Computer No. 139866 Government of India Ministry of Environment, Forest and Climate Change CENTRAL ZOO AUTHORITY

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Dated 21/09/2020

OFFICE MEMORANDUM

Sub: Guidelines for sample collection from captive wild animals in suspected cases for diagnosis of SARS CoV-2 – regarding

The undersigned is directed to refer to this office advisory to zoos vide letter F.No.25-1/2002-CZA(Vol-II)(AK)/2469/B/2020 dated 6th April, 2020 wherein zoos were advised to collect sample from captive animals in suspected cases and send to designated institutions as mentioned below to test for SARS CoV-2:

- a. National Institute of High Security Animal Diseases (NIHSAD), Bhopal, Madhya Pradesh
- b. National Research Centre on Equines (NRCE), Hisar, Haryana
- c. Centre for Animal Disease Research and Diagnostic (CADARD), ICAR-Indian Veterinary Research Institute (IVRI), Bareilly, Uttar Pradesh
- 2. In addition to the above-mentioned three institutions, the Centre for Cellular and Molecular Biology Laboratory for Conservation of Endangered Species (CCMB-LaCONES) has recently initiated testing of samples from zoos for SARS CoV-2 as informed by Dr R. K. Mishra, Director, CCMB vide email dated 20th July 2020. Further, copy of the Guidelines prepared by the CCMB-LaCONES for **collection**, **preservation and transport of samples** collected for SARS CoV-2 testing and related information sheet is enclosed herewith for information and use of zoos.
- 3. Also, as a resource for reference of zoos, copy of the "Guidelines for working with free-ranging wild mammals in the era of the COVID-19 pandemic" issued by the World Organisation for Animal Health (OiE) in collaboration with the IUCN-SSC Wildlife Health Specialist Group is enclosed herewith.

Encls. As above

(Sonali Ghosh)

Dy Inspector General Of Forest

To

The Chief Wildlife Warden of all States / Union Territories,

Copy forwarded for information / necessary action to:

- 1. PS to Hon'ble Minister (EF&CC), Government of India
- 2. PS to Hon'ble Minister of State (EF&CC), Government of India
- 3. Sr. PPS to the Secretary to the Government of India (EF&CC), Government of India
- 4. Sr. PPS to the Director General of Forests and Special Secretary, Government of India
- 5. The Director, Centre for Cellular and Molecular Biology
- 6. The Director, Indian Veterinary Research Institute, Izatnagar
- 7. The Member Secretary of the concerned State Zoo Authority,
- 8. The Officer in-charge of all recognised zoos
- 9. DPA CZA to upload on website of the CZA.

(Sonali Ghosh)

Dy Inspector General Of Forest

Guidelines for Collection, Packing and Transport of Wild Animal Samples for Covid-19 Test

In view of the pandemic outbreak of SARS-Cov-2 (COVID-19) and the incidences of transmission of COVID-19 from zoo animal keepers to zoo animals, particularly among carnivores, CSIR-CCMB has initiated a program to test wildlife for COVID-19. All zoo veterinarians and staff are well versed in sample collection, packing, and transport of samples for various investigations. Guidelines are given below for safe and efficient collection, packing and transport of zoo animal samples for COVID-19 test. IT IS IMPORTANT TO ADHERE TO THESE INSTRUCTIONS TO ENSURE PROPER TESTING OF SAMPLES.

