	+	+	-	+	-
M10	+	-	-	C10	+	+	+	-	+	-
M11	+	-	-	C11	+	+	+	-	+	+
M12	+	-	-	C12	+	+	-	-	-	-
M13	+	-	-	C13	+	+	-	-	+	+
M14	+	-	-	C14	+	+	+	-	+	-
M15	+	-	-	C15	+	+	-	-	+	+
Total	15	0	0	Total	11+2	15	5	0	11	4

M = Mother C = Calf + = Positive ± = Dubious - = Negative x= Discharge from nose (C3)

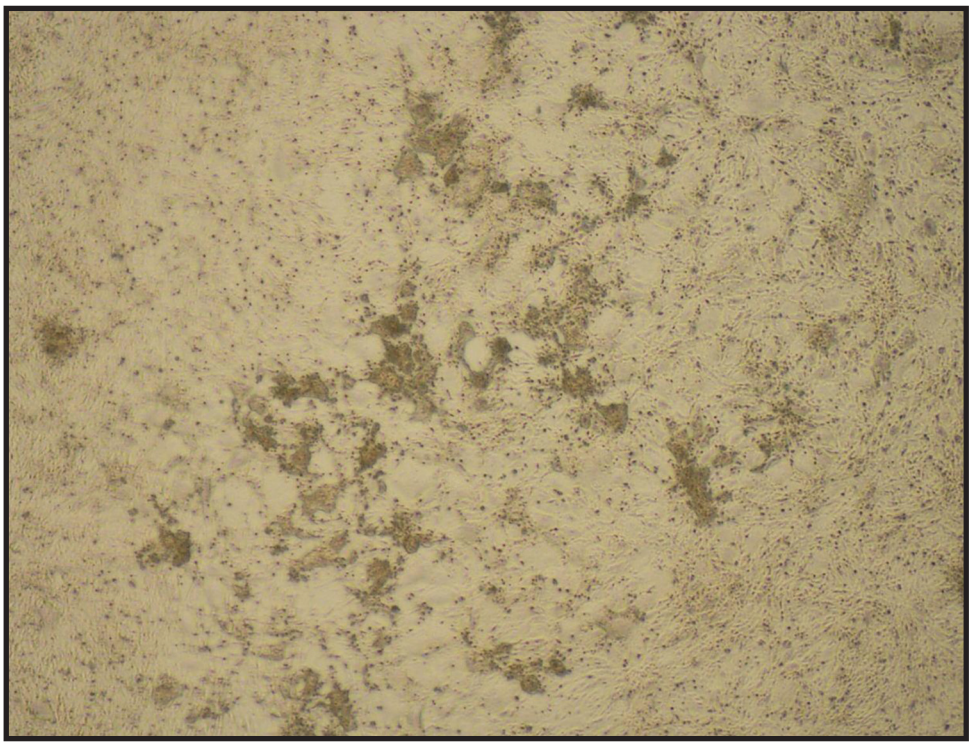

Fig 1. MERS-CoV isolated from a dromedary nose showing a CPE on Vero cells after 48h incubation at 37°C.

and Kolodziejek, 2014) and Saudi Arabia (Hemida *et al,* 2014). The infection was caused in dromedaries less than 1 year-old. Our investigations seem to show that mainly young dromedaries are subjected to MERS-CoV infection. It is not clear yet how and from where they get infected. During the next delivery season which is in spring 2015 intensive investigation should concentrate on a diaplacental infection route. It is also conceivable to infect pregnant MERS-CoV antibody negative dromedaries to elucidate the epidemiology in pregnant camels and their offsprings. No nasal discharge was observed in 5 virus-positive dromedary calves from our investigation. Only camel calf No 3 showed nasal discharge, was PCR-positive but no virus was isolated. This seems to show that these camel calves either did not develop a rhinitis or the rhinitis was even shorter than the presence of virus in the nasal cavity; another fact which may explain the low transmission rates to humans.

Young dromedaries less than 1 year-old are ''semiwild'' and are only handled when they are sick (see the above mentioned Saudi Arabian case) which is seldom the case. This clearly indicates that close contact to young dromedaries is rare and therefore the transmission to humans as well. In contrary adult camels, however are more or less handled on a daily basis for example when they are milked. They do not seem to transmit the virus to people most probably because over 90% have antibodies to MERS-CoV.

It has been experimentally proven that dromedaries excrete the virus for not more than 8 days (Adney *et al,* in press). This is confirmed by our field investigations. After 8 days all 5 calves from which the virus was isolated became negative. However, they remain PCR-positive for longer period of time, in the experiment for 35 days.

On the Arabian Peninsula where dromedaries play an important role for their owners, thousand and thousand camel caretakers have contact to their ''beast of burden'' on a daily basis but only few have contracted the disease. We tested 230 sera from camel caretakers of the Emirate of Dubai for MERS-CoV antibodies. These were all negative (unpublished data).

However, it is known that 60% of all infectious diseases (bacteria, virus, fungus, protists – formerly known as protozoans) are zoonotic diseases including SARS and MERS and spillovers are unpredictable. Dynamics of transmission is unknown and may depend on many factors like geographical patterns, reservoirs seasonal factors, gender and age.

Burke (1998) listed the criteria that might implicate certain viral families as possible candidates to

cause new pandemics. He chose the Orthomyxoviridae (including influenza), the Retroviridae (including HIV) and the Coronaviridae (such as SARS) as the most dangerous virus families because most of them are ready to mutate and recombine (or reassort). He considers especially coronaviruses as serious threats to human health. This statement came 6 years before the SARS epidemic.

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