SWAB AND TISSUE SAMPLE COLLECTION PROCEDURES ENHANCING MERS-CoV DETECTION IN CAMELS

An illustrative guideline

SUMMARY

- Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging zoonosis affecting dromedary camels especially in Africa and the Arabian Peninsula, with potential spillover into human populations.
- In order to improve understanding of virus transmission dynamics and the zoonotic potential of different virus clades, the Food and Agriculture Organization of the United Nations (FAO), the World Organization for Animal Health (OIE) and the World Health Organization (WHO) recommended MERS-CoV ribonucleic acid (RNA) detection with subsequent gene sequencing and virus isolation as a priority research area.
- Despite the high sero-prevalence of MERS-CoV in camels in Africa, the detection of RNA in nasal swabs has been comparatively low.
- FAO considers appropriate procedures for sample collection from live and slaughtered camels are key in order to optimize MERS-CoV RNA detection.
- This guideline describes improved sampling procedures that reach the posterior respiratory epithelium inside the nasal cavity of camels and allow for collection of good quality samples for optimized MERS-CoV detection, isolation and genetic characterization.

BACKGROUND

Middle East respiratory syndrome coronavirus (MERS-CoV) is a global emerging public health threat. MERS-CoV was first reported in 2012 in Saudi Arabia (FAO, 2017). In the Arabian Peninsula, the virus is thought to spillover repeatedly into humans via direct or indirect contact with infected dromedary camels. Limited, non-sustained human-to-human transmission continues to occur mainly in health care settings, primarily in Saudi Arabia (WHO, 2019).

The close phylogenetic similarity between MERS-CoV isolated from people and dromedary camels strongly suggests the existence of cross-species transmission (Ferguson and Van Kerkhove, 2014). However, recent virus genetic studies suggested that camels act as sources of MERS-CoV for humans rather than vice versa (Dudas et al., 2018; Corman et al., 2014).

Although the majority of dromedary camels are in Africa, zoonotic spillover events were reported in the Arabian Peninsula.
In 2019, the World Organization for Animal Health (OIE) declared that positive real-time polymerase chain reaction (RT-PCR) MERS-CoV results or isolation of the MERS-CoV from dromedary camels are notifiable (OIE, 2019a). The 87th OIE General Session Report (May 2019) stated that the OIE Scientific Commission advised that MERS-CoV should be added to the OIE list of reportable diseases and recommended the Code Commission amend the Terrestrial Animal Health Code Chapter 1.3, accordingly (OIE, 2019b).

The Food and Agriculture Organization of the United Nations (FAO), OIE and the World Health Organization (WHO) recommended that MERS-CoV ribonucleic acid (RNA) detection with subsequent gene sequencing and virus isolation be a priority research focus in cross-sectional and longitudinal surveillance studies involving camels. This will enhance understanding of virus transmission dynamics and the zoonotic potential of different MER-CoV clades (FAO, OIE, WHO, 2018). Since 2016, with support from the United States Agency for International Development (USAID), FAO is implementing MERS-CoV applied research projects in dromedary camels in Egypt, Ethiopia, Kenya and Jordan. The various country projects mainly focus on cross-sectional and longitudinal surveillances in selected value chain nodes (including slaughterhouses) aimed at understanding virus shedding dynamics, patterns of infection, and phylogeny of circulating clades and their zoonotic potential.

Despite the reported high sero-prevalence of MERS-CoV in African camels, observed prevalence of MERS-CoV RNA is low (Kandeil et al., 2019; Dighe et al., 2019). Such low or sometimes completely absent molecular detection rates, despite high seroprevalence, led to extensive speculation, including the possibility of sampling errors related to procedures that do not take into account the unique anatomical features of the camel’s upper respiratory tract. Commercially available swabs are relatively short and most sample collectors lack experience in restraining camels. FAO considers collecting good quality samples for molecular detection of MERS-CoV to be highly dependent on swab collection from the appropriate site where virus replication takes place. To achieve this, proper animal restraint is essential. This guideline describes optimized procedures to facilitate swab sampling from the deeper part of the upper respiratory tract inside the camel’s nasal cavity and to collect good quality specimens from slaughtered camels. In addition, we describe a simplified camel restraining method and highlight how to avoid some common errors that occur during the sampling process.

This guideline focuses on good practices for sample collection procedures to enhance MERS-CoV detection in camels. More generally, it recommends following the OIE guidelines for collection, submission and storage of diagnostic specimens (OIE, 2013), FAO guidelines for the use of personal protective equipment (FAO, 2011) and respective national standard operating procedures for transportation of samples for MERS-CoV testing.

The anatomy of the camel nasal cavity and relevance for MERS-CoV sampling

Nasal swabs are the sample of choice for MERS-CoV RNA detection in camels, using RT-PCR technology (Mohran et al., 2016). However, the nasal cavity of camels is the longest among ruminant species, reaching up to 16.5 cm in young camels (Yahaya et al., 2012). It is, therefore, important to understand the unique anatomy of the camel nasal cavity and the predilection tissues where MERS-CoV replication is highest in order to optimize collection of quality swab samples.

