Wild Tiger Health Centre Information Sheet

Disease threat: Canine distemper virus - CDV

Hazard description: Canine distemper is caused by a paramyxovirus within the Morbillivirus genus, with a single-stranded RNA genome (Greene & Appel, 2006; Frölich 2012). Infection in free-ranging tigers was first identified in 2003 from the Russian Far East (Seimon *et al*. 2013) where it has since been shown to threaten small isolated populations (Gilbert *et al.* 2014). Reports of infections have also been made in wild tigers outside Russia but are less established in the scientific literature.

Distribution: Worldwide.

Host species: Originally described in dogs, it is now thought most carnivores are likely to be susceptible to CDV. There are a few exceptions to this, including small felids (e.g. *Felis* spp.).

In carnivores, clinical disease has been documented in the following families: *Ailuridae, Canidae, Felidae, Hyaenidae, Mephitidae, Mustelidae, Otariidae, Phocidae, Procyonidae, Ursidae, Viverridae* (Martinez-Gutierrez and Ruiz-Saenz 2016). Serological evidence of exposure has also been reported from *Odobenidae* (Nielsen *et al*. 2000).

Among the non-carnivores infections have been recorded in rodents, primates and artiodactyls including members of the *Caviidae, Cebidae, Cercopithecidae, Cricetidae, Muirdae, Tayassuidae* and *Sciuridae* families (Appel *et al*. 1991; Martinez-Gutierrez & Ruiz-Saenz, 2016). However, most of these involved captive animals, some of which were experimentally infected. Serological evidence has been found in an even wider range of hosts (Martinez-Gutierrez & Ruiz-Saenz, 2016), but the significance is unclear (i.e. if antibody production followed exposure without viral replication, or potentially even cross-neutralization with other as yet unidentified morbilliviruses).

In captivity, clinical disease and mortalities have been reported in all five of the *Panthera* species (Appel *et al.* 1994; Nagao *et al*. 2012; Chinnadurai *et al.* 2017). In wild populations large outbreaks and mortality events have been documented in African and Asiatic lions *(P. leo)* (Roelke-Parker *et al.* 1996; Munson *et al*. 2008; Mourya *et al.* 2019) and sporadic cases reported in leopards *(P. pardus)* (Sulikhan *et al.* 2018; Nayak *et al*. 2020) and tigers (see below).

Free-ranging tiger occurrence: CDV infection and mortality has been confirmed in Amur tigers in the Russian Far East (RFE) (Quigley *et al.* 2010, 2012; Seimon *et al.* 2013), and in free-ranging tigers in India (ProMED-mail 2013; Nigam *et al.* 2016; Rajput *et al.* 2018). Details of cases from the RFE are given in Gilbert *et al.* 2015. Further unconfirmed cases of CDV are suspected to have occurred in Sumatra (D. Martyr 2019, pers. comm; Mulia *et al,* in prep.) and Malaysia (R. Pickles 2020, pers. comm.). Serological evidence of infection reported from clinically healthy Amur tigers in the wild (Goodrich *et al.* 2012) suggests that some individuals recover following infection.

Transmission and pathogenesis: Transmission of CDV primarily occurs through the respiratory tract, via aerosol or saliva, during close contact with an infected individual. Transmission between species is also presumed to occur through predation. Other opportunities for transmission include contact with infectious material, such as urine or faeces. However, the risk from these other routes is thought to be relatively limited, as the virus cannot survive for long in the environment. In dogs, CDV may be shed prior to the onset of clinical signs and as soon as five days post infection. Shedding can continue long after clinical signs have resolved and, in some cases for as long as four months (Sykes 2014). In captive tigers, virus has been detected in the urine as much as 150 days after infection (Gilbert *et al.* 2015), although as this was determined by RT-PCR it is unclear whether the detected material was viable and infectious virus.

