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MISS HARRIET PETERS (Orcid ID : 0000-0002-1175-3951)

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Corresponding author mail id:hpeters6@rvc.ac.uk

Risks from disease caused by *Mycobacterium orygis* as a consequence of Greater one-horned Rhinoceros (*Rhinoceros unicornis*) translocation in Nepal

Harriet Peters (Royal Veterinary College), Amir Sadaula (National Trust for Nature Conservation), Nick Masters (Zoological Society of London) and Anthony Sainsbury (Zoological Society of London, Insititute of Zoology)

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Risks from disease caused by Mycobacterium orygis

Summary

The greater one-horned rhinoceros (*Rhinoceros unicornis*) is listed as vulnerable by the IUCN Red List. *Mycobacterium orygis* associated disease was identified in a single greater one-horned rhino in Chitwan National Park in February 2015 prior to a planned translocation of five greater one-horned rhinoceros from Chitwan National Park to Bardia National Park for conservation purposes. This paper describes a qualitative disease risk analysis conducted retrospectively post-translocation for *Mycobacterium orygis* and this translocation, with the aim to improve the understanding of disease threats to the conservation of greater one-horned rhino. The disease risk analysis method used was devised by Sainsbury & Vaughan-Higgins (2012) with modifications by Bobadilla Suarez et al (2017) and Rideout et al (2017), and included the use of a scenario tree, and an analysis of uncertainty as recommended by Murray *et al*. (2004), the first time this combination of methods has been used to assess the risk from disease in a conservation translocation. The scenario tree and analysis of uncertainty increased the clarity and transparency of the analysis. Rideout *et al*.'s (2017) criteria were used to assess the source hazard and may be useful in comparative assessment of source hazards for future conservation translocations. The likelihood of release into the destination site of *Mycobacterium orygis* as a source hazard was estimated as of low risk, the risk of exposure of populations at the destination was of high risk and the likelihood of biological and environmental consequences was low. Overall the risk from disease associated with *Mycobacterium orygis* as a result of this translocation was found to be low. Recommendations on disease risk management strategies could be improved with a better understanding of the epidemiology including the presence/absence of *Mycobacterium orygis* in greater one-horned rhino to develop effective disease risk

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management strategies.

Key words

Disease risk analysis, Translocations, *Mycobacterium orygis*

Ethical statement

An ethical statement was not applicable as neither sample collections or questionnaires from animals/human had been gathered.

Introduction

The greater one-horned rhinoceros (*Rhinoceros unicornis*) (hereafter, 'GOH rhino'), also known as the Indian rhinoceros or the Asian one-horned rhinoceros, listed in Appendix I of the Convention on International Trade in Endangered Species (CITES) and classified vulnerable on the International Union for the Conservation of Nature's (IUCN's) Red List, is the only rhinoceros species found in Nepal (Talukdar *et al*., 2008; WWF, 2017). The range of the GOH rhino originally spread across the northern Indian sub-continent but, due to urbanisation, loss of habitat, poaching and hunting for sport (WWF 2017), they are now restricted to a few small, isolated populations in India and Nepal (Subedi *et al*., 2013). GOH rhino numbers had decreased to 600 individuals in 1975 but increased to approximately 3500 individuals by 2008 (Talukdar *et al*., 2008). The ban on sport hunting in the early 1900's and protection in National Parks were considered important factors in this increase (Talukdar *et al*., 2008). All GOH rhino live within National Parks, which have been vital to their survival, and the Kaziranga National Park in India is home to 70% of the global population. Attempts to increase the population in other National Parks are important because the population is vulnerable to a catastrophic event, such as a disease outbreak (Kaziranga National Park, 2017). Buffer zones surrounding the National Parks in Nepal were set out in 1996 in an attempt to provide additional habitat, encourage community participation in conservation, improve the economic benefits to the community and reduce human and GOH rhino conflict (WWF Nepal, 2006; Chitwan National Park, 2015).

Adult females and males are solitary except when dams are caring for calves, and adult males maintain overlapping territories of up to 8 km² (Pradhan *et al*., 2008). The GOH rhino is semi-aquatic, spending up to 80% of its time in water and feeds on floating and submerged vegetation in addition to grasses, fruit and leaves (Save the Rhino, 2017). The GOH rhino plays an important ecological role through providing grazing

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opportunities for smaller herbivores that rely on cropped foliage (Dinerstein, 2003) and by dispersing seeds and fruit of over 30 different plant species (Dinerstein and Price 1991; Steinheim *et al*., 2005).

Prior to the translocation analysed in this paper, 83 GOH rhino had been translocated from Chitwan National Park to Bardia National Park, both located in southern Nepal, between 1986 and 2003 (Chaudhary, 2016; Das Shrestha, 2016). Poaching of GOH rhino between 2002 and 2006 led to their decimation in Bardia National Park and only 25 remained in 2016 (Chaudhary, 2016). Translocations between National Parks are increasingly being used to prevent encroachment on human settlements and reduce the risk of species extinction (Sinha *et al*., 1993; Cedric *et al*., 2016). Translocations between these fragmented populations also reduce or slow the loss of genetic diversity and inbreeding (Armstrong & Seddon, 2008; Dinerstein, 2003). Despite GOH rhino numbers dropping to 60-80 individuals in 1962 in Chitwan National Park, heterozygosity levels remained high in 1990 (Dinerstein & McCracken, 1990; Rajshekhar *et al*., 2003).

