Animal and environmental investigations to identify the zoonotic source of the COVID-19 Virus

Zoom conference, 1pm (Paris time) Friday 31 January 2020

Participants: Billy Karesh (Chair – President OIE Wildlife Working Group), Casey Barton Behravesh (Centers for Disease Control, USA), Peter Ben Embarek (WHO), Etienne Bonbon (FAO), Stephane de La Rocque (WHO), Keith Hamilton (OIE), Hiroshi Kida (Hokkaido University), Jean-Claude Manuguerra (Institut Pasteur, Paris), Stefano Messori (OIE), Misheck Mulumba (OIE Scientific Commission for Animal Diseases), Malik Peiris (Hong Kong University), Dirk Pfeiffer (Hong Kong University), Primal Silva (Canadian Food Inspection Agency), Changchun Tu (Changchun Veterinary Research Institute) Sophie VonDobschuetz (FAO), Linfa Wang (Duke University, Singapore), Zheng Zengren (OIE Scientific Commission for Animal Diseases)

The purpose of the call was to discuss what is known about the role of animals in the emergence of Coronavirus Disease 2019, COVID-19 (caused by the SARS-CoV-2 virus (otherwise known as the COVID virus)) and to make preliminary recommendations relating to investigations at the human-animal ecosystems interface.

1. **Review of current knowledge base:**

   Many important questions remain unanswered about the animal origin of the COVID-19 virus. Although an animal source is probable, the scarcity of information leaves significant knowledge gaps, which leaves the door open for speculation and rumours. The lack of evidence also leads to, and in some ways necessitates, several assumptions being made.

   From what is known, the COVID-19 virus isolated from humans shares 96% homology with beta coronaviruses isolated from multiple species of bats in the genus Rhinolophus (Yunnan, 2013). SARS-CoV isolated from humans shared 92% homology with SARS-like viruses that were circulating in bats. 90% of the SARS-like viruses from bats have been isolated from the Rhinolophus genus. The comparatively strong genetic sequence homology between the COVID-19 virus and beta coronavirus isolated from bats suggests that ancestors of the COVID-19 virus were circulating in bats in the Rhinolophus genus. Bats belonging to the Rhinolophus genus are widely distributed across Asia, the Middle East, Africa and Europe.

   There is evidence that the transmission of SARS-CoV from an animal reservoir to humans involved an intermediate host (civets were implicated as an intermediate host for SARS-CoV). Owing to similarities between SARS-CoV and the COVID-19 virus, including the circumstances around their emergence, and considering the absence of other plausible theories an alternative assumption is being made that the transmission route of the COVID-19 virus to humans involved an intermediate animal host which has yet to be identified as opposed to direct bat to human transmission. The epidemiology of MERS shows how the role of an intermediate host can be more significant at the human animal interface than the original animal source of the virus. Thus, it is important to investigate the involvement of an intermediate host and to identify it.

   Human epidemiological data links a high proportion of first- and second-generation human cases of COVID-19 to the Huanan Seafood Wholesale Market in Wuhan. An assumption is made that the COVID-19 virus was introduced to humans who visited or worked in the market. In the absence of detailed epidemiological data, several hypotheses exist for the introduction of the

COVID-19 virus from animals to humans at the market. These include that 1. the virus was introduced to the human population from an animal source at the market and 2. that a human introduced the COVID-19 virus to the market (following exposure to the virus outside the market) and the virus was then amplified in animals which then infected humans.