Like other morbilliviruses, CDV is lymphotropic. Inhaled viral particles initially colonise the upper respiratory tract and tonsils, infecting monocytes by binding to a surface protein known as the signalling lymphocyte activation molecule (SLAM). Following initial replication in the tonsils, the virus spreads throughout the body via the lymphatic and blood systems (Ludlow *et al.* 2013). SLAM receptors are expressed on the surface of several cells involved in the immune system, including lymphocytes, macrophages, thymocytes and dendritic cells. During this initial viraemic phase the virus enters and destroys these cells resulting in leukopenia and immunosuppression. The only symptom that may be detected at this stage is a transient fever. Whether disease progresses from here depends on the extent of infection and degree of immunosuppression (Greene & Appel 1990). In mild cases, where the immune system has not been too severely compromised, the host may be able to eliminate the virus. However, in more severe cases, the virus will overpower a weakened immune system and infection will progress to a second viraemic phase. In dogs, this second phase typically occurs ~ 8-9 days after infection and it is during this time that animals will start to become overtly symptomatic (Sykes 2014).

During the second phase of viremia, the virus makes use of another cell receptor, known as nectin-4. Cell entry using nectin-4 is thought to be relatively inefficient compared to the SLAM mechanism used in the initial phase, but it does allow the virus to infect a much broader range of cells, including epithelial cells such as those in the gastrointestinal and respiratory mucosa (Noyce *et al.* 2013). The infection of these tissues gives rise to the respiratory and gastro-intestinal signs described below. This is also the stage that the

characteristic intranuclear and intracytoplasmic inclusion bodies start to form and become visible within the cells (Sykes 2014).

The third and final phase of infection occurs once the virus has gained entry to the central nervous system (CNS). Several mechanisms have been proposed for this including the movement of infected mononuclear cells across the blood brain barrier and anterograde infection from olfactory neurons. Once within the CNS, infection spreads through cerebrospinal fluid causing a demyelinating leukoencephalomyelitis resulting in progressive neurological signs (Lempp *et al,* 2014). Not all animals which enter the second phase, will go on to the third phase. The factors which influence whether this occurs are not well understood.

Clinical signs: In captive tigers, reported symptoms include respiratory signs, which may be accompanied by oculo-nasal discharge, and gastrointestinal signs such as anorexia, vomiting and diarrhoea (Appel *et al,* 1994; Konjević *et al,* 2011; Nagao *et al,* 2012). The course of disease is varied, and individuals can differ widely in their presentation. In some cases, death can occur at this early stage. Animals which survive the initial phase of disease may either make a full recovery or go on to develop progressive neurological signs. Neurological signs include blindness, ataxia, paresis and seizures. Once neurological signs develop death is almost inevitable.

In free-ranging tigers the initial symptoms are likely to go unnoticed. However, as neurological signs develop affected tigers appear to lose their fear of people and may be found inappropriately close to areas of human habitation. At this stage signs can include fearlessness, sensory deficits (*e.g.* blindness), ataxia and muscular tremors (Gilbert *et al,* 2015). Many of these animals also appear to be in poor body condition (Seimon *et al,* 2013). Tigers are often taken into captivity at this stage where despite supportive care their condition deteriorates until they die.

Differential diagnoses: Infection with CDV can result in a wide range of symptoms, and differential diagnoses are likely to vary depending on the stage of disease. In wild tigers most animals are not detected until they develop neurological signs. Similar clinical symptoms can be seen in a range of other conditions including rabies, poisoning with organochlorine and organophosphorus compounds, cerebral or spinal trauma (*e.g.* following vehicular collisions), cerebral space-occupying lesions (*e.g.* cysticercosis (Phutenshok *in prep*), tumours and abscesses), the dry form of FIP, toxoplasmosis, liver failure (Crook & Carpenter 2014), and Borna virus disease.

Treatment of affected tigers: Suspected cases should be treated as infectious and should be barrier nursed, ideally away from any other captive animals. Virus is often shed in the urine and particular care should be taken to ensure this does not run off in to neighbouring enclosures or get transmitted on keepers' boots. The use of disinfectant foot dips is strongly advised. Similarly, all efforts should be made to ensure enclosures

are bio-secure and free from pests which might transfer material between enclosures. A full clinical examination should be conducted under anaesthesia during which diagnostic samples can be obtained and supportive treatment administered. All animals should be sampled as they are taken into captivity, with additional samples taken every time the animal is handled. For a detailed list of samples see Tables 1 & 2 below.