The nasal cavity (Figure 1) starts with a rostral section (nasal vestibule), which leads into a middle part (respiratory) that includes the turbinates, and ends in the caudal (olfactory) part (Moussa and Mokhtar, 2005). The nasal septum divides the nasal cavity into two equal sides; each side includes narrow dorsal and medium nasal meatuses, and a larger ventral nasal meatus.

The camel turbinate has a dorsal, ventral (anterior) and middle (posterior) part (Figure 2) and consists of a scroll-like...
structure protruding from the lateral wall of the nasal cavity, restricting the wall-to-wall distance in the air passage to only 1-2 mm (Schmidt-Nielsen et al., 1981).

MERS-CoV replication in the nasal cavity is limited to the respiratory and olfactory epithelium of the turbinates (Haverkamp et al., 2018). Within the respiratory part, samples collected from the posterior nasal turbinate revealed the highest viral load (Adney et al., 2014) and the turbinates are thus the optimal target for MERS-CoV detection.

Unlike in other animals (e.g. buffalo and donkey), the nasal turbinates of camels do not extend to the rostral third of the nasal cavity but are located in the caudal two-thirds (Metwally et al., 2019). The anterior tip of the ventral turbinate is approximately 10 cm away from the narial opening (Figure 3-A), whereas the deepest turbinate surface that the swab can reach in a live camel (Figure 3-B) is approximately 15 cm away from the narial opening. Therefore turbinate swabbing in live camels requires deep swab insertion due to the relatively short length of commercially available nasopharyngeal swabs (15 cm), taking into account that the surveillance technician has to use 2-3 cm of the handle length to hold the swab.

In cases of MERS-CoV infection with nasal discharge, sampling any part of the nasal cavity may sufficiently capture MERS-CoV RNA. However, this situation is rarely observed in the field, and therefore the turbinate should be the targeted site for quality swab and specimen collection for enhanced molecular characterization. The term "turbinate swabbing" should be used instead of "nasal swabbing" as several structures in the nasal cavity such as the nasal vestibule, nasal septum, nasolacrimal canal and ventral nasal meatus are accessible by the swabs but are not the target sampling sites.

**CAMEL RESTRAINING**

**Note**
- Camels can kick with their hind limbs in a semicircular direction, and kick forward with one forelimb even if the other is restrained. Therefore camels should be forced into sternal recumbency before sampling.
- If samples are being collected from a standing camel with a tied forelimb, the surveillance technician should stand to one side of the restrained leg.
- An assistant should hold the camel's head using a rope (head halter), or by gripping the ears and lower lip/jaw (if a halter is not available). **Avoid restraining the camel by holding the nose.**
- The assistant(s) and the surveillance technician should use protective gloves and change them each time a new camel is being handled to avoid cross-contamination of samples.

**Procedure**
1. Ask the owner/worker to tie up one of the camel’s forelimbs and push it into sternal recumbency.
2. Place a 4 metre long rope around the camel's neck, leaving the rope twice as long on one side as the other.
3. Twist the two sides around each other three times keeping close to the neck (Figure 4-A).
4. Cross the long side over the nasal bone (Figure 4-B).
5. Finally using one hand hold the two sides together at the level of the pharynx (Figure 4-C).
This technique creates two pressure points, one on the dorsal nasal bone and one on the upper neck. In this way one person can fully restrain and hold the camel during sampling (Figure 5). The rope can then be removed quickly and easily once the sampling process is complete.

**BIOSECURITY CONSIDERATIONS DURING RESTRAINING OF CAMELS PRIOR TO AND DURING SAMPLING**

- A common biosecurity flaw observed, mainly during attempted restraining of camels prior to or during blood sampling, is to hold the narial openings without using protective gloves (Figure 6).
- Such practices may lead to cross-contamination of the nasal vestibule and the spread of infection at the time of sampling.
- If a rope (halter) is not available, the owner/assistant should use protective gloves prior to holding the nostrils, and replace with a new pair of gloves before handling the next camel.
COLLECTING QUALITY SAMPLES FROM CAMELS FOR MERS-COV RNA DETECTION

Turbinate swabbing from a live camel

**Important note**
- Flocked swabs are better than commonly used tipped swabs due to their enhanced contact surface and higher pathogen recovery (see details on recommended swabs in the Annex).
- Hold the swab in a way that comfortably allows you to rotate it laterally.

**Procedure**
1. To estimate the point (site) of sampling, draw an imaginary line, starting approximately 2 cm (for young camels) and 4 cm (for adult camels) behind the lip junction up towards the nasal bone (Figure 7).
2. Hold and raise up the nasal wing using your thumb and index finger (Figure 7).
3. Insert the swab gently through the upper half of the nasal vestibule alongside the nasal septum, as deeply as possible until the entire swab handle including your fingers are completely inside the nasal vestibule (Figure 8).
4. On reaching the deepest point, rotate the swab laterally, at least five times, against the turbinate wall to loosen and collect cellular material.
5. Retract the swab and insert it in a tube containing enough of the virus transport medium (VTM) to cover the tip completely.
6. Rotate and then squeeze the swab against the wall of the tube to release as much of the collected material into the VTM as possible.
7. If you have to cut the swab with a scissors, make sure to clean the scissors with alcohol (minimum concentration 70 per cent) before using it to cut the next swab.

