Food and Agriculture Organization of the United Nations

November 2021

ANIMAL HEALTH **RISK ANALYSIS** MANAGEMENT No. 3

INVESTIGATING POTENTIAL RECOMBINATION OF MERS-CoV AND SARS-CoV-2 OR OTHER CORONAVIRUSES IN CAMELS

Supplementary recommendations for the epidemiological investigation of SARS-CoV-2 in exposed animals

SUMMARY

- Middle East Respiratory Syndrome (MERS) is caused by a zoonotic coronavirus (zCoV), a severe or fatal disease in humans.
- Dromedary camels are the main reservoir species for MERS-CoV and can also be infected by other human or animal CoVs.
- With the pandemic spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in humans, it is not a matter of if, but rather when, camels will be exposed to SARS-CoV-2.
- Co-circulation of both viruses in the same host for extended periods can favour virus recombination, potentially leading to the emergence of new, recombinant viruses with increased virulence in animals and/or humans.
- These recommendations will assist national authorities and research institutions to systematically investigate the susceptibility of camels to SARS-CoV-2 using a step by step approach.
- This document contains detailed guidance on One Health field epidemiology investigations and laboratory protocols to detect recombination of MERS-CoV and SARS-CoV-2 or other coronaviruses in camels.

BACKGROUND

Dromedary camels are the main reservoir species for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) (Sikkema *et al.*, 2019). Genetic analysis of thousands of MERS-CoV isolates from humans and dromedaries revealed that direction of transmission is from camels to humans, rather than vice versa (Dudas *et al.*, 2018). Several studies reported evidence of camel infection by other human CoV (HCoV-229E) (Corman *et al.*, 2016), animal CoV (bovine-like coronavirus) (Vlasova and Saif, 2021) or unknown coronaviruses (Alraddadi *et al.*, 2019). There is evidence of recombination between different betacoronaviruses in camels (So *et al.*, 2019). Analysis of dromedaries' Angiotensin-converting enzyme 2 receptor (ACE2) predicted potential binding affinity to the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) receptor binding domain (RBD), however some other studies predicted the contrary (El Masry *et al.*, 2020).

With the pandemic spread of SARS-CoV-2, countries such as Saudi Arabia, United Arab Emirates, Qatar and Kuwait with high density of dromedaries and/or use of camel products as well as reports of human cases of MERS-CoV infection and positive findings in camels also reported thousands of COVID-19 human cases. Other countries with high camel densities and positive MERS-CoV cases in camels but not up to now in humans have reported COVID-19 in humans as well: Kenya, Ethiopia, the Sudan, Mauritania and Mali. Therefore, it is not a matter of if, but rather when, camels will be exposed to SARS-CoV-2 in these countries. Although there has been progress in the search for candidate vaccines against MERS-CoV based on the spike protein (Al-Amri *et al.*, 2017), no vaccine is available to date for use with either camels or humans.

Co-circulation of both viruses in the same host for extended periods can favour virus recombination (Baddal and Cakir, 2020) and may lead to increased virulence in animals and/or humans if the recombinant virus incorporates the pathogenic characteristics of MERS-CoV with the highly transmissible SARS-CoV-2. Further investigations into camel susceptibility to SARS-CoV-2, possible recombination between MERS-CoV and SARS-CoV-2 or other coronaviruses in camels, with associated zoonotic risks are urgently required to ensure early detection.