Unfortunately, once neurological signs have developed there is no known treatment, and most cases will prove fatal. However, it is important to keep an open mind until a firm diagnosis has been reached. During this time supportive care should be provided by maintaining adequate hydration and nutrition and administering broad-spectrum antibiotics and nonsteroidal anti-inflammatory drugs. Although extremely rare in tigers, rabies should always be considered a potential differential, and as a result all personnel should wear gloves and ensure they avoid any direct contact with potentially infectious material such as blood or saliva.

Diagnosis: Considering the potential significance of CDV for the conservation of tigers in the wild, all agencies involved in the management of tigers are encouraged to introduce protocols for assessing population exposure and confirming individual infections. We advocate sampling all live tigers handled for routine purposes (e.g. research or conflict resolution) and the consideration of CDV as a potential factor in all cases of mortality even when the primary cause is readily apparent (i.e. in road traffic accidents or poaching incidents). Of course, this should not be at the expense of broader clinical investigations, particularly when tigers are showing signs of ill health.

To prioritise appropriate diagnostic tests, it is important to understand the reasons for testing and what the investigator intends to detect. There are two main test approaches - those which assess exposure to virus (through detection of antibodies, also known as serology), and those that diagnose active infections (through detection of the presence of virus, primarily using the molecular test RT-PCR).

Serological assays are the most appropriate technique for conducting routine surveillance or investigating population level exposure (Goodrich *et al,* 2012). The main advantage of a serological approach is that it dramatically extends the window of detection which is particularly relevant for species like tigers which are seldom handled. Animals that recover from CDV are protected from further infection by antibodies that remain detectable for extended periods, potentially the remainder of the animal's life. By comparison, the window for detecting active infection via RT-PCR may only be a matter of weeks. The chance that an animal will be captured and sampled during this period is far lower, particularly if it recovers prior to developing neurological signs. It is important to remember that a positive serological result does not necessarily indicate current infection. In animals that appear clinically well, it is more likely to be the result of a historic infection which the animal has overcome.

In domestic dogs, the detection of antibodies to CDV is made easy by using commercially available ELISA kits. These bench top tests are easy to use, requiring no specialist equipment, and produce results with reasonable sensitivity and specificity (Litster *et al,* 2012). However, these tests rely on secondary marker antibodies, which are species-specific (*i.e.* anti-dog IgG) meaning that tests which are developed for dogs are only reliable for detecting antibodies in dog serum. At present there are no commercial ELISA kits validated for use in tigers and the use of these tests cannot be relied upon. Virus neutralisation tests (VNT) remain the most appropriate technique for antibody detection in this species. Unfortunately, this is a more complex technique, involving cell culture and the ability of the tested serum sample to neutralise live virus. The main advantage of this approach is that it is not species-specific and, once established, the same assay can be used to test samples from any species of animal. Although VNTs are available commercially in many veterinary laboratories worldwide, few are in tiger range countries. Range country government restrictions and CITES requirements can present challenges to the export of diagnostic samples and in some countries prevent it altogether. In response to this, a Cornell-led research project has recently sought to establish VNT capacity in research laboratories within several tiger range states. The protocol has already been established in Indonesia and Thailand, with testing in Nepal due to be completed as soon as current travel restrictions are lifted. **Anyone interested in establishing this protocol elsewhere should contact Dr Martin Gilbert at the Cornell Wildlife Health Centre: m.gilbert@cornell.edu**