Sample collection

- 1. Follow the appropriate biosafety protocols while sample collection and packing. Use appropriate personal protection equipment (PPE): Face Shield, Face mask, N-95 mask, head cover, goggles, hand gloves, shoe cover and coverall or gown.
- 2. Use proper sanitization/disinfection protocols when sick animals are handled for sample collection. Give sufficient time to sanitize the restraining devices (chute/cages), if the same devices are used to be for next animal.
- 3. Sample collection materials: (**Please note Naso/Oral swab is mandatory**, blood and saliva could be sent additionally)
 - Sterile flocked polyester/nylon swabs. These swabs are used to facilitate and enhance the absorption of virus. Sterile Polyester swabs are available commercially. **DONOT USE COTTON SWABS.**
 - 2. Use Sterile Collection Vials for collecting discharges/secretions
 - 3. Avoid the collection of soiled samples (eg saliva/discharges from the floor).
 - 4. Label the sample vials/containers with sample ID, sample type, animal details, and name of the zoo).
 - 5. Samples should not be contaminated with blood.
 - 6. Blood should be collected in sterile EDTA vacutainers.
 - 7. For post-mortem samples: Collect the samples as early as possible after death of animal. Take deep throat swabs and nasopharyngeal swabs. Avoid sampling if the trachea or nasal passages are contaminated with stomach contents. Take a swab from the lung.
 - 8. Send the samples to testing laboratory as early as possible after collection. Send dry swabs, if the time to reach testing lab will take less than 24-48h. If it will take more than 48h send the samples in Viral Transport Medium (2-3ml). Viral Transport Medium is available commercially.

Sample Packing

Packing Material:

- 1. Adsorbent material to prevent the contamination from any leakage: Cotton/ tissue
- 2. Leak proof container (2nd): 50 ml centrifuge tubes/ plastic containers to place the swab collection vials/containers (1st container).
- 3. Cool packs: frozen gel packs
- 4. Leak proof container (3rd): plastic containers/cryo vial boxes

- 5. Outer box: thermocol box/ vaccine carrier/ice box/hard card board box to place the 3rd container (with 1st and 2nd) and ice packs.
- 6. Parafilm to seal the sample collection vials.
- 7. Cello tape (transparent) and Scissors
- 8. Permanent markers (micro tip marker for labelling sample details on sample vial/container; large tip markers for labelling outer box.

Packing of samples (VTM)

- 1. Samples collected **beyond 48h** should be send the samples in VTM (2-3 ml)
- 2. After swab collection, place the swab into the same container provided with the swab and tight the cap (1st container). Please ensure that swab does not touch the outer surface of the container while keeping the swab into the container.
- 3. Seal the neck of sample tube with parafilm and wrap the tube with cotton/tissue paper
- 4. Place the 1st container in 50 ml centrifuge (2nd) tube and seal the neck with parafilm.
- 5. Keep the centrifuge tube in the sterile zip lock plastic cover
- 6. Place the sterile zip-lock cover with sample in a plastic container (3rdcontainer)
- 7. Keep the 3rd container along with ice packs in thermocol box/ice box/card board box (outer box) and seal the outer container with cello tape properly.
- 8. Label the outer box with sender's address and contact details, and consignee's address and contact details.
- 9. Send the samples with person to the testing laboratory.

Packing of samples (dry swabs)

- Samples (Dry Swabs) should reach the testing lab within 24-48h after collection
- 2. Follow steps, from 2 to 9 given above

Transport of Samples

Send the samples with authorized person to the testing lab. Samples should reach the testing laboratory within 24-48h after collection. If it takes more than 48h, preserve the samples in VTM (2-3 ml). Ensure that the sample is maintained at refrigeration temp (4- 5° C) during the transport.

Note:

Please try to send dry swabs. **Don't add formalin/normal saline/any other medium to the samples until unless prescribed.** Use isopropanol for sterilization of packing material eg.2nd, 3rd packing containers and the outer box. Use the abbreviations for labeling the samples vials. NP: Nasopharyngeal; OP: Oropharyngeal; NS: Nasal Swab; S: Saliva; B: Blood. Use the packing containers as 1st container: sample/swab holding container; 2nd container: 50 ml centrifuge tube / 3rd container: plastic container; Outer box: Thermocol box/cool box/vaccine carrier/ hard card board box.