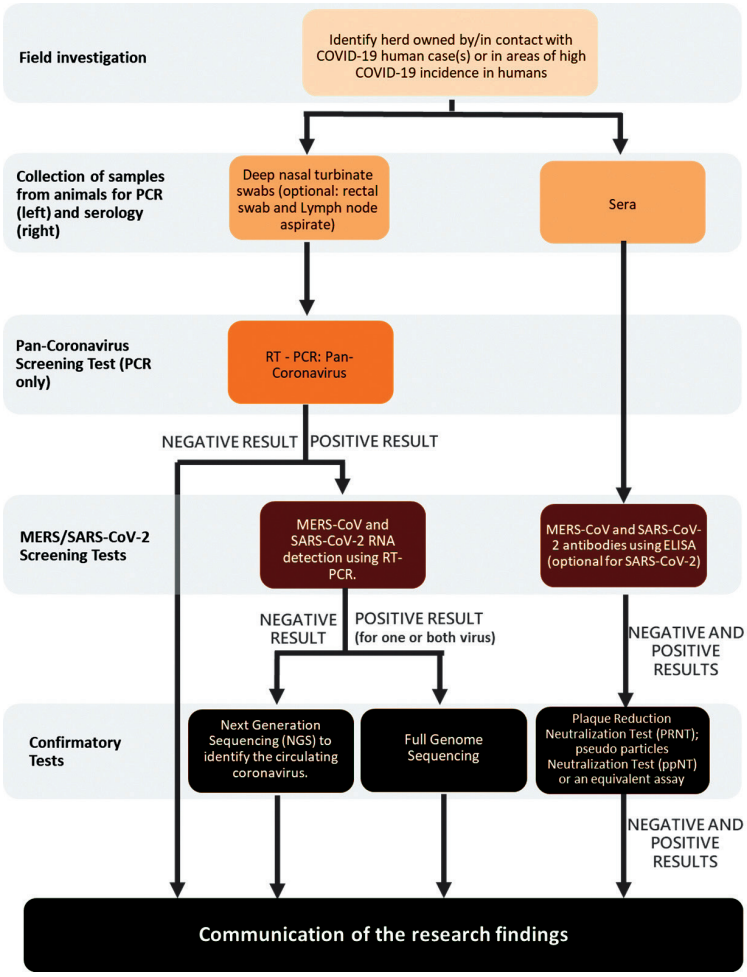
OBJECTIVES OF THIS DOCUMENT

- To provide guidance on investigating the susceptibility of camels to SARS-CoV-2 using a step by step approach.
- To provide recommendations on One Health field epidemiology investigations and laboratory protocols to detect recombination of MERS-CoV and SARS-CoV-2 or other coronaviruses in camels.

Note: Assessing the zoonotic potential of recombinant viruses, if detected, is an additional step not covered by this protocol that will involve international reference laboratories and the tripartite organizations Food and Agriculture Organization of the United Nations (FAO), World Organisation for Animal Health (OIE) and World Health Organization (WHO).

For guidance on all other farmed or companion animal species, please refer to this document: Recommendations for the epidemiological investigation of SARS-CoV-2 in exposed animals.

FIGURE 1. *Flowchart of the different steps in the detection of MERS-CoV and SARS-CoV-2 or other coronaviruses in camels and investigation of recombination*



A. LABORATORY RESEARCH TO INVESTIGATE THE SUSCEPTIBILITY OF CAMELS TO SARS-CoV-2

(This work can be done in preparation or in parallel with the field investigation studies described under B.)

	Objectives	Description	Assay or virus type
Research Study 1 (optional) cell transfection	• Assess if cells of camel-origin are permissive to the SARS-CoV-2*	• HeLa or other relevant cells (negative to ACE2) • Insert plasmid carrying full length cDNA of camel ACE2 • Infect cell expressing full-length camel ACE2	• Pseudotype virus** • SARS-CoV-2 (preferred)
Research Study 2 (recommended) tissue explant	• Assess SARS-CoV-2 replication in camel tissues and production of infectious particles	• Experimental infection of camel tissue organoid culture (upper and lower respiratory tract tissues)	• SARS-CoV-2

* Transfected cells expressing full length amino acids of natural isoform (with one mutation Y217N) of Rhesus monkey ACE2 were not permissive to SARS-CoV-2 despite proven susceptibility of Rhesus monkeys after experimental infection (Zhang *et al.*, 2021), suggesting that the virus might behave differently in different breeds of the same species. Therefore, if transfected cells expressing camel ACE2 result as "not permissive", it is advisable to perform tissue explant studies. If the latter results are negative, experts should be consulted on whether to hold further investigations.