RT-PCR assays detect the pathogen itself, or its derivatives, and as such are the best method for detecting active infection. The products from positive assays can also be sequenced, potentially yielding valuable epidemiological information. During the course of infection, CDV may be detectable in a wide variety of bodily fluids and tissues. However, as viral shedding is transient, and occurs from different sites at different stages of disease, some caution must be given to the interpretation of results. All positive RT-PCR results should be considered as true positives (unless there is evidence of contamination), but a negative result based on swabs, blood or urine cannot conclusively rule out ongoing infection and further investigation is warranted, particularly if the animal is showing signs suggestive of CDV. Post-mortem tissue samples are more consistent and to date the most reliable of these has been brain. Given the potential significance of CDV to tiger populations (Gilbert et al, 2014), brain should be considered a standard tissue and routinely sampled during all tiger necropsies. As some animals may succumb prior to neural involvement, it is advisable to collect and screen a full set of tissues. However, if brain tissue from an animal with a history of neurological disease is negative on RT-PCR, it is unlikely CDV was causing its neurological symptoms.

Unlike VNT, most range-states will have the capacity to run RT-PCRs in-country, and a point of care assay has recently been developed to aid diagnosis in the field and overcome the challenges of remote working (Tomaszewicz Brown *et al,* 2020). If RT-PCR tests are positive for CDV, sequencing of the products should be pursued wherever possible.

Other diagnostic approaches such as direct immunofluorescence assays and histopathology may yield valuable information. In brain tissue, reported histological findings include a nonsuppurative viral encephalitis with severe demyelination and eosinophilic viral inclusions in neuronal and glial cells (Blythe *et al,* 1983; Seimon *et al,* 2013). Both immunohistochemistry (IHC) which targets proteins and in-situ hybridisation (ISH) which targets viral RNA have been successful in identifying CDV as the causative agent of these lesions (Seimon *et al,* 2013). In dogs suffering with distemper, a variety of changes have been seen in other tissues, including lung, stomach, spleen, lymph node and tonsils, the most characteristic finding being the presence of eosinophilic inclusion bodies (Kubo *et al,* 2007). To date this has not been the case in tigers (Quigley *et al,* 2010) but only a limited number of cases have been reviewed and reported and it is possible that changes may be seen in other organs in future cases.

Whilst CDV is clearly a pathogen of concern, clinicians attending sick tigers must ensure they keep an open mind and conduct a thorough investigation. Identifying and reporting any co-morbidities present will be particularly important, as these could influence the outcome of infection (Munson *et al,* 2008).

The following tables detail the specific samples for collection for the diagnosis of CDV (Table 1.) and the additional samples to be collected as part of a wider investigation (Table 2.). It is recognised that the resources available for such investigations vary widely. As such in each case the optimal storage conditions are described alongside alternative methods which can be used if optimal conditions are not available. Wherever possible a full sample set should be collected each time the animal is handled. It should be borne in mind that the samples listed in Table 2. represent a suggested minimum, rather than an exhaustive list, and additional samples may be indicated depending on the individual presentation and/or post-mortem findings.

Table 1. Specific samples for collection for the diagnosis of CDV, alongside guidance on their storage. (best) = Optimal storage method, alternative options follow in descending order of preference

<table>
<tr><td colspan="3">Live animals:</td></tr>
<tr><td>Sample</td><td>Test</td><td>Collection and storage</td></tr>
<tr><td>Nasal and conjunctival swabs</td><td>RT-PCR</td><td>Wet swabs in PBS (best) or normal saline
Preserved in RNA-later or Guanidine (best) or 95% ethanol (70% ethanol if 95% not available)
Frozen at -80°C (best) or -20°C</td></tr>
<tr><td>Whole blood</td><td>RT-PCR</td><td>Collected in Heparin or EDTA (ideally min. 5 ml in multiple tubes)
Frozen at -80°C (best) or -20°C</td></tr>
<tr><td>Serum</td><td>RT-PCR
VNT</td><td>Collected in plain tubes (ideally min. 5 ml in multiple tubes)
Separated by centrifuge (pref. prior to freezing)
Frozen at -80°C (best) or -20°C</td></tr>
<tr><td>Urine</td><td>RT-PCR</td><td>Collected by catherization (best) or expression
Frozen at -80°C (best) or -20°C</td></tr>
<tr><td colspan="3">Dead animals, as live animals plus:</td></tr>
<tr><td>Brain</td><td rowspan="6">RT-PCR