Wildlife translocations may increase the risk of disease to the translocated animals and the recipient population (Sainsbury and Vaughan-Higgins 2012). Hazards include (i) source hazards that may be transferred to the destination to which destination populations are immunologically naive, (ii) destination hazards, parasites at the destination site to which the translocated animals are immunologically naive, (iii) carrier hazards, commensal infectious agents which trigger disease when stress compromises immune function (iv) transport hazards, novel infectious agents to which translocated animals are exposed during transportation (v) zoonotic hazards are carried by the translocated GOH rhino and of a potential risk to human health. Non-infectious hazards are also a risk and include exposure to toxins and physical injury risking the health and survival of the reintroduced population (Woodford & Rossiter, 1994; Cunningham, 1996; Leighton, 2002).

The IUCN recommends that a disease risk analysis is carried out prior to every translocation to determine the risks from disease and allow for mitigation measures (Jakob-Hoff *et al*., 2014). Several authors have set out methods of disease risk analysis suitable for wild animal translocations (Leighton, 2002; Miller, 2007; Sainsbury & Vaughan-Higgins, 2012; IUCN, 2013; Bobadilla-Suarez *et al.,* 2017). Following hazard identification, the framework most often followed in disease risk assessment is; release assessment, exposure assessment and a consequence assessment for each hazard (Sainsbury *et al*., 2012; Jakob-Hoff *et al*., 2014). A common approach is to use the scientific literature to evaluate the likelihood of release of the pathogen, the likelihood of animals at the destination becoming exposed to the pathogen and the consequences if exposure was to occur. Amalgamation of these three risk assessments allows the overall risk to be estimated. Management methods to reduce the risk from disease are evaluated to determine whether the risk can be reduced to the point where the benefits of translocation outweigh the costs. Scenario trees can be used as a visual framework to lay out the biological pathways of a hazard during a

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translocation and can be completed for the release, exposure and consequence assessment (Murray *et al*., 2004). To allow the problem of uncertainty in the disease risk assessment to be made explicit and transparent some authors choose to write a statement of uncertainty in an attempt to improve transparency, facilitate development of the assessment once new information emerges and allow readers to make their own decisions on the hazards (Hartley *et al*., 2012).

During this disease risk analysis the level of risk was categorised as being or high, medium, low or negligible risk to GOH rhino and other species as a result of the translocation. High risk was defined as a high risk of extinction due to significant population decline, or the mortality of a single human. Medium risk was defined as a population decline without the risk of extinction. Low risk was defined as individual mortality but without any effect on the overall population. Negligible risk was defined as no consequence occurring. The term likelihood was used to describe the likelihood that any of the translocated individuals were acting as a carrier of *Mycobacterium orygis* and were able to transfer it to another animal (Murray *et al*., 2004).

In this study we investigated the risk of disease from *Mycobacterium orygis* in translocating five GOH rhino from Chitwan National Park to Bardia National Park in 2016. This translocation was of concern because the first case of disease associated with *Mycobacterium orygis* in GOH rhino had been identified in Chitwan National Park in 2015 and further translocations of GOH rhino in Nepal are planned. The disease risk analysis was carried out after the translocation took place.

In 2015 a GOH rhino was found listless and depressed in Chitwan National Park and died the following day. It was necropsied and granulomatous lesions were discovered,, the only pathological changes identified. Spoligotyping and gene sequencing were used to identify the bacterium associated with these lesions as *Mycobacterium orygis* (Thapa *et al.,* 2016). *Mycobacterium orygis* (previously called *Oryx bacillus*), a bacterium which can chronically infect mammals, was only recently (in 2012) identified as pathogenic (van Ingen *et al*., 2012). The isolate of *Mycobacterium orygis* from the GOH rhino was found to differ from other *Mycobacterium orygis* isolates, including those previously identified in Nepal, in a single locus (MIRU 424) (Thapa *et al*., 2016). *Mycobacterium orygis* has been isolated from other Nepalese species including spotted deer (*Axis axis*), nilgai (*Boselaphus tragocamelus*), rhesus monkeys (*Macaca mulatta*), and domestic cattle and outside Nepal from Arabian oryx (*Oryx leucoryx*), waterbuck (*Kobus ellipsiprymnus*) and African buffalo (*Syncerus caffer*) (Pittius *et al*., 2012; van Ingen *et al.,* 2012; van Ingen *et al*., 2013; Thapa *et al*., 2015; Rahim *et al*., 2016). The case of *Mycobacterium orygis* associated disease in GOH rhino in Chitwan National Park was exceptional because (i) *Mycobacterium orygis* had previously only been detected in captive animals in South Asia (van Ingen, 2012; Thapa *et al*., 2017), (ii) *Mycobacterium orygis* has not previously been found in rhino species or other free-living Perissodactyl species (van Ingen, 2012) and should it spread to the remainder of the potentially immunologically naïve,

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isolated and vulnerable GOH rhino it could potentially have an impact on population numbers (Thapa *et al.,* 2016; Bardia National Park, 2017).