** The work described here can be performed in BSL-2 conditions if pseudo particles are used; these can be supplied by a reference laboratory.

Camels can be infected with other animal CoVs when in close contact with other species

B. FIELD INVESTIGATION STUDIES TARGETING SARS-CoV-2 AND MERS-CoV DETECTION IN CAMELS

Actions	Description	Responsibility
1	Identify camel herd(s) owned by/in contact with COVID-19 human case(s) or in areas of high COVID-19 incidence in humans*	MoH and veterinary services
2	Conduct field investigation by collecting: • Sera to be screened for MERS-CoV and SARS-CoV-2 antibodies using: – ELISA (optional): however, both negative and positive ELISA results, especially for SARS-CoV-2, should be confirmed by VNT (virus neutralization test) or equivalent assay – VNT, PRNT (plaque reduction neutralization test), ppNT (pseudo particles neutralization test) or equivalent assay • Deep nasal turbinate swabs** (according to FAO guidelines) to be screened for MERS-CoV and SARS-CoV-2 RNA using RT-PCR (see protocol in Annex 3) • Additional collection of rectal swab and lymph node samples** using fine needle aspiration from inferior cervical lymph node of a live camel or a post-mortem specimen from retropharyngeal lymph node is recommended.	National veterinary laboratory in collaboration with international laboratories, with support from the FAO reference centres for zoonotic coronaviruses
3	Communication of the research findings to safeguard camel trade, livelihoods and public health: • to policymakers • to camel keepers and other camel value chain stakeholders • to the international community	

* The highest priority to be given to camel herd(s) owned by/in contact with COVID-19 human case(s).

** FAO can provide practical training on these sampling techniques if needed. Please note that archived camel sera, swabs or lymph node aspirates (e.g. taken during MERS-CoV surveillance) can be tested at a later stage if camel susceptibility to SARS-CoV-2 is confirmed by natural or experimental infection.

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Dromedary camels are the main reservoir species for MERS-CoV and can also be infected by other human CoVs

C. DIAGNOSTIC PROTOCOL FOR THE DETECTION OF SARS-CoV-2 RECOMBINATION WITH MERS-CoV OR OTHER CORONAVIRUSES

Actions	Description	Responsibility
Step 1: **Screening**	RT-PCR: Pan coronavirus*	National laboratory in collaboration with international laboratories, with support from FAO reference centres
Step 2: **Confirmation of MERS-CoV and/or SARS-CoV-2 shedding**	• Samples testing positive in Step 1 to be subjected to MERS-CoV and SARS-CoV-2 RNA detection using RT-PCR	
Step 3: **Confirmation of circulating coronaviruses, including recombinant viruses**	• Samples testing positive in Step 2 (with adequate shedding level) to be subjected to full genome sequence** • Samples testing negative in Phase 2 to be subjected to NGS (next generation sequencing) to identify the circulating coronavirus	

* PanCoV is recommended as an initial screening test to make sure the algorithm is able to detect: (i) MERS-CoV and SARS-CoV-2 recombinant virus that might carry changes in the PCR target genes affecting PCR detection. Furthermore, it will help target some specimens by NGS to detect any potential recombination between other known or unknown CoVs with MERS-CoV.

** In a family cluster investigation, it is important to determine if the cluster results from common exposure to a zoonotic source or if one animal-to-human transmission preceded human-to-human transmission.