Histology</td><td rowspan="6">All samples should be preserved for <u>both</u> RT-PCR and histology

For RT-PCR:
Preserve in RNA-later or Guanidine (best) or 95% ethanol or 70% ethanol
Frozen at -80°C (best) or -20 °C

For histology:
10% formalin (at least ten times volume of formalin to tissue)</td></tr>
<tr><td>Lung</td></tr>
<tr><td>Spleen</td></tr>
<tr><td>Lymph nodes</td></tr>
<tr><td>Intestine</td></tr>
<tr><td>Urinary bladder</td></tr>
</table>

Table 2. Additional samples to be taken as part of a routine investigation which may aid in the exclusion of differentials or identification of co-factors.

Live animals:		
Sample	**Test**	**Collection and storage**
Whole blood	Haematology	Collected in EDTA (ideally 2 ml + in multiple tubes) Frozen at -80°C (best) or -20°C
	Biochemistry	Collected in Heparin (ideally 2 ml + in multiple tubes) Frozen at -80°C (best) or -20°C
	Parasitology	Either in EDTA or Heparin (ideally 2 ml + in multiple tubes) Frozen at -80°C (best) or -20°C
Fresh blood smear	Differential Cell Count	Prepared from plain whole blood using a glass slide Air dried and fixed using methanol (best) or ethanol
Urine	Microscopy	Collected by catherization (best) or expression Centrifuged
	Toxicology Bacteriology	Collected by catherization (best) or expression (ideally 30ml +) Plastic container without preservative Frozen at -80°C (best) or -20°C
Dead animals:		
Tissue samples as above plus: Heart Liver Gall bladder Stomach Kidney	Histology Toxicology	For histology: 10% formalin (at least ten times volume of formalin to tissue) For toxicology: Plastic container without preservative Frozen at -80°C (best) or -20°C
Stomach contents	Toxicology	Plastic container without preservative Frozen at -80°C (best) or -20°C

Epidemiological notes: In wild tigers, CDV infection is presumed to represent spill over of disease from a local reservoir. Knowledge and understanding of these reservoirs are therefore critically important.

CDV is commonly found wherever domestic dog populations are not routinely vaccinated (Ng *et al.* 2019; Nayak *et al.* 2020). This often leads to the assumption that domestic dogs are the reservoir of infection for other species, but this may not be true. It is increasingly accepted that CDV may be maintained by mixed species populations made up of a matrix of susceptible hosts (Almberg *et al.* 2010), and that the contribution and significance of domestic dogs to these populations will vary from case to case (Gilbert *et al.* 2015). Where reservoirs do include wildlife, it is likely to be the smaller, more numerous, mesocarnivores that play the biggest role in maintaining disease (Kapil & Yeary, 2011).

CDV has a relatively short infectious period and results in prolonged, if not lifelong, immunity if an infected animal survives. Pathogens exhibiting these characteristics are vulnerable to density dependent fade-out and maintaining them typically requires an abundance of susceptible hosts. Few wild carnivores have populations capable of meeting this criterion. However, as a multi-host pathogen, CDV can infect a wide variety of animals and cross species barriers. These characteristics allow it to escape the density dependent fade-out that would be expected were it operating in more single-host-pathogen systems (Gilbert *et al.* 2014) resulting in a number of potential scenarios for maintenance and transmission, some of which are given below and illustrated in figure 1 below.

Scenario one: Domestic dogs act as a reservoir

Where numbers allow, CDV may be maintained by a single reservoir species. Few wild carnivores exist at the densities required to support this, but domestic dog populations can often reach these levels. Circulating infection within a domestic reservoir may be accompanied by occasional transmission to other hosts resulting in sporadic disease and intermittent outbreaks in wildlife. This classical model of spill-over is thought to have underpinned the 1994 outbreak of CDV in the Serengeti National Park (SNP) (Viana *et al.* 2015).