--SAMPLES WILL NOT BE OPENED AND WILL BE DESTROYED IF THEY ARE NOT IN PROPER CONDITION--

Chec	Check List for Sample Collection, Packing and Transport							
Step	Action	Points to remember						
COLL	ECTION							
1.	Wear appropriate PPE	Face Shield, Face mask, N-95 mask, head cover, goggles, hand gloves, shoe cover and coverall or gown.						
2.	Use appropriate collection material	Flocked polyester/nylon swabs						
3.	properly	Use permanent markers and cover the label with cello tape. Check the information provided in the sample information form with sample label.						
	Collect samples aseptically	Avoid sampling of soiled samples						
5.	Preferred samples: Dry swabs of NP,OP,NS, Saliva (Dry swab: without VTM)	Dry samples: If transit time is <48h. Use VTM(2-3 ml), if transit time >48h.						
6.	Use EDTA coated vacutainers for blood	EDTA vacationers are purple in colour.						
7.	Post-mortem samples: NP, OP, NS, Deep throat	Avoid sampling if air passages are contaminated with stomach contents						
PACK	ING							
1.	Wear appropriate PPE	Face Shield, Face mask, N-95 mask, head cover, goggles, hand gloves, shoe cover and coverall or gown.						
2.	Seal the neck of the sample vial with parafilm and wrap with cotton/tissue paper (1 st or primary container)	Check the label information with the information provided in the specimen information form.						
3.	Place the swab container/vial into the sterile 50 ml centrifuge tube (2 nd container)	Seal 2 nd container with parafilm.						
4.	Place the 2 nd container (with sample container/vial) into sterile plastic container (3 rd container).	Label the outer box with zoo name and testing lab name.						
5.	Keep the 3 rd containers along with ice packs in the outer box.	Seal the outer box with cell tape.						
6.	Samples should accompany with specimen information forms	Check the specimen information forms the forms						
TRAN	SPORT							
	Samples should reach the testing laboratory within 24-48h after collection.	If it is >48h, send the samples in VTM (2-3 ml).						
	Send the samples preferably with person to the testing lab.							

Note:

Please try to send dry swabs. Don't add formalin/normal saline/any other medium to the samples until unless prescribed. Use isopropanol for sterilization of packing material eg. 2nd, 3rd packing containers, and the outer box. Use the abbreviations for labeling the samples vials. NP: Nasopharyngeal; OP: Oropharyngeal; NS: Nasal Swab; S: Saliva; B: Blood. Use the packing containers as 1st container: sample/swab holding container; 2nd container: 50 ml centrifuge tube / 3rd container: plastic container; Outer box: Thermocol box/cool box/vaccine carrier/ hard card board box.

⁻⁻ SAMPLES WILL NOT BE OPENED AND WILL BE DESTROYED IF THEY ARE NOT IN PROPER CONDITION--



CASE No. For use at CCMB

Sample Information Sheet for Covid-19 (Only For Zoo Animals) Please fill the form and submit along with sample(s). If required enclose additional information with this form

Name & Address of the							
Details of the animal							
Species					Sex		
Animal I.D (Name/No)					Age		
Nature of sample (tick a	appropriate box	c)					
A. Live animal scree	ning						
B. Post-mortem*							
* sample collected in Clinical Symptoms: Y/	•		cording:				
Anorexia	Cough			V	omition		
Fever	Dyspnoea			D	iarrhoea		
Pre existing medical co	onditions please	e specify	with date (eg. Bacte	erial , viral.	, paras	sitic
diseases, tumors etc.)							
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Date:







Guidelines for Working with Free-Ranging Wild Mammals in the Era of the COVID-19 Pandemic

SUMMARY

The SARS-CoV-2 virus, the cause of COVID-19, emerged as a human pathogen in 2019. While it is thought to have a zoonotic source, the original wildlife reservoir and any potential intermediate hosts have not yet been identified. Phylogenetic analyses suggest the progenitor virus is related to beta-coronaviruses previously identified in bats. At this time, SARS-CoV-2 should be considered a human pathogen with people acting as reservoir and sustaining transmission. There is a possibility SARS-CoV-2 will become endemic in the human population and thus be considered as a potential reverse zoonosis to wildlife as with infectious diseases such as tuberculosis and influenza.