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People stop by the well in the Fort Bentily area to water their camels, Westen Sahara, 2003

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A veterinarian collecting a deep nasal turbinate swab from a camel

Annex 1
EPIDEMIOLOGICAL INVESTIGATION FORM (2 pages)

EPIDEMIOLOGICAL INVESTIGATION FORM 1/2

Date: …./…./……. **Type of interview:** ☐ Individual ☐ Group (indicate number of people)

Interviewee/s: ☐ COVID-19 human case ☐ Others (specify relationship with the human case and role in the household)

……

COVID-19 case ID/code: ……

COVID-19 human case data (collected from PH authorities, human case/s and/or cohabitants)

1. Interviewee name		**2. Address** (site of investigation)	
3. Family size	☐ Human case alone ☐ Specify number ……	**4. Family cluster of infection**	☐ Yes ☐ No ☐ NA
5. Number of confirmed/probable infections among family members		**6. Clinical status of the initial human case/s**	☐ Asymptomatic ( ) ☐ Symptomatic ( )
7. Isolation place	☐ In the family's house ☐ Hospital	**8. Starting date of isolation**	……../……/…… ☐ NA
9. Date of first clinical sign/s observed	……../……/……	**10. Date of first laboratory confirmation / type of test used**	☐ PCR ……../……/…… ☐ Virus isolation ☐ Serology
11. Human case/s occupation/s		**12. Type** (and species) **of in-contact animal/s prior to symptom/s onset**	☐ Wildlife (……..……..) ☐ Companion (……..……..) ☐ Farmed (……..……..)
13. Location of potential human case exposure from animals (if applicable)	☐ Market ☐ Farm ☐ House ☐ ……	**14. Date of last visit to the location specified in question 13 before symptom/s onset**	……../……/……

Data on camel/s

15. Live camels present	☐ Yes ☐ No*		**16. If yes, number of animals** (If No, skip to number 30)	Males: ( ) Females: ( )	Age of the camels: ☐ Juvenile ( ) ☐ Young ( ) ☐ Adult ( )
17. Primary purpose of camels (select all that apply)	☐ Meat	☐ Milk	☐ Trade only	☐ Event (racing, religious gathering, camel shows)	☐ Other - please specify: ……
18. Medications or vaccines currently or recently used (over the past 4 weeks)					
19. Underlying health issues of camels					
20. Camel health status 14 days prior to human case infection	☐ Mortality ☐ Sick ☐ Apparently healthy		**21. Physiological status of females** (specify number in case of having > 1 animal)		☐ Gravid ( ) ☐ Lactating ( ) ☐ NA
22. Clinical signs (specify number of camels)	☐ Asymptomatic ( ) ☐ Symptomatic ( )		Complete Standard Sampling Sheet (Annex 2) for details**		
23. Date of onset of clinical signs	……../……/…… ☐ NA		**24. Date of full recovery** (or death)		……../……/…… ☐ NA
25. How many camels are affected?	Males: ( ) Females: ( ) ☐ NA		**26. How long has the human case owned the camels?**		……
			27. Age of the affected camels		☐ Juvenile ( ) ☐ Young ( ) ☐ Adult ( )
28. Date of sampling			**29. How many camels were sampled?**		Males: ( ) Females: ( )

30. Any other species owned by the human case/s*** (number)	☐ Feline (…….)	☐ Canine (…….)	☐ Bovine (…….)	☐ Ovine/Caprine (…….)	☐ Equine (…….)	☐ Mustelids (…….)	☐ *Cricetidae* (…….)	☐ Other (…….)

(Cont.)

* If there are no live camels on the date of investigation (i.e slaughtered or died etc.), postmortem samples can be collected.

** Such as fever, coughing, difficulty breathing or shortness of breath, lethargy, sneezing, nasal discharge, ocular discharge, vomiting, diarrhoea.