There is only preliminary and incomplete information from investigations into the animal source at the market. This is understandable considering the importance and urgency of focussing on the public health response to contain the disease. **However, information from these investigations is critical because it may hold the key to preventing further introductions of the virus into the human population, and it may also provide useful insights to reduce the risk of future spill over events from animals to humans.**

In the absence of detailed information, the following assumptions are made. That a spill over event from animals to humans occurred at the Huanan Seafood Wholesale Market. The fact that wildlife was being sold at a seafood market indicates a possible route of introduction by wild species being brought into the market. It is likely that many different animal species were present in the market. Sampling investigations would likely have taken place several days (at least one incubation period) after animal-human exposure had occurred, by which time the source animals may no longer have been present in the market. It is known that samples were taken from several species of animal and that none of these samples tested positive, however information about the number of samples and species sampled is not available. However, several environmental (swab) samples did test positive and virus was isolated from environmental sample(s). It is not clear exactly how animals were incriminated from the positive environmental samples (apart from knowing that the swabs were taken from areas adjacent to where animals had been kept). The fact that the COVID-19 virus was easily isolated from environmental specimens taken at the Huanan Seafood Wholesale Market suggests that survivability of the virus in the environment is good and/or that viral load in the environment was high. In general, the COVID-19 virus and other SARS-like viruses appear to be stable; this has implications for contamination of and persistence in the environment and on fomites. Available information also suggests that it is relatively easy to culture and isolate the COVID-19 virus from specimens and that the virus grows well in Vero cells.

It is critical that important epidemiological and virological information which may explain the emergence and transmission of the COVID-19 virus from animals to humans is collected and preserved. The opportunity to understand this event must not be lost.

General immediate recommendations:

- The Advisory Group offers technical collaboration to support investigations into the animal source
- Multisectoral one health collaboration including animal health, public health, wildlife experts should be encouraged
- Immediate sharing of information from field investigations so far (including positive and negative results) should be encouraged

2. Research priorities (broad categories):

Surveillance and risk assessment

Strategic objective: To develop a better understanding of the key determinants of COVID-19 virus infection and transmission dynamics in animals (including at ecosystem level) and to humans to inform research, surveillance, and control.

Suggestions:

- **Identify the animal reservoir and intermediate host through surveillance/investigation strategies which consider:**
  - Evidence that ancestors of the COVID-19 virus circulate in bats from Rhinolophus genus
  - The absence of information about an intermediate host, which could be any number of animal species (including wildlife, pests/vermin, domestic animals (companion or livestock), stray/feral animals)
  - In the absence of specific information, studies into the role of animals may need to consider a broad range of animal types and species. Where possible and appropriate, scientific information (epidemiological, virological, genetic etc.) may guide and support targeting of the investigations.
  - Broad serological surveillance is more likely to detect the COVID-19 virus in animals than virological surveillance alone (virological surveillance is too narrow). Serological studies can guide more specific targeted virological surveillance
  - Targeting surveillance to selected locations may improve likelihood of detection e.g. markets/farms where wildlife and other animal species (including domestic animals/livestock) are gathered (particularly markets with a link to the Huanan Seafood Wholesale Market). Sampling locations may include other points identified along the supply chain to and from the market. Investigation around markets should also consider that, many markets have already been closed to support control efforts
  - Other types of animal (free ranging, feral, vermin) found in proximity to markets (and other relevant locations) should also be considered in investigations.
  - Strategies could include testing archived animal samples (serum, faeces etc.) collected from recent surveillance projects
  - Positive environmental samples could be tested for genetic material belonging to animal species (using metagenomics or DNA bar coding techniques (DNA bar coding may be more efficient than sequencing the whole genome)). This approach may guide investigations to identify the source of environmental contamination.
  - Rhinolophus group has extensive range, concerted research in China has found >50 SARS-like CoV's. Using biodiversity and host-phylogenetic diversity data sets to model targeting of sampling to increase likelihood of identifying range of reservoirs across Asia, Middle East and Europe.
- **Transmission pathways**
  - Investigate potential transmission pathways from animal reservoirs to intermediate hosts to humans
  - Evaluate the role of intermediate hosts in amplifying the virus
  - Investigate routes and duration of viral shedding from potential hosts
  - Investigate viral persistence under a variety of environmental conditions