Our qualitative disease risk analysis was carried out using the method devised by Sainsbury & Vaughan-Higgins (2012) as modified by Bobadilla Suarez *et al.* (2017), including use of a scenario tree to visually explain the complex analysis, using the method described by Rideout *et al.* (2017) to predict the importance of a source hazard, and by carrying out an analysis of uncertainty as recommended by Murray *et al*. (2004), the first time this combination of methods has been used to assess the risk from disease in a wild animal translocation.

Materials and Methods

A qualitative disease risk analysis was carried out to determine the risk from disease associated with *Mycobacterium orygis* in undertaking the wild to wild translocation of three female (one approximately 12 months into her gestation) ("WWF", 2016a) and two male GOH rhino from Chitwan National Park to Bardia National Park in March 2016 and written in a logical, reasoned manner. Literature on the taxonomy, biology, ecology of GOH rhino and the epidemiology of mycobacterial diseases affecting this, and closely related, species was accessed using Science Direct, Web of Knowledge and Google Scholar. The translocation pathway was written in detail to identify the exact route and timing the GOH rhino took from their source to final destination and how they were captured and transported.

Geographical and ecological barriers

By assessing the geography of the two Parks, the land between them, the source of the GOH rhino and their ecology we thoroughly examined the possibility of geographical and ecological barriers crossed during translocation, and therefore whether *Mycobacterium orygis* was likely to be novel to the destination site or whether there was a possibility of parasite transfer between the two populations.

Disease risk assessment

A qualitative disease risk assessment was completed in four steps: release assessment, exposure assessment, consequence assessment, and risk estimation. In the release assessment we described how the GOH rhino would have been exposed and infected with *Mycobacterium orygis* and the likelihood that GOH rhino were infected at the time of release. In the exposure assessment we assessed the likelihood

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that GOH rhino and other species in Bardia National Park became exposed and infected by *Mycobacterium orygis* and the likelihood that infection disseminated through the populations in Bardia National Park. In the consequence assessment the biological, environmental and economic consequences of the effects of exposure and infection were assessed including the likelihood that disease occurred and the magnitude of the effects. Lastly the risk estimation combined the results from the release, exposure and consequence assessments to create an overall estimation of risk, which was described as being from negligible to high risk.

Scenario tree

A scenario tree was used to gain a graphical, logical and transparent depiction of the range and types of biological pathways involved in space and time as described by Murray *et al.* (2004). The scenario tree allows readers to visualise the chain of events and assist in the understanding of the analysis, in addition to aiding in identifying measures for risk management. This model was constructed by mapping the biological pathways possible for the release of *Mycobacterium orygis* into Bardia National Park.

Level of uncertainty

We measured the uncertainty in our disease risk analysis by assessing the quality of information available on the epidemiology of *Mycobacterium orygis* in free living GOH rhino and the number of documented cases of infection or disease in other species of rhino or captive GOH rhino. The quality of information was assessed for each stage of the disease risk analysis including the translocation pathway, geographical barriers, justification of hazard, release assessment, exposure assessment and consequence assessment.

Disease risk management

Management actions that were carried out during this translocation which would have reduced the risk of disease associated with *Mycobacterium orygis* were described. Other methods that potentially could have been applied to reduce the risk from disease were identified and evaluated for the benefit of any future translocations of GOH rhino between Chitwan National Park and Bardia National Park, and possibly other translocations of GOH rhino.

Disease risk communication

During the completion of the disease risk analysis the findings were discussed at each stage with the National Trust for Nature Conservation. On completion, the findings will be sent to all stakeholders involved in translocation of GOH rhino in Nepal: WWF Nepal, Nepal's Ministry of Forests and Soil Conservation, the Department of National Parks and Wildlife Conservation and the National Trust for Nature Conservation.

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Results

Translocation pathway

The source of the three female and two male, adult GOH rhino was the Chitwan National Park, located in south-central Nepal. Between the 1st and 5th of March 2016 the GOH rhino were translocated to their release site, Bardia National Park in south-west Nepal, 400km to the west of Chitwan National Park. The rhino were sedated with 3-4mg etorphine hydrochloride and 12-15mg acepromazine delivered by remote injection, a radio collar was attached and each rhino was loaded into a crate and the sedation reversed (Das Shrestha, 2016). The GOH rhino were individually transported directly to Bardia National Park by truck, the journey took nine hours without a break on route, and on arrival they were immediately released into the Park, without a period of quarantine ("WWF", 2016b; Sadaula, 2017). At the time of release there were approximately 25 GOH rhino in Bardia National Park and 601 in Chitwan National Park ("WWF", 2016c). The personnel involved in the translocation may have had contact with GOH rhino and other species in both Chitwan National Park and Bardia National Park (Sadaula, 2017).

Geographical and ecological barriers

The GOH rhino populations in the Chitwan National Park and the Bardia National Park are separated by 400km of land, including mountains, rivers and human settlements and a stretch of uninhabited natural wilderness that GOH rhino could potentially cross. The Parks are not fenced, but water bodies and mountain ranges mark the boundaries to the National Parks with the Rapti and Narayani Rivers bordering the north and west boundaries of the Chitwan National Park ("Chitwan National Park", 2015) and the Bardia National Park bordered by the Karnali River to the west and the Siwalik Hills to the north. Human settlements also surround the borders of the National Parks ("Bardia National Park", 2017). GOH rhino were originally distributed across the whole of Nepal and rivers do not act as a barrier to their distribution because they are successful swimmers (Hutchins & Kreger, 2006; Subedi *et al*., 2013). In addition, human settlement does not create an unbroken physical barrier between the Parks. Both National Parks are tourist attractions, so tourists could potentially act as a carrier of parasites between the Parks. Likewise translocations of other species between the Parks do occur, so animals, Park staff and vehicles act as a potential route for parasite transfer, in addition birds migrating between the ParksThe Parks are not fenced and there are no biosecurity measures to prevent parasite transfer between the Parks (Sadaula, 2017). Both Parks are managed by The National Trust for Nature Conversation and Trust workers could transfer parasites (Sadaula, 2017).