Scenario two: Multiple wildlife species act together as a reservoir

Following the 1994 outbreak in the SNP, vaccination programs were initiated targeting dogs all around the park. This strategy effectively removed domestic dogs from the system. Whilst this approach altered the disease dynamics, it has failed to eradicate disease (Viana *et al.* 2015) which clearly demonstrates that in the absence of an abundant host, such as domestic dogs, sylvatic cycles of CDV can still persist. This has led to a review of the way in which reservoirs are defined (Haydon *et al*. 2002). In some instances, they may still be comprised of, or dependent on, a single species, but in other cases they may be mixed populations made up of several species. Occasional spill-over to other wild carnivores (outside the maintenance reservoir) may still occur and result in outbreaks, but their scale and frequency may differ from outbreaks originating under scenario one (Viana *et al.* 2015). There may also be spill-overs from sylvatic cycles to domestic dogs (Kapil

& Yeary 2011). Widespread vaccination of dogs is not the only situation in which this scenario could exist - in some instances the local population of dogs may simply be too small to act as a reservoir.

Scenario three: Domestic dogs and wildlife act together as a reservoir

There is nothing to suggest a reservoir could not include both domestic and wild species. Expanding feral dog populations (Young *et al.* 2011) coupled with the ability of some carnivores to exploit human dominated landscapes (Bateman & Fleming 2012) leads to a greater level of interaction between species providing ample opportunity for virus transmission. In such scenarios the contribution of each species may be difficult to discern. The most important question from a control point of view is to establish whether removing domestic dogs from the system through vaccination will be sufficient to cause the reservoir to collapse and disease to fade out (Prager *et al.* 2012a).

Scenario four: Domestic dogs and wildlife act independently as reservoirs for separate cycles

In situations where there is limited opportunity for transmission between domestic dogs and wildlife, it is feasible that two distinct cycles could run parallel to one another, i.e. one domestic and one sylvatic. Sequencing viral DNA recovered from wildlife cases and comparison with samples recovered from domestic dogs can be useful in exploring this possibility. If the same strain is circulating in both communities, it would suggest they retain some connection. If samples from the two groups formed distinct clusters it would support a theory of separation. Spill overs to species outside those reservoirs may still occur, and on some occasions, there may be spill overs between reservoirs. If disease is identified in a non-reservoir species such as a tiger, it may be possible to use the same sequencing approach to infer where the virus was acquired (Trebbien et al. 2014).

Scenario five: There are multiple meta-reservoirs

In this scenario there is no identifiable reservoir and no single population can sustain the virus. Instead it is passed between so called meta-populations, which are loosely linked to one another to form a network that spreads across the landscape. Each meta-population experiences epidemic waves, followed by localised fade out. However, during each epidemic phase spill-overs seed outbreaks in other nearby populations, causing the virus to leapfrog across the landscape (Prager, Mazet *et al*. 2012b).