Currently the risk of human-to-animal transmission to non-captive wildlife species warrants concern. A number of cases have demonstrated natural human-to-animal transmission of SARS-CoV-2 in felids, canids and mustelids, the majority due to close and prolonged contact with infected households or people, and none has involved free-ranging wildlife. The identification of close phylogenetically-related viruses (e.g. in bats and pangolins), the presence of important cell receptor proteins (ACE2 receptors) and infection following natural exposure or experimental inoculation suggest that a wide range of mammalian species may be susceptible to SARS-CoV-2. Knowledge and experience with human-to-animal transmission with other human respiratory pathogens (e.g. metapneumovirus, measles, other human coronaviruses and tuberculosis) indicates that some species taxonomically closely related to humans (e.g. non-human primates) would likely be susceptible to infection and/or clinical disease caused by SARS-CoV-2.

There are valid concerns about the health of individuals or populations if infected with the virus and/or a wildlife population becoming a reservoir for SARS-CoV-2. Any wildlife species/taxa that becomes a reservoir for SARS-CoV-2 could pose a continued public health risk of zoonosis, a risk for the transmission of SARS-CoV-2 to other animal species, and the risk of negative perceptions of that species resulting in human threats to the species or their populations.

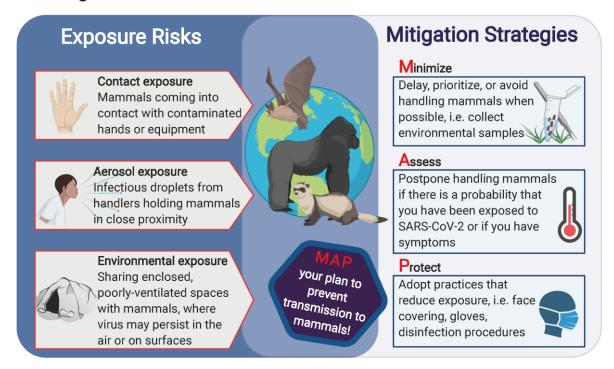
Efforts that require working with free-living wildlife are vital to professional management and conservation as well as the health of wildlife, people and ecosystems. The recommendations below were developed to minimize the risk of SARS-CoV-2 transmission from people to free-ranging, wild mammals. Specifically, these recommendations are for people engaged in **wildlife work*** in the field, either in direct contact (e.g. handling) or indirect contact (e.g. within 2 meters or in a confined space) with free-ranging wild mammals, or working in situations in which free-ranging wild mammals may come in contact with surfaces or materials contaminated by infected personnel.

Version: 25 August 2020

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^{*} These recommendations are provided for trained biologists, conservationists, researchers, veterinarians, etc who work with free-living wildlife in situ. They are not intended for people who interact with wild mammals under different circumstances, such as rehabilitators or ecotourists, etc.

Preventing transmission of SARS-CoV-2 from humans to wild mammals



This figure was adapted in collaboration with the IUCN Bat Specialist group. This work by IUCN SSC,Bat Specialist Group is licensed under CC BY-NC-ND 4.0.

RECOMMENDATIONS

These recommendations are based on first principles of biosecurity and hygiene, current knowledge of human-to-animal SARS-CoV-2 transmission and the precautionary principle.

Minimize

In line with ethical considerations for working with wildlife, we recommend that the three "R's" be considered. If postponement is not possible, it is recommended to "Replace" work that involves animals with alternatives that do not require handling free-living wildlife (i.e. environmental sampling, remote monitoring); "Reduce" the number of animals required to conduct the work and "Refine" the methods used to minimize the impact of the handling on the individual animal and on that animal's population. The recommendations given below are focused on "Refine" however, "Replacing" and "Reducing" work with animals should also be considered at all times.

The primary aim of "Refining" work to be done with wild mammals is to reduce transmission of SARS-CoV-2 from a person to wild mammals. Like tuberculosis and measles, SARS-CoV2 may pose a serious threat of transmission from people to wild mammals. Thus, these additional refinements are recommended for those working indirectly with wild mammals within an enclosed space as well as those working directly with/handling free-living wild mammals.