**Deep turbinate swabbing (post-slaughter)**

**Important note**
- The method previously described for turbinate swabbing is not appropriate for slaughtered camels.
- After slaughtering, the narial opening relaxes, and the nasal vestibule may contain blood and body fluids from the camel or other animals (Figure 9), especially when pressure washers are used to remove the blood accumulated during slaughtering, leading to the greater likelihood of cross-contamination.

**Procedure**
1. Use absorbent tissue paper to remove any fluids or coagulated blood from the skin surrounding the nasal cavity.
2. Disinfect the incision area (around the margins of the nasal bone) with a suitable antiseptic to avoid cross-contamination of samples.

3. Use a sterile scalpel to incise the skin horizontally (1 cm) immediately rostral to the nasal cartilage, starting from the nasal septum.

4. Press the skin over the nasal vestibule area to expose the incision opening (Figure 10).

5. Insert the swab deeply into the incision opening alongside the nasal septum, rotate it laterally at the level of the ventral and middle turbinates (Figure 11).

6. Withdraw the swab carefully (avoid touching the outer skin) while following the same procedures to add VTM and squeeze the swab as indicated for turbinate swabbing procedures described above (points 5 and 6 for live camels).

7. Use a new blade for each animal to avoid cross-contamination of samples.

Note that swabs collected using this technique will contain blood. It is therefore necessary to inform the laboratory so they can adopt the appropriate real-time polymerase chain reaction (RT-PCR) protocol to avoid the inhibitory effects of blood in the sample.

Taking turbinate specimen (post-slaughter)

Important note
- The spongy bone of turbinates becomes harder in adult camels. Use forceps (not your fingers) to hold the tip of the turbinate as excessive pressure on the scalpel could release the blade from the handle and expose the practitioner to injury.
- Avoid areas of abnormal swelling or necrosis that may be caused due to myiasis (Cephalopenia titillator).

Procedure
1. Disinfect the incision area along the entire nasal cavity with suitable antiseptic to avoid cross-contamination of samples.

2. Use a sterile blade and scalpel to incise the skin starting from the lateral nasal wing towards the maxilla and nasal bone, then flip the skin over the nasal bone to expose the ventral turbinate (Figure 12).

3. After incising the skin, change the blade to avoid cross-contamination and begin incising the soft tip of the anterior turbinate.

4. Use sterile forceps to twist and break the turbinate bone and to obtain the specimen (Figure 13).

5. Note that the lining tissue is delicate and the underlying bones are spongy and fragile, therefore the specimen obtained will consist of small irregular pieces of bone with the lining epithelium (Figure 14).
6. Place the specimen in a suitable container. If the specimen will reach the laboratory within six hours, place the container in an ice box before storing at -80 °C. Avoid the -20 °C step if possible. However, if in the field where delivery is likely to take more than six hours, a liquid nitrogen (N2) transport canister should ideally be used.

7. As an alternative, add tissue stabilizing solution (e.g. RNAlater®) to prevent RNA degradation at room temperature for an extended period (one to four weeks).

8. Use new gloves and a sterile scalpel for each animal to avoid cross-contamination of samples.

HOW TO AVOID COMMON ERRORS DURING SAMPLING

Three errors commonly observed during sampling are as follows.

1. Swab insertion into the nasolacrimal canal: the orifice of the nasolacrimal canal located laterally within the nasal vestibule stretches when raising the nasal wing. This can occur when inserting the swab through the lateral side of the nasal vestibule (Figure 15).
2. Swabbing the nasal vestibule or septum instead of the turbinate (Figure 16).
3. Swabbing the base or sides of the ventral nasal meatus (Figure 17) after swab insertion through the ventral part of the nasal vestibule.

In order to avoid these errors, make sure the swab is inserted into the upper half of the nasal cavity (approximately 15 cm deep) alongside the nasal septum, and then rotate it laterally against the turbinate wall.

REFERENCES


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Annex

LIST OF MATERIALS REQUIRED FOR MERS-COV SAMPLING IN CAMELS

1. Nasopharyngeal swabs, preferably flocked swabs commonly used for virology research (minimum length 15 cm). Note: If tipped swabs are used, avoid those with wooden handles and/or cotton tips.
2. Virus transport medium (VTM).
3. Tissue stabilizing solution (RNA later*).
4. Tubes (to keep the collected swabs in VTM).
5. Specimen container (to keep the collected tissue specimen in VTM or tissue stabilizing solution).
6. Scissors (to cut the swab handle).
7. Alcohol (minimum concentration 70 per cent).
8. Cotton or absorbent tissue paper.
9. Disposable scalpels (two for each camel).
10. Tissue forceps, preferably the finger ring type (one for each camel).
11. Portable cooler.
12. Sharps disposal bin.
13. Personal protective equipment (PPEs).
15. Permanent marker pens.
16. Data collection sheets – either printed on paper, on a portable computer or as an app on a smartphone or tablet.
17. Strong, non-stretchable rope (4 metres) for use as a camel halter).

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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, 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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