*** See Glossary for description

EPIDEMIOLOGICAL INVESTIGATION FORM 2/2			
Movement tracing of camels*			
31. Date of last visit by vets	...../...../..... ☐ NA	**32. Date of last introduction of new animals (any species)**	...../...../..... ☐ NA Species ……..……..
33. Do you allow your camels to roam freely outside the household/farm?	☐ Yes ☐ No	**34. If yes, specify the date of last interaction with other animals outside the household/farm**	...../...../..... ☐ NA
35. Did your camels visit a public place (markets, pasture areas, racing track, show etc.) up to 14 days prior to confirmation of human case infection?	☐ Yes ☐ No	**36. If yes, specify the place/s and date/s**	
		37. If yes, specify the preventive measures taken upon their return	☐ Isolation /quarantine ☐ Other …… ☐ No action taken
38. Camels' rearing/housing place			
Risk behaviour and practices (camels)			
39. Animal interactions with human case (select all that apply)	☐ Licking ☐ Sniffing ☐ Other ……		
40. Human case interactions with animal/s (select all that apply)	☐ Kissing ☐ Calving ☐ Slaughtering ☐ Other ……..	☐ Eating ☐ Feeding ☐ Contact with faeces/urine (e.g. cleaning pen)	☐ Sharing food ☐ Drinking milk

Notes

……

……

……

Interviewee name: …….. **email** …… **Tel**……

Interviewee name: …….. **email** …… **Tel**……

Interviewee name: …….. **email** …… **Tel**……

Interviewee name: …….. **email** …… **Tel**……

Names of investigation team members ……

**** The form can be customized to fit the country specific situation (e.g. pastoralism is not detailed here).

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A farmer transporting harvested sorghum by camel, Niger

Annex 2
STANDARD SAMPLING SHEET FOR INVESTIGATION IN CAMELS
(to be used together with the Epidemiological Investigation Form)

STANDARD SAMPLING SHEET FOR ANIMAL DISEASE SURVEILLANCE

Date ……/…..…/……..……	**Governorate/County**	**District**	**Clan/township/village**	**Latitude** ..... **Longitude**	**Linked to COVID-19 case ID/code:** .....

Site
☐ Market ☐ Quarantine ☐ Abattoir
☐ Farm ☐ Household ☐ Pastoralist

Surveillance method
☐ Repeated cross sectional ☐ Cohort ☐ Active, randomized ☐ Outbreak investigation
☐ Active, risk-based ☐ Passive ☐ Syndromic ☐ Other ……..…..……

Owner name ……..……
Owner tel ……..……

Herd size (farm, household, pastoralist)
……..…..……..…..……..…..……

Other species present on site
……..…..……..…..……..…..……

Number of camels on date of disease onset
(Not applicable in abattoir, quarantine, market)
……..…..……..…..……

Number of dead camels since disease onset
(Not applicable in abattoir, quarantine, market)
……..…..……..…..……

Number of sick camels on date of visit
……..…..……..…..……

Animal ID	**Sex**				**Age group***			**Husbandry**		**Clinical signs** *(insert the corresponding number)*	**Samples****				
	Male	Female			Neonatal	Juvenile	Adult	Open	Closed	**1.** No signs **2.** Death **3.** Fever **4.** Lethargy **5.** Poor appetite **6.** Poor hair coat **7.** Coughing/wheezing **8.** Sneezing **9.** Shortness of breath **10.** Nasal discharge **11.** Other resp. signs **12.** Ocular lesions **13.** Ocular discharge **14.** Weight loss **15.** Vomiting **16.** Diarrhoea **17.** Oral lesions **18.** Other GI signs **19.** Neurological signs **20.** Other (specify)	B	N	T	L	Other***
		Gravid	Lactating	Dry											
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															.....
															.....
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* Neonatal: 1-6 months; Juvenile: 6-24 months; Adult: more than 24 months ** B=blood, N=nasal swab, T=trachea, L= Lymph node *** Other (specify in table)

Notes

……

……

……

Reporting officer(s): …… **Position:** ……..…… **Tel:** ……..……

Annex 3
LABORATORY DIAGNOSTIC PROTOCOLS AS PER SECTION C

Sample storage: Swab homogenates should ideally be stored at -80°C. If this is not possible, -20°C can be considered, but only for a limited period of time, up to one week.*