Assess

The SARS-CoV-2 virus will likely be endemic in many human populations for the foreseeable future, making the potential for transmission of SARS-CoV-2 to wild mammals from people an on-going risk. It is recognised that as the local rate of transmission of SARS-CoV-2 in human

Version: 25 August 2020

populations in different localities fluctuates, the subsequent risk of transmission to wildlife will also vary, requiring continuous and adaptive risk assessment. As the level of community transmission (as defined by WHO) increases and decreases according to implemented control measures, so too will the level of risk. When community transmission rate increases, the potential that at least one person on the field team will be infected (even if they don't have symptoms) also increases. This is important as currently almost half of human infections are asymptomatic, which increases the risk of unknowingly transmitting the virus to wild mammals. These factors make it impossible to estimate the exact quantitative risk of human-to-animal SARS-CoV-2 transmission that working with wildlife represents. Thus, when assessing whether to proceed or postpone work it is recommended that one:

- 1) Postpone the work, unless it is urgent for the health and wellbeing of the animal, if there is known or suspected COVID-19 community transmission, as defined by the WHO, in the area around the site of the wild mammal work or in an area where the team members have been in the past two weeks. Wildlife work should be postponed at least until the transmission rate of COVID-19 has been limited to clusters of cases instead of community transmission (WHO).
- 2) Confirm that local authorities currently permit this type of work and always follow local public health guidelines regarding COVID-19 prevention; if the work is permitted,
- 3) Use one's best judgement as to when to work with wild mammals, erring on the side of the precautionary principal (i.e. uncertainty must be resolved in favor of prevention); if one decides to continue,
- 4) Assess the field team or individual:
 - If someone on the team tests positive for SARS-CoV-2 or has COVID-19 symptoms (WHO), they should follow public health advice on quarantining and avoid working with wild mammals for 2 weeks (WHO) after symptom onset and if symptoms persist, for at least three days after symptoms have resolved without the use of fever-reducing medications. In the case of an asymptomatic infection, avoid working with wild mammals for 2 weeks after the last positive test date.
 - If someone on the team has had contact with a confirmed or suspected case in the past 2 weeks, they should follow public health advice on quarantining and should not work with wildlife for 2 weeks since the potential/known exposure or once they are cleared by public health authorities.
 - This may mean the whole team needs to be quarantined if they were in contact with the team member that tested positive.
 - No one who is currently showing <u>symptoms of SARS-CoV-2</u> (fever of 38°C [100.4 °F] or greater, cough etc.) should work with wild mammals.
 - o Implement daily temperature checks on the days you will be in contact with wild mammals.
 - It is important to avoid taking fever-modifying medicine prior to the temperature check to prevent masking a fever.

Version: 25 August 2020

o If possible, each person on the field team should be tested for SARS-CoV-2 with negative confirmation at least 24-48 hours prior to fieldwork commencing, understanding that this may not be feasible in all circumstances/locations.

Protect

If, upon assessment of the local situation, it is determined that work with free-ranging wild mammals may proceed, it remains the team's duty to minimize the risk of asymptomatic transmission of SARS-CoV-2 to the wild mammals (and each other) by using the proper protective equipment and biosecurity measures. To do this, it is recommended that you

- Follow local public health recommendations.
- Limit the number of personnel to the minimum necessary to safely complete the task and minimize the number of personnel who actually handle or come into close contact (within 2 meters [6 feet]) with wild mammals.
 - o Maintain the same field team for the duration of the operation to minimize the number of different people contacting one another and animals.
 - o To the extent possible, maintain physical distancing between personnel, particularly during transportation and activities in closed spaces.
- Minimize the amount of time people are in close or direct contact with wild mammals.
- Ensure the people on the team that will have direct contact with wild mammals have been properly trained in using personal protective equipment, infection control and animal handling.
- Wear clean, dedicated clothing (e.g. disposable (Tyvek coveralls) or clothing that will be removed and properly cleaned immediately after sampling, at the site).
- If working **indirectly** (e.g. >2m or in a confined space) with wild mammal species that are considered to be particularly susceptible* (e.g. bats, felids, mustelids, non-human primates and any species with the same ACE2 receptor):
 - Wear a face mask or covering, preferably a surgical mask or a more protective covering (e.g. fit-tested N95 without an air release valve).
 - Note a mask or other cloth face-covering is used to prevent the spread of respiratory droplets from your nose and mouth. If surgical masks or respirators are not available locally, it is recommended to use a fitted face covering to improve the ability of the mask to catch respiratory droplets.
 - If working with a team, team members should wear face coverings regardless of the susceptibility of the animal species as recommended by local public health officials.