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The GOH rhino population in Bardia National Park were either originally from Chitwan National Park or are descendants of GOH rhino from Chitwan, as GOH rhino were absent from Bardia until their re-introduction from Chitwan in 1986. Therefore there is a medium likelihood that the two populations harbour similar compliments of parasites.

We conclude that direct contact and parasite transfer between GOH rhino in the two populations (Chitwan National Park and Bardia National Park) is possible. However the lack of competition between GOH rhino for resources in the Parks due to the low population densities, the distance between the populations and the fact that GOH rhino sightings outside the reserves are rare, suggests that the free movement of GOH rhino between the Parks is very unlikely. Although there is movement of animals and humans between the Parks and both populations of GOH rhino are from the same source, we cannot rule out differences in parasite complement between the populations. Therefore we conclude there is a low likelihood of geographical and ecological barriers between the two Park populations.

Disease risk analysis for Mycobacterium orygis in translocating GOH rhino from Chitwan National Park to Bardia National Park in 2016

If geographical and ecological barriers are present between Chitwan and Bardia National Parks, *Mycobacterium orygis* would be a source hazard (Sainsbury and Vaughan-Higgins 2012), while if the barriers were absent it would be designated a carrier hazard. The disease risk analysis has been carried out for *Mycobacterium orygis* as a source hazard (Table 1) and a carrier hazard (Table 2).

Table 1 - Disease risk analysis for *Mycobacterium orygis as a source hazard*

Justification of hazard A confirmed case of disease associated with *Mycobacterium orygis* was identified in a female GOH rhino in February 2015 in Chitwan National Park, and there have been no known cases of the disease in Bardia National Park. Although screening for *Mycobacterium orygis* has never been carried out (Thapa *et al.,* 2016; Sadaula, 2017) in either National Park, the distribution of *Mycobacterium orygis* may be localised to Chitwan National Park and other areas within Nepal. There is a low likelihood of geographical and ecological barriers to parasite transmission between the source and destination populations, as described in the text, and therefore we have evaluated *Mycobacterium orygis* as a source hazard.
Release assessment for Mycobacterium orygis as a source hazard GOH rhino are exposed to *Mycobacterium orygis* from other infected wild mammals. GOH rhino become infected with *Mycobacterium orygis* via (i) inhalation of airborne *Mycobacteria* carried on droplets expelled from an infected animal and (ii) ingestion from contaminated food or water, (iii) percutaneous transmission,

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through breaks in the mucosal membrane, (iv) via fomites, (v) venereal transmission and (vi) vertical transmission; calves becoming infected via ingestion of contaminated milk (Coggin, 2006; Kramer *et al*., 2006; McVey *et al*., 2013) (Figure 1).

During necropsy of the GOH rhino found dead, several encapsulated granulomatous lesions were observed in the lungs, on examination of the other organs no other lesions were found (Thapa et al., 2016). The risk of pathogen release is minimal compared to if numerous, widespread, open granulomatous lesions had been found on multiple organs. Evidence suggests the exposure of GOH rhino to *Mycobacterium orygis* and susceptibility to infection are both low: infection and disease associated with *Mycobacterium orygis* has only been identified in one GOH rhino in Chitwan National Park, despite their relatively high density in Chitwan (601 individuals in 932km²), while no artiodactyls have been found infected in the same Park (Thapa *et al.,* 2017). The susceptibility of Rhinocerotidae to infection and disease from other *Mycobacterium* species is apparently low, judging by the sporadic cases of *Mycobacterial* disease reported in these species, for example only one case of *Mycobacterium bovis* infection in an adult black rhino in the Kruger National Park, South Africa, had been reported by 2016, despite *Mycobacterium bovis* - associated disease having been present in other species in the Park since 1950-1960 (Michel *et al*., 2006; Miller *et al*., 2016; Miller *et al*., 2017). A second reported case of *Mycobacterium bovis* infection in free-ranging black rhino was detected in Mkuzi game reserve in South Africa (Michel *et al*., 2006; Espie *et al*., 2009). On the assumption that GOH rhino have as low a susceptibility to infection and disease from *Mycobacterium orygis* as the African rhino species have to *Mycobacterium bovis* then the likelihood of infection in the translocated GOH rhino is low. In addition, tuberculosis testing has been completed on GOH rhino at the source and destination sites with the DPP VetTB test, with no individual testing positive for infection, however this test has not been validated in rhino and is unreliable at detecting the early stages of disease (De Lisle *et al*., 2002; Sadaula, 2017). Therefore, the likelihood of a released GOH rhino being infected with *Mycobacterium orygis* is low.