Step 1. RT-PCR: Pan coronavirus (Goldstein *et al.*, 2016)

Reverse-complementation

Samples to be tested for RNA viruses must first be reverse-transcribed (RT) to provide a suitable cDNA template for PCR. We recommend that cDNA is generated prior to PCR in a separate reaction, and primed by random hexamers (ie. perform two-step PCR), using Invitrogen's SuperScript III First-strand cDNA synthesis kit. This approach has been taken as a sample quantity is often limited and budgetary restrictions and/or the availability of reagents also influence the preference for target cDNA (RT performed prior to PCR in a separate reaction).

Coronaviruses

Note: Please use both coronavirus protocols for screening all samples. These two assays target non-overlapping regions of the RNA-dependent RNA Polymerase in ORF 1b and it is useful to have both regions for phylogenetic discrimination.

- **PROTOCOL P-001** (Quan *et al.*, 2010)
  Notes: Reverse-transcription performed separately using Superscript III, followed by nested PCR. On the human coronavirus genome (strain 229E) it roughly amplifies the region 17 480-17 820.
  Target: RNA-Dependent RNA Polymerase (RdRp)
  Primers:
  – *Round 1:*
    CoV-FWD1:
    CGTTGGIACWAAYBTVCCWYTICARBTRGG
    CoV-RVS1:
    GGTCATKATAGCRTCAVMASWWGCNACATG
  – *Round 2:*
    CoV-FWD2: GGCWCCWCCHGGNGARCAATT
    CoV-RVS2: GGWAWCCCCAYTGYTGWAYRTC
  PCR master mix: Primers were applied at 0.2 µM concentrations with 1µl cDNA and Hot-Star polymerase (Qiagen, Valencia, CA).
  Protocol: 95°C 5 minutes, then 15 cycles of 95°C for 30 seconds, 65°C for 30 seconds and 72°C for 45 seconds, then 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 45 seconds. Finish with 72°C for 7 minutes.
  Same protocol for Rounds 1 and 2.
  Amplicon: Round 1: 520 bp. Round 2: 328 bp
  Control: Universal Control 1, or appropriate Coronavirus cDNA

- **PROTOCOL P-002** (Watanabe *et al.*, 2010)
  Note: Like the Quan protocol, this assay also targets the polymerase. However, it targets a different region slightly more upstream. On the human coronavirus genome (Strain 229E) it targets roughly nucleotides 14 370-14 750. If you are looking for coronaviruses in bats, this may be a good protocol to use because many of the partial sequences published on CoV (in bats) have been generated using these primers. Please note that this assay has been modified from the original publication. Initial primer sequences have been modified to increase the ability of the assay to detect widely variant coronaviruses. A second, heminested step has also been added to increase sensitivity. This step can be performed using a forward primer that is optimized for bat viruses, or other coronaviruses, depending on the sample being investigated.
  Target: RNA-Dependent RNA Polymerase (RdRp)
  Primers:
  – *Round 1:*
    CoV-FWD3: GGTTGGGAYTAYCCHAARTGTGA
    CoV-RVS3: CCATCATCASWYRAATCATCATA
  – *Round 2:*
    CoV-FWD4/Bat: GAYTAYCCHAARTGTGAYAGAGC
    (or CoV-FWD4/Other:
    GAYTAYCCHAARTGTGAUMGWGC)
    CoV-RVS3: Same reverse primer as round 1
  PCR master mix: Primers were applied at 0.2 µM concentrations with 2× GoTaq PCR Master mix (Promega) in 25µL reaction mixture
  Protocol: 94°C for 2 minutes, then 35 cycles of 94°C for 20 seconds, 50°C for 30 seconds and 72°C for 30 seconds. Finish with 72°C for 7 minutes.
  Same protocol for rounds 1 and 2.
  Amplicon: Round 1: 440 bp, Round 2: 434 bp
  Control: Universal Control 1, or appropriate coronavirus cDNA

Step 2. Preliminary confirmation: Samples testing positive in Step 1 to be subjected to MERS-CoV and SARS-CoV-2 RNA detection using real time RT-PCR.