4 Version: 25 August 2020

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^{*} Note: as new information becomes available any other taxa / species in which SARS-CoV-2 transmission is demonstrated via natural or experimental inoculation should also be considered "potentially susceptible".

- If **directly** handling wild mammals:
 - Wear a face mask or covering, preferably a surgical mask or a more protective covering (e.g. fit-tested N95 without an air release valve) when handling/transporting wild mammals.
 - When handling particularly susceptible species* (e.g. bats, felids, mustelids, non-human primates and any species with the same ACE2 receptor) wear an N95 respirator (without an air release valve) or other equivalent/increased respiratory protection.
 - Wash your hands with soap and water and/or apply hand sanitizer (>60% alcohol applied to clean hands) before and after handling wild mammals.
 - Wear disposable or clean reusable gloves, and change gloves between sampling events or handling individuals of solitary species.
 - Do not blow on mammals to see anatomical features or ectoparasites.
 - Keep captured animals separate from each other to greatest extent possible when capturing and handling.
 - Avoid touching your face or mask, and if contact occurs, change/disinfect your hands/gloves.
 - Clean and disinfect all reusable field gear and equipment that may come into contact with wild mammals prior to starting the work and after each field-work shift or between handling individuals of solitary species.
 - When selecting a disinfectant consider its efficacy against SARS-CoV-2 (<u>EPA</u>), its effectiveness against other pathogens (<u>The Center for Food Security and Public Health</u>) that the animal being sampled may carry, and its potential effect on the equipment that will be used and its environmental impact.
 - 70% isopropyl alcohol or a 10% solution of household bleach are recommended for disinfection against COVID-19 (<u>WHO</u>).
 - For both disinfectants, the surface must be cleaned before they are applied, and your working solution of bleach must be made fresh every day.
 - Properly dispose of used materials and biological and hazardous waste.
 - Follow more specific guidelines produced for each specific taxa group when available (see links below).
 - In settings where peri-urban work is required, ensure that any onlookers from the public remain at least 10 meters away and be upwind from the work that is ongoing with the wild mammals.

Version: 25 August 2020 5

^{*} Note: as new information becomes available any other taxa / species in which SARS-CoV-2 transmission is demonstrated via natural or experimental inoculation should also be considered "potentially susceptible".

These recommendations are deliberately broad to apply to multiple taxa of wild mammals. Some expert groups have developed their own recommendations (see below), which should be used in addition to these. The situation with the COVID-19 pandemic is continually evolving. As we learn more about the effects of SARS-CoV-2 in more species and transmission risks, these recommendations may change or be superseded by species or taxa-specific recommendations. As the SARS-CoV-2 will likely become endemic in human populations, it is our responsibility to prevent the same thing from occurring in the wild, free-ranging mammal species that are in contact with people.

ADDITIONAL RESOURCES

IUCN Great Apes Specialist Group Statement:

http://www.internationalprimatologicalsociety.org/docs/COVID-

19 Advisory for conservation field teams.pdf

IUCN Bat Specialist Group Statement:

 $\underline{https://www.iucnbsg.org/uploads/6/5/0/9/6509077/map_recommendations_for_researchers_v._1_0_final.pdf$

AZA Felid Statement: https://zahp.aza.org/felid-tag-statement-on-sars-cov-2/

AZA Small Carnivore Statement: https://zahp.aza.org/wp-content/uploads/2020/04/AZA-Small-carnivore-TAG-SARS-CoV-Statement_8Apr2020.pdf

AFWA Statement: https://wildlifedisease.org/Portals/0/Covid-19%20Information/AFWA%20Statement%20on%20COVID-19%20and%20Mustelids%20Felids%20and%20Canids%20June%209%202020.pdf

European Association of Zoo and Wildlife Veterinarians – Transmissible Disease Handbook, Chapter 4.4 SARS-CoV2 and COVID-19. https://www.eazwv.org/page/inf_handbook

6 Version: 25 August 2020