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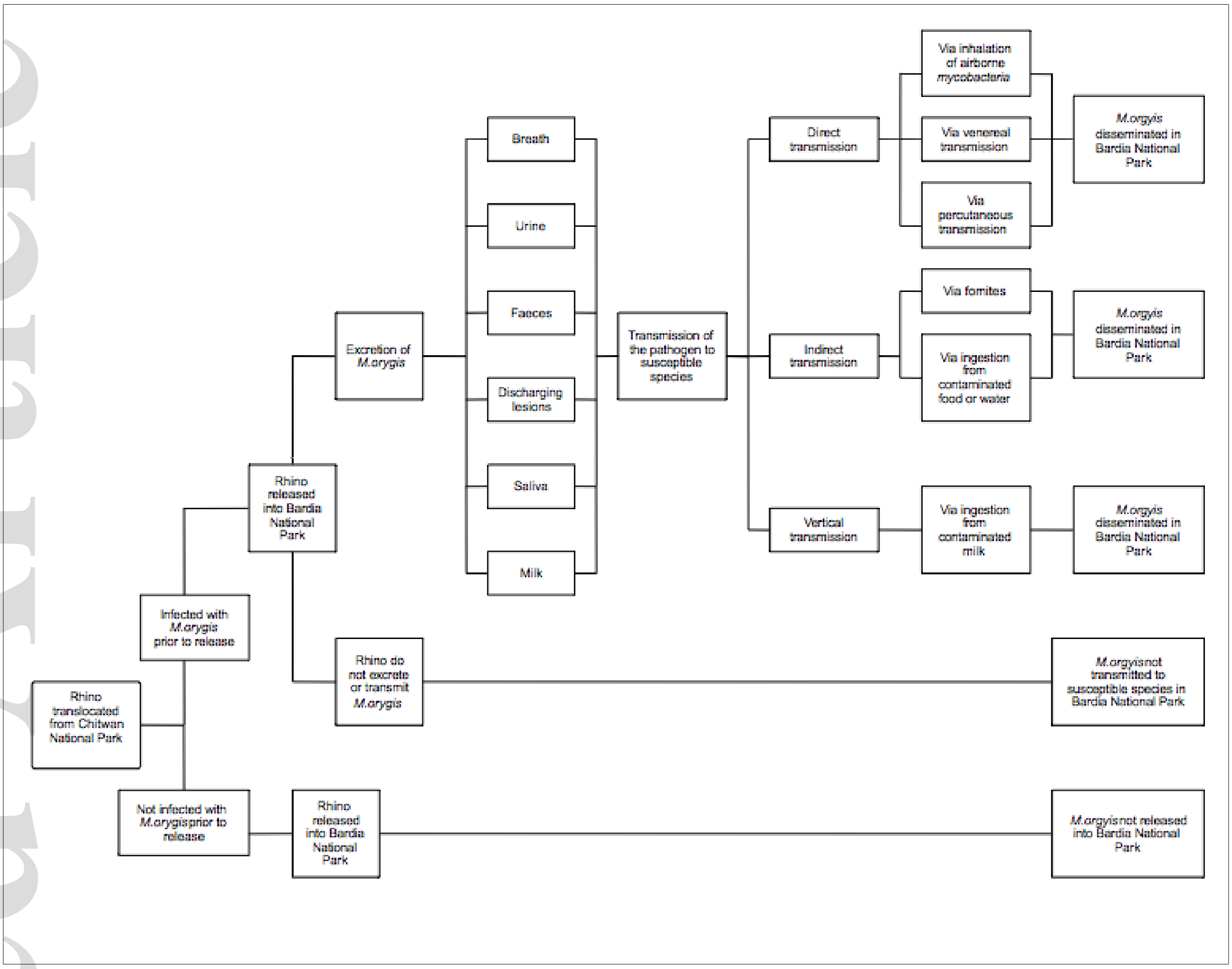

Figure 1: A scenario tree displaying the pathways for release, exposure and dissemination of *Mycobacterium orygis* in Bardia National Park through greater-one horned rhino translocation from Chitwan National Park.[1]

Exposure assessment for Mycobacterium orygis as a source hazard

GOH rhino and other susceptible species already present in Bardia National Park may be exposed to *Mycobacterium orygis* via direct and indirect transmission from infected released animals by (i) air droplets, which are inhaled by the susceptible animals, and once the *Mycobacteria* reach the pulmonary alveoli they invade and replicate within endosomes (McVey *et al*., 2013), (ii) ingestion, (iii) percutaneous transmission, through breaks in the mucosal membrane, (iv) via fomites, where the pathogen can have a survival time of over three months (Kramer *et al*., 2006), (v) venereal transmission or through (vi) vertical transmission; calves becoming infected via ingestion of contaminated milk (Coggin, 2006). The outcome of an infection with *Mycobacterium orygis* depends on the host's immune response, the dose of infection, whether the

1 Fomite: An inanimate object that is capable of transmitting infectious organisms from one individual to another

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infection occurs repeatedly or is a one off and the genetic strain. Male and female GOH rhino are equally susceptible to infection but males are more likely to meet and have close contact with other GOH rhino to fight over territory or mates and so may be more likely to be exposed (Laurie, 1982). GOH rhino calves have a higher likelihood of exposure than other age groups if their dam is infected. Other susceptible species and possible carriers of infection in Bardia National Park include domestic cattle (*Bos taurus*), spotted deer (*Axis axis)*, nilgai (*Boselaphus tragocamelus*)*,* buffalo (*Bubalus arnee*) and rhesus monkeys (*Macaca mulatta*) (van Ingen *et al.,* 2012; Thapa *et al*., 2015; Rahim *et al*., 2016) and the number of susceptible animals from these species is numerous and their density is high ("Chitwan National Park", 2015). Therefore there is high likelihood that other animals will be exposed at the destination.