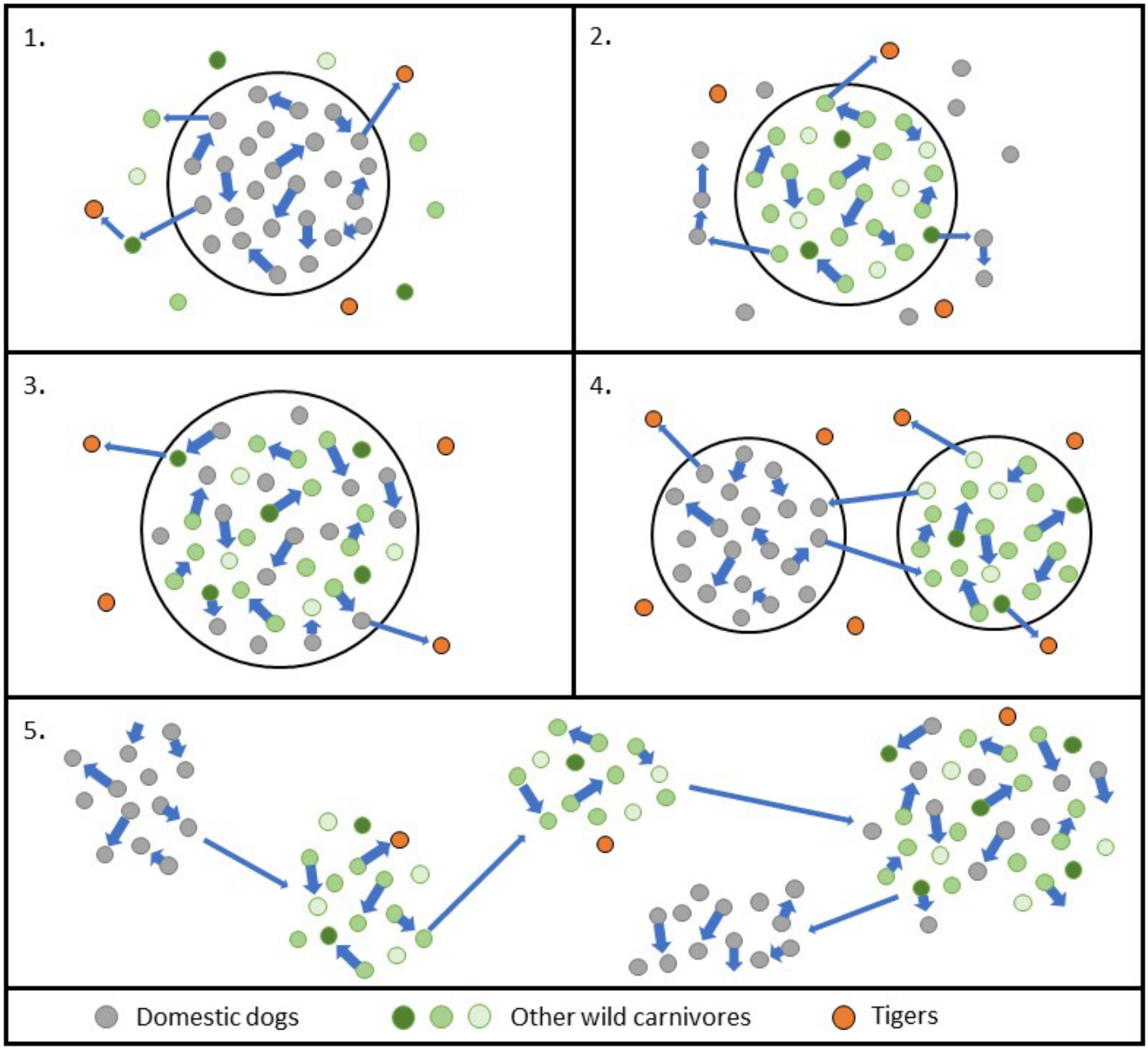

Figure 1. Graphic illustration of five potential reservoir scenarios (1-5) and the spill over to infect tigers:

Scenario 1. Domestic dogs act as a reservoir.

Scenario 2. Multiple wildlife species act together as a reservoir.

Scenario 3. Domestic dogs and wildlife act together as a reservoir.

Scenario 4. Domestic dogs and wildlife act independently as reservoirs for separate cycles.

Scenario 5: There are multiple meta-reservoirs.

The large black circles delineate reservoirs, within which disease is endemic. Animals within the circle contribute to the maintenance of the virus, animals outside the circle are infected as a result of spill-over events. Arrows indicate the direction of transmission. The weight of the arrows indicates the likelihood of transmission, with narrower arrows indicating transmission is less likely.

Another important epidemiological consideration is the potential role of co-infection. Infection with CDV has resulted in mortality in a wide range of species and contexts (Roelke-Parker *et al*. 1996; Seimon *et al.* 2013; Trebbien *et al.* 2014). However, a review of serological evidence has identified, so called, 'silent' outbreaks, which were not accompanied by any identified mortality (Munson *et al.* 2008). The reasons for this variation are unclear. Although there is some evidence to suggest certain strains of CDV may be more pathogenic than others (Carpenter *et al.* 1998), an alternative explanation in some cases could be the role played by co-infections. *Babesia* was implicated as a co-factor in a fatal outbreak in Tanzania (Munson *et al.* 2008) although in other equally serious outbreaks no co-factor has been identified (Seimon *et al*. 2013). What is clear is that the epidemiology of CDV is extremely complicated and that the outcome of disease may be dependent on a wide range of factors which may differ between each ecosystem studied.