1. MERS-CoV RNA detection using real time RT-PCR:

- **Protocol for UPE Real Time RT-PCR** (Corman *et al.*, 2012)
  20-µl master mix reaction, per sample:
  – 12.5 µl of 2 X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each dNTP and 3.2 mM magnesium sulfate)

* Sample storage should follow international guidelines as recommended by WHO, 2020.

- 1 µl of reverse transcriptase/Taq mixture from the kit
- 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen)
- 3.6 µl of RNase/DNase free water
- 1 µl Non-acetylated BSA (1mg/ml)
- 1 µl (400 nM final concentration) of primer upE-Fwd (GCAACGCGCGATTCAGTT)
- 1 µl (400 nM final concentration) primer upE-Rev (GCCTCTACACGGGACCCATA)
- 0.5 µl (200 nM final concentration) of probe upE-Prb (6-carboxyfluorescein[FAM])-CTCTTCACATAATCGCCCCGAGCTCG-6-carboxy-N,N,N,N´-tetramethylrhodamine [TAMRA])
- 5 µl of extracted RNA from samples (freeze remaining extracted RNA at -80°C) or 5 µl positive control (UpE RNA) or 5 µl negative control (bi-distilled water)

Thermal cycling: 55°C for 20 minutes, 95°C for 3 minutes, then 45 cycles of 95°C for 15 seconds, 58°C for 30 seconds

- **Protocol for ORF 1b Real Time RT-PCR** (Corman *et al.*, 2012)

20-µl master mix reaction, per sample:

- 12.5 µl of 2 X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each dNTP and 3.2 mM magnesium sulfate)
- 1 µl of reverse transcriptase/Taq mixture from the kit
- 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen)
- 2.6 µl of RNase/DNase free water
- 1 µl Non-acetylated BSA (1mg/ml)
- 1 µl (400 nM final concentration) of primer ORF1b-Fwd (TTCGATGTTGAGGGTGCTCAT)
- 1 µl (400 nM final concentration) primer ORF1b-Rev (TCACACCAGTTGAAAATCCTAATTG)
- 0.5 µl (200 nM final concentration) of probe ORF1b-Prb (6-carboxyfluorescein[FAM])-CCCGTAATGCATGTGGCACCAATGT-6-carboxy-N,N,N,N´-tetramethylrhodamine [TAMRA]).
- 5 µl of extracted RNA from samples (freeze remaining extracted RNA at -80°C) or 5 µl positive control (UpE RNA) or 5 µl negative control (bi-distilled water)

Thermal cycling: 55°C for 20 minutes, 95°C for 3 minutes, then 45 cycles of 95°C for 15 seconds, 58°C for 30 seconds

For further reading on MERS-CoV diagnostic techniques, please consult the OIE Terrestrial Manual chapter on MERS-CoV: https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.05.02_MERS-CoV.pdf

2. SARS-CoV-2 RNA detection using real time RT-PCR

- **Protocol for E gene assay Real Time RT-PCR** (Corman *et al.*, 2020)

25 µl master mix reaction, per sample:

- 12.5 µl of 2 X reaction buffer provided with the SuperScript III Platinum One-Step Quantitative RT-PCR System (Invitrogen ref. 11732-088)
- 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen)
- 2.6 µl of RNase/DNase free water
- 1 µl Non-acetylated BSA (1mg/ml)
- 1 µl Enzyme mix
- 1 µl (400 nM final concentration) of primer E_Sarbeco_F1 5'-ACAGGTACGTTAATAGTTAATAGCGT-3'
- 1 µl (400 nM final concentration) primer E_Sarbeco_R2 5'-ATATTGCAGCAGTACGCACACA-3'
- 0.5 µl (200 nM final concentration) of probe E_Sarbeco_P1 5'--ACACTAGCCATCCTTACTGCGCTTCG-BBQ-1-3'
- 5 µl of extracted RNA from samples (freeze remaining extracted RNA at -80°C) or 5 µl positive control or 5 µl negative control (bi-distilled water)