    - Testing of farmed wildlife, wildlife markets and wild animals of species other than bats that could be intermediate hosts to identify potential CoVs and possible transmission pathways to humans
    - Investigate the possibility for transmission from humans to animals (domestic animals)
- **Host range**
    - Investigate the possible animal host range of the COVID-19 virus (including use of field (serology) and laboratory studies).
- **Dynamics of wildlife trade**
    - Better understand the dynamics of wildlife trade e.g. origin of different wildlife species in markets, diversity of species, husbandry/production practices, contact/mixing of groups, supply chains etc.
- **Possible role of livestock**
    - As well as assessing the possible role of other types of animal (wildlife, stray animals), it will be important to consider the possible role of livestock, including the possibility for them to become infected by humans
- **Possible role of companion animals in epidemiology of human disease**
    - Assess the potential role of pets and companion animals in the epidemiology of the disease in countries affected with human cases. Consider investigations/sampling of pets of humans suspected or confirmed with disease.

Diagnostics

Strategic objective: To develop diagnostic tools (for use in animal species) that provide consistent optimal results in any setting.

Suggestions:

- **Serology**
    - A fit for purpose serology test for use in different species would be a powerful tool in surveillance for the COVID-19 virus in animals (the utility of serology was demonstrated in SARS-CoV and Hendra virus investigations)
    - Adapt and validate current serology test for antibodies to the COVID-19 virus used in humans to animal systems
    - Consider developing laboratory and field serology kits for animal investigations
    - Assess cross reactivity between the COVID-19 virus and other SARS-like viruses
    - Recombinant protein techniques can play a role in developing serological techniques
- **RT-PCR**
    - RT-PCR platforms for the COVID19 virus have been developed and disseminated for use in humans
    - RT-PCR platforms for the COVID-19 virus need to be adapted to animal systems
    - RT-PCR tools need to be adapted to be fit for purpose e.g. For initial screening of animal surveillance samples, sensitivity will be more important than specificity, therefore for RT-PCR screening tools, primers which span the whole subgroup of SARS-like viruses could be used (with SARS as a positive control). RT-PCR which are more specific to the COVID-19 virus could be used to differentiate viruses when samples are positive on screening
- **Other tests**

- Virus neutralisation, pseudo particle VN, and other tests may also be useful for detection in animal samples

Prevention and control interventions

Strategic objective: To guide targeted and effective evidence-based interventions

Suggestions: In addition to the priorities listed under Surveillance and Risk Assessment above,

- **Collect baseline data to inform prevention and control strategies**
  - Conduct studies to develop a better understanding of the dynamics around illegal wildlife capture, transport, and trading, and current prevention strategies, considering,
    - Social science around criminal behaviour
    - Social/marketing studies on consumer demand
    - Existing international standards, agreements, legislation, and guidance around wildlife trade, markets etc.
    - Relevant stakeholders – NGOs, IOs, national government, public, criminals, traders
    - Coordination between law enforcement, veterinary services, market inspectors/regulators
    - Effectiveness of various interventions e.g. law enforcement, legislation, prosecution, risk communication, incentivisation of legal practices, certification
    - Use of innovation and technology in criminal surveillance/prosecution – cameras, drones, identification of animals
    - The management of wet markets in China, particularly in Wuhan
  - Identify high-risk practices and behaviours (for spill over events) along the food/wildlife supply chain
- **Assess drivers of high-risk practices**
  - Social and economic drivers of legal and illegal activities
  - Value chains leading to human animal/wildlife/environmental exposure.
- **Develop strategies to reduce risk of spill over events**
  - Research to determine the most effective risk communication strategies which avoid stigmatisation and other unintended consequences
  - Research to determine the most effective social and behavioural change (SBC) practices to improve hygiene practices at wet markets
  - Research to determine most effective SBC practices to implement realistic and feasible strategies to encourage a high level of compliance at wet markets
  - Research to determine the strategy to strictly manage the wild animal farming and to stop the illegal transportation and trading as well as smuggling.
- **information from laboratory studies**
  - In the absence of field data, animal laboratory studies could help to inform prevention and control strategies e.g. animal models

Host-pathogen interaction

Strategic objective: To improve understanding of virus-host interactions and factors that impact on the interactions such as disease pathogenesis, transmissibility, and immune responses to better inform infection control.