Potential control measures: Given the complexity of its epidemiology, designing effective control measures for CDV is rarely straightforward. In simple terms there are three potential approaches:

1. Remove the reservoir
2. Reduce spill-over
3. Block infection progressing to disease by vaccinating the species concerned - in this case tigers.

To make progress with options 1 and 2 an understanding of which species contribute to the reservoir and how and where spill-over occurs is needed. To make progress with option 3 a safe and effective vaccine plus an efficient method of delivery are needed.

In domestic dogs, vaccination can be highly successful, reducing prevalence within a population and protecting the individuals concerned. Previously it was thought that the vaccination of dogs may also protect wildlife, by removing the reservoir or reducing the environmental burden of virus. However, there is increasing evidence to suggest that this is not always the case (Prager *et al.* 2012a). As already discussed, domestic dogs may not necessarily contribute significantly to a reservoir, and, even where they do, wild species may still be able to support the virus in their absence (Viana *et al.* 2015). If wild species are thought to be contributing to the reservoir, then one option would be to consider vaccination. Oral baited rabies vaccines have been used to successfully target several wild carnivores (Maki *et al.* 2017), but unfortunately an orally delivered CDV vaccine is not yet available. Therefore, any current wildlife vaccination program against CDV would require the trapping and vaccination of each animal separately.

Reducing the risk of spill-over could be a useful approach, particularly if investigations suggest tigers were acquiring infection from a specific source such as domestic dogs. However, much like attempts to target the reservoir, the situation is unlikely to be straight-forward. Any approaches which limited the movement of wildlife, such as the use of fencing, would have to be balanced against the impact this could have on

connectivity. Therefore, if tigers were acquiring infection from unvaccinated dogs, vaccination may be the better option.

These combined challenges have led many authors to conclude that the optimal route for protecting endangered species may be direct vaccination of the species of concern (Cleaveland 2009; Prager *et al. 2012)*. Given that most free-ranging tigers are rarely seen let alone handled, widespread vaccination programmes would present massive logistical challenges. However, whenever tigers are handled for research purposes, conflict resolution, rehabilitation etc, low-level, opportunistic vaccination could still be useful - particularly for smaller more vulnerable populations (Gilbert *et al.* 2015). Vaccination programmes targeting free ranging tigers of any sort would require further vaccine development and trials.

Vaccination of tigers: Most zoological collections routinely vaccinate their tigers against a range of feline viruses and in some cases where the risks are judged high, also against CDV. Research into the development of vaccines for use in wild tigers is ongoing.

Broadly speaking there are two main classes of vaccine against CDV - modified live and recombinant. Modified live vaccines contain live CDV virus which has been attenuated to make it less virulent. These typically elicit a strong and lasting immune response but can carry a greater risk of the vaccinal virus reverting to virulence. Recombinant vaccines only incorporate selected elements of CDV into a canarypox vector virus which is harmless to tigers. Recombinant vaccines are less immunogenic and typically require regular boosters.

Unfortunately, the need for annual booster vaccination makes recombinant products effectively innappropriate for free ranging animals. However, recent trials of a modified live vaccine based on the Onderstepoort strain of CDV have shown promise, invoking strong antibody responses without clinical side effects in domestic cats and tigers (Sadler *et al.* 2016; Ramsay *et al.* 2016). Unfortunately, commercial monovalent vaccines (i.e. those containing only modified CDV virus) may not always be readily available. When deliberating on the use of more readily available multivalent products, practitioners will have to carefully consider the safety of the other vaccine components. The inclusion of live, attenuated parvovirus should be viewed with particular caution, as the vaccination of pregnant female cats has previously been associated with foetal abnormalities.

Further trials of these products, and investigation into remote delivery systems, are now a priority.

Assumptions: None.

Limitations: Incomplete epidemiological pictures in tiger range states.

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