The likelihood of dissemination of *Mycobacterium orygis* within the GOH rhino population and other species in Bardia National Park is high due to (i) the large number of susceptible species in the Park (Duffield & Young, 1985; Kramer et al., 2006) and (ii) the resistant nature of *Mycobacterium* tuberculosis complex in the environment, for up to three months, which allows for a persistent source of infection even if the population size of the hosts drop below the threshold for animal to animal transmission (Duffield & Young 1985, Public health agency, 2010; Gog et al., 2002; Kramer et al., 2006, Fine *et al.*, 2011). However, a wildlife maintenance host is required in order for *Mycobacterium orygis* to persist in the ecosystem, and in the absence of any known maintenance host in Bardia National Park the likelihood of persistence is reduced.

Consequence assessment for Mycobacterium orygis as a source hazard

The site of predilection of pathogenic mycobacteria is the lung tissue (McVey *et al*., 2013). The strength of the individual's immune response towards the pathogen will determine whether the infection spreads to other organs in the body or whether it remains contained within the lungs, and in the latter situation, the animal may not show clinical signs (Bercovier & Vincent, 2001). The clinical signs of disease associated with the Mycobacterium tuberculosis complex vary depending on the species of *Mycobacterium* and the species of the host, but include weakness, depression, anorexia, weight loss, coughing and dyspnoea, which may not appear until the advanced stages of the disease (WHO, 2010). On the basis of the single case of disease associated with *Mycobacterium orygis* in GOH rhino, the infection is capable of leading to severe disease and death.

Other species present in Bardia National Park that are known to be susceptible to infection by the *Mycobacterium tuberculosis* complex include elephants (*Elephas maximus*) (endangered), blackbuck (*Antilope cervicapra*) (near threatened), gaur (*Bos gaurus*) (vulnerable), hog deer (*Axis porcinus*) (endangered) barking deer (*Muntiacus muntjak*) (least concern), sambar deer (*Cervus unicolor*) (vulnerable) and the Bengal tiger (*Panthera tigris tigris)* (endangered) (Rao & Acharjyo, 1992; Priya *et al*., 2014).

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Mycobacterium tuberculosis has been confirmed to cause mortality in wild Asian elephants (Zachariah *et al*., 2017), hog deer, barking deer and sambar deer (Rao & Acharjyo, 1992). *Mycobacterium tuberculosis* has been recorded in captive but not wild gaur and blackbuck (Ahasan & Rahaman, 2007; Podhade *et al*., 2013). Tuberculosis has never been observed in free living Bengal tigers, however a case of *Mycobacterium avium* associated disease was identified in a captive Bengal tiger (Cho *et al*., 2006). Bengal tigers do not prey on adult GOH rhino, but will prey on other susceptible species in the Park including buffalo or deer, and the transmission of the parasite to the tiger's prey species will result in a medium risk of the tigers becoming infected. In an analogous situation African lions (*Panthera leo*) in Kruger National Park, became infected with *Mycobacterium bovis* harboured by African buffalo (*Syncerus caffer*) which resulted in the decline of both lion and buffalo numbers (Ferreira & Funston, 2010). The risk that Bengal tigers will become infected with *Mycobacterium orygis* through routes other than consuming infected prey is low. Other endangered species that might be affected should *Mycobacterium orygis* be introduced into the Park include; barasinga or swamp deer (*Rucervus duvaucelii*) (vulnerable), leopard (vulnerable), snow leopard *(Panthera uncia*) (endangered) and the striped hyena (*Hyaena hyaena*) (near threatened).

There is a low likelihood that disease associated with *Mycobacterium orygis* will make susceptible species vulnerable to stochastic events because mycobacterial disease weakens the immune system, increasing the vulnerability to other diseases, including, for example, canine distemper, which is sporadically observed in tigers and leopards (*Panthera pardus fusca*) and elephant herpesvirus, and diseases associated with these agents have a high mortality rate (Deem *et al*., 2000; Goodrich *et al*., 2008; Schaftenaar *et al*., 2010). Deaths from disease associated with *Mycobacterium orygis* may lead to (i) a reduction in genetic diversity and lowered ability to adapt to future stochastic events and (ii) reduced population growth rates (Leberg, 1993). At the time of translocation the small population of 29 GOH rhino, with a potentially small gene pool, in Bardia National Park had a high probability of failure to adapt. The small global GOH rhino population (WWF, 2017) was considered to have a high species genetic variation in 1990 (Dinerstein & McCracken, 1990).