Suggestions:

- **Host pathogen studies**
  - Animal susceptibility - host range determination, receptor specificity/distribution in different species etc.
  - Cell line infections and animal experimental infections to understand transmission and pathogenicity.
  - Epidemiology of CoV in animal reservoirs, i.e. from bats to other species (viral load, routes of transmission).
- **Behavioural risk**
  - Identify communities with high levels of exposure to bats and other key wildlife; analyse their risk behaviours; test samples from wildlife and people in these communities for serological evidence of the COVID-19 virus and other CoV spill over.
  - Inserting standardized key questions on wildlife exposure to be used during interview with suspected cases.

Socio-economics and policy

Strategic objective: To improve the effectiveness of detection, prevention and control measures through the integration of social, economic and institutional analyses of the environment affected

Suggestions:

- **Wildlife trade**
  - Define what is meant by wildlife (i.e. farmed wildlife vs. domestic animals/livestock etc.) in different contexts
  - Characterize the wildlife trade value chain globally and regionally and how it is linked with China.
  - Policy/social research to regulate wildlife trading – innovation (cameras, drones etc.), collaboration with social scientists, law enforcement/ behaviour/demographic patterns
  - Study of economic impact of removing wildlife from markets and market closures
  - Analyses of the social impacts and economic analyses of different degrees of limiting wildlife trade for food: 1) complete ban; 2) partial ban (select species); 3) regulating and testing animals; 4) promoting only farmed wildlife as a source of food
- **Wildlife capture vs. production**
  - Scenario analysis of whether or not farming wildlife reduces the risk of CoV emergence as compared to wild caught wildlife
- **Wildlife consumption**
  - Survey of public to assesses knowledge, attitudes, and practices around wildlife consumption, geographic variation, and changing demographics.
- **Domestic animals**
  - Draw on research/risk communication already existing in this area relating to other zoonotic diseases (e.g. zoonotic influenza, Nipah, SARS, etc.) related to the breeding, keeping, selling and consumption of livestock.
  -

3. Additional general notes:

There is a need to learn lessons from the introduction of the COVID-10 virus to the human population and from similar past events. A similar event in the future is inevitable.

Research

There is a need to highlight the limitations of research objectives in order to manage expectations on outcomes

Risk mitigation strategies

It will be important to take a comprehensive long-term approach to risk mitigation strategies which aim to reduce the risk of spill over events.

Risk mitigation strategies need to feasible and consider cultural importance of certain high-risk practices. They need to adopt a multidisciplinary approach (vets, economists, food hygienists, microbiologists, social scientists, communication experts) and could include a package of risk mitigation measures targeted to the right stakeholders.

In risk communication there is a need to be clear about the current uncertainties around the role of animals in human outbreaks or animal species involved and it will be important to manage expectations e.g. risk can be reduced but not eliminated.

For short term, a key message is that the highest risk for COVID-19 virus infection is human to human transmission; identifying animal hosts is only an additional measure so that other (rare) spill over events can be reduced and similar human outbreaks prevented in future

Risk communication can also build on material developed for other risk mitigation strategies (Ebola and wildlife/bushmeat, zoonotic avian influenza and live bird markets).

The spectrum of people at risk in different systems (field scientists, farmers, traders, consumers) needs to be considered in risk communication and other risk mitigation strategies.

Interventions need to be targeted for maximal positive impact (e.g. HACCP) and policies should avoid or manage unintended negative consequences (regulatory impact assessment).

Studies and guidance on wildlife trading and consumption should be adapted to both the global and regional levels i.e. global coverage whilst considering regional characteristics and specificities.

Strategies should be realistic and focus on risk reduction rather than elimination and should take lessons from other successful policy initiatives which led to behaviour change e.g. seat belts, smoking, diet.