A FAO team member vaccinates a camel belonging to a nomadic herder in Kabkabyia, North Darfur

Thermal cycling: 55°C for 10 minutes, 95°C for 3 minutes, then 45 cycles of 95°C for 15 seconds, 58°C for 30 seconds

- **Protocol for RdRp gene assay Real Time RT-PCR** (Corman *et al.*, 2020)
  25 µl master mix reaction, per sample:
  - 12.5 µl of 2 X reaction buffer provided with the SuperScript III Platinum One-Step Quantitative RT-PCR System (Invitrogen ref. 11732-088)
  - 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen)
  - 1,1 µl of RNase/DNase free water
  - 1 µl Non-acetylated BSA (1mg/ml)
  - 1 µl Enzyme mix
  - 1,5 µl (600 nM final concentration) of primer RdRp_SARSr-F 5'- GTGARATGGTCATGTGTGGCGG -3'
  - 2 µl (800 nM final concentration) primer RdRp_SARSr-R 5'- CARATGTTAAASACACTATTAGCATA -3'
  - 0.25 µl (100 nM final concentration) of probe RdRP_SARSr-P1 5'-- FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ -1-3'
  - 0.25 µl (100 nM final concentration) of probe RdRP_SARSr-P2 5'-- FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ-1-3'
  - 5 µl of extracted RNA from samples (freeze remaining extracted RNA at -80°C) or 5 µl positive control or 5 µl negative control (bi-distilled water)

Thermal cycling: 55°C for 10 minutes, 95°C for 3 minutes, then 45 cycles of 95°C for 15 seconds, 58°C for 30 seconds

Step 3: Confirmation:

- **Samples testing positive in Step 2 to be subjected to full genome sequence**
- **Samples testing negative in Step 2 to be subjected to NGS (next generation sequencing) to identify the circulating coronavirus**

Validated and recommended protocols for full genome sequence and NGS analysis are not yet available, therefore this confirmatory test should define case by case, the epidemiological situation, geographical area of sampling, etc. based on the results obtained during phase 1 and phase 2.

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A camel herder getting water for his camels, Chad

NOTES

RISK ANALYSIS IN ANIMAL HEALTH

Risk analysis is a procedure, which we all do intuitively in our everyday life as we also do in our professional work to assess the risk of any hazard or threat. In animal health, risk analysis has been most widely used as a decision tool to help select the most appropriate health interventions to support disease control strategies, guide disease surveillance and support disease control or eradication strategies.

It should be remembered that risk is not equal to zero and never stays static. Risk changes as drivers or factors of disease emergence, spread or persistence change such as intensification of livestock production, climate change, civil unrest and changes in international trading patterns. Risk analysis should therefore not be seen as a "one off" but as good practice for animal health systems as part of their regular activities. Therefore, the risk analysis process should be repeated and updated regularly.

Risk analysis comprises the following components:

Hazard identification: the main threats are identified and described.

Risk assessment: risks of an event occurring and developing in particular ways are first identified and described. The likelihood of those risks occurring is then estimated. The potential consequences or impact of the risks if they occur are also evaluated and are used to complete the assessment of the risk.

Risk management: involves identifying and implementing measures to reduce identified risks and their consequences. Risk can never be completely eliminated but can be effectively mitigated. The aim is to adopt procedures that will reduce the level of risk to what is deemed to be an acceptable level.

Risk communication: an integrated process that involves and informs all stakeholders within the risk analysis process and allows for interactive exchange of information and opinions concerning risk. It assists in the development of transparent and credible decision-making processes and can instil confidence in risk management decisions.

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