The introduction of *Mycobacterium bovis* into the southern Kruger National Park between 1950 and 1960 led to a high disease prevalence , but the disease has had little effect on the overall population numbers of other herbivores species in the Park found to be infected with *Mycobacterium bovis* (Michel *et al*., 2006; Rodwell *et al*., 2001). A reduction in the number of herbivores will result in a reduction in prey for the predators and a shift in grazing patterns resulting in a change in flora growth and diversity which could result in impacts on flora and smaller animals (Olff & Ritchie, 1998). Assuming that a similar pattern, to that seen in Kruger National Park, is likely to occur following the introduction of *Mycobacterium orygis* into Bardia National Park, there is a negligible likelihood that *Mycobacterium orygis* will decrease the biodiversity within the Park in the long term.

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Compensatory reproduction has prevented a decline in numbers of European badgers (*Meles Meles*) which harbour *Mycobacterium bovis* (McDonald *et al*., 2016). The ability of mycobacteria to persist in reservoir species and the environment potentially enables the pathogen to persist until one or more hosts become extinct (McCallum & Dobson, 1995; zu Bentrup & Russell, 2001). The presence of a suitable reservoir host is a requirement for M.orygis to persist, this requirement further lowers the risk of the translocation. However there have been no cases of species extinction as a result of *Mycobacterium* spp disease, and the likelihood that disease associated with *Mycobacterium orygis* results in extinction is considered negligible.

Mycobacterium orygis has been isolated from people in Asia, and transmission from people to cattle has been documented (Dawson *et al*., 2012; Thapa *et al*., 2017). The difficulty in detecting the bacterium and the potential for misidentification may have led to an artificially low number of reports (Thapa *et al.*, 2017). *Mycobacterium orygis* has not been associated with disease in people and therefore the of disease in humans associated with translocation of GOH rhino is considered very low. There is a very low likelihood that the release of GOH rhino infected with *Mycobacterium orygis* into Bardia National Park will be associated with disease in people (Dawson *et al*., 2012). The Park is surrounded by human settlements, and people who live and farm livestock within the buffer zone may be in contact (Michel *et al*., 2006). There was an estimated 32,000 cases of *Mycobacterium tuberculosis* cases in 2016 in Nepalese people resulting in 6,500 deaths (WHO, 2015). The release of ~~another~~ *Mycobacterium tuberculosis* strains into the population carries a lower consequential risk to the public compared to releasing the infectious agent into a tuberculosis free area (NTC, 2017).

There is a very low likelihood of economic consequences due to (i) a reduction in tourism because of public concern leading to a reduction in funding of the protection and management of the Park, including anti-poaching, and result in a loss of jobs (ii) a reduction in income for local people which may result in a transition to unfavourable methods to gain income, including poaching (Studsrod & Wegge, 1995). The economic consequences of the introduction of *Mycobacterium orygis* into Bardia National Park are very low because *Mycobacterium tuberculosis* is already present in this region of Nepal. It is noted that an eradication programme would have a high economic consequential risk, for example the cost of attempted eradication of *Mycobacterium bovis* in the UK between 2004 and 2014 was £500 million (DEFRA, 2014), and methods to attempt to rid tuberculosis from Kruger National Park, including testing and culling infected animals, has proved costly and time consuming (De Vos *et al*., 2001).

The likelihood that at least one animal will become infected with *Mycobacterium orygis* is medium. There is a low likelihood that the introduction of *Mycobacterium orygis* into Bardia National Park and the subsequent maintenance of the organism will cause (i) mortality resulting in a decline in susceptible species populations, the loss of genetic diversity and (ii) failure of this translocation, and subsequent translocations of GOH rhino,

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reducing GOH rhino viability in Nepal. There is a negligible likelihood that the introduction of *Mycobacterium orygis* into Bardia National Park will cause extinction of one of the susceptible species present.

Risk estimation for Mycobacterium orygis as a source hazard

The likelihood of the release of *Mycobacterium orygis* from translocated GOH rhino is low, the risk of exposure of GOH rhino and other species in Bardia National Park to *Mycobacterium orygis* is high, there is a high risk of dissemination within the Park, and there is a low likelihood of mortality and decline of susceptible species and failure of the translocation of GOH rhino. Overall the risk estimation for *Mycobacterium orygis* as a source hazard is considered to be low. The criteria set out by Rideout *et al*. (2017), to evaluate the relative risk of source hazards, supported *Mycobacterium orygis* being of relatively high risk compared to other parasites because (i) it is a microparasite, has a relatively short generation time and rapid evolution, (ii) it is a generalist parasite able to invade a diverse array of (reservoir) hosts and (iii) it persists relatively long term in the environment.

Level of uncertainty

Information on the natural history, biology and epidemiology of *Mycobacterium orygis* was limited in all species, with less than ten documented cases of *Mycobacterium orygis* associated disease being discovered in free living wildlife and less than twenty documented cases of *Mycobacterium orygis* in captive wildlife. *Mycobacterium orygis* has never been identified in any species of captive rhino.

Mycobacterium bovis, Mycobacterium avium and *Mycobacterium tuberculosis* were found to be the only other *Mycobacterium* species identified in rhino species, with *Mycobacterium avium* only isolated in captivity (Byrant *et al*., 2012). Apart from the case of *Mycobacterium orygis* associated disease described above, no other documented cases were found of *Mycobacterium tuberculosis complex* infection in either free living or captive GOH rhino.

In interpreting the release and exposure assessments, we were heavily reliant on predicting the epidemiology of *Mycobacterium orygis* on the basis of our understanding of other *Mycobacteria* including *Mycobacterium tuberculosis, Mycobacterium bovis* and *Mycobacterium avium*, specifically in the susceptibility to infection, in the transmission of the bacterium between animal populations at the destination and the effect of *Mycobacterium orygis* on host immunity. An improved understanding of the epidemiology in the future would improve the transparency of this assessment.

Risk management for Mycobacterium orygis as a source hazard

If evidence continues to suggest that Bardia National Park is free of *Mycobacterium orygis* then it would be

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preferable for GOH rhino free of the infection and disease to be translocated into Bardia National Park. Clinical signs of the disease, including coughing, sneezing, nasal discharge, fever and respiratory issues, identify infected individuals in the later stages of the disease (Valandikar & Raju, 1996) but such sick animals are already prevented from translocation. Therefore, a prevention strategy will require testing for the presence of *Mycobacterium orygis.* Testing and monitoring of *Mycobacterium tuberculosis complex* is difficult due to long infectious and incubation periods (Cleaveland *et al*., 2005). Survey tuberculosis testing has been completed on GOH rhino in both Chitwan and Bardia Parks using the Dual Path Platform (DPP) VetTB test (which detects antibodies to *Mycobacterium bovis* and *Mycobacterium tuberculosis* (Chembio, 2014; Miller *et al*., 2015) and all rhino tested negative, however the ante-mortem detection of tuberculosis is often unreliable (De Lisle *et al*., 2002; Sadaula, 2017). GOH rhino infected with *Mycobacterium orygis* may produce a positive reaction to an intradermal tuberculin test (Pittius *et al*., 2012), which could be followed by PCR or gene probe tests on blood or biopsies to distinguish between different *Mycobacterium* strains (Lyashchenko *et al*., 2008). The intradermal tuberculin test is the most commonly used test to determine whether an individual is exposed to *Mycobacteria* spp. Other available tests include an ELISA assay, which proved accurate and reproducible in identifying *Mycobacteria* spp infection in elephants (Mikota & Maslow, 2011) and the rapid (immunochromatographic) test (RT) used for detecting *M.tuberculosis* in elephants and *M.interjectum* in pgymy hippopotamus (*Hexaprotodon liberiensis*) (Bouts *et al.*, 2009; Lyashchenko *et al.,* 2006). Tests successful in identifying *Mycobacterium* spp*.* in rhinos include the IFN-γ based ELISA designed specifically for detecting *Mycobacterium bovis* infection in white rhino (Morar *et al.*, 2007) and the Elephant TB Stat-Pak Assay and the Multi-Antigen Print Immunoassay (Mapia) which have successfully detected *M.tuberculosis* in black rhinos (Duncan *et al*., 2009). Ante-mortem diagnosis has proved difficult in other Perissodactyla including tapirs (*Tapirus* spp*),* where a combination of tests is most commonly used in captive animals including the tuberculin skin test, nasal and gastric washes and radiographs (Mangini *et al*., 2012). The inaccuracy of *Mycobacterium* tests, the expense of completing multiple tests and the practical problems of testing free-living wild animals prior to translocation affect the feasibility of this prevention strategy.

Quarantine of translocated GOH rhino at the destination, and repeated testing for *Mycobacterium orygis,* would reduce the risk of release of *Mycobacterium orygis* infected individuals. However, the incubation period for tuberculosis in rhino might be many months, and quarantine periods used in captive elephants have been up to one year (Elephant TAG, 2012; Simpson *et al*., 2017). Quarantine enclosures would be expensive and their use would increase stressors on translocated rhino. Given the difficulty of implementing feasible measures of risk management, gathering evidence on the presence or absence of *Mycobacterium orygis* in Bardia National Park is crucial to future decision making.

Table 2 - Disease risk analysis for *Mycobacterium orygis as a carrier hazard*

Justification of hazard

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A carrier hazard is a commensal organism that causes disease when stressors reduce immunocompetence and alter the host–parasite relationship (Bobadilla Suarez *et al*., 2017). *Mycobacterium orygis* may be present in GOH rhino, or other species, in both Chitwan National Park and Bardia National Park. Translocated GOH rhino, infected with *Mycobacterium orygis*, will be subjected to stressors which may precipitate disease.

Release assessment for Mycobacterium orygis as a carrier hazard

The likelihood that GOH rhino translocated to Bardia National Park will be exposed to and infected with *Mycobacterium orygis* is low for the same reasons stated in Table 1.

Exposure assessment for Mycobacterium orygis as a carrier hazard

There is a low likelihood that translocated GOH rhino will already be exposed and infected with *Mycobacterium orygis*. There is a high likelihood that resident GOH rhino and other susceptible species already present in Bardia National Park will be exposed to *Mycobacterium orygis* via direct transmission from infected released animals, and the infection will disseminate through susceptible populations at Bardia National Park through the mechanisms stated in Table 1.

Consequence assessment for Mycobacterium orygis as a carrier hazard

Evidence demonstrates that translocations are a stressor for mammals and reduce the competence of the immune system (Dickens *et al.,* 2010) and increase the risk from disease in the destination (Reeder & Kramer, 2005). The strength of the immune response is important in determining the outcome of infection with *Mycobacteria* spp and immune-suppression can increase the likelihood that *Mycobacterium orygis* will be pathogenic (Sheridan *et al*., 1994), and infection may spread systemically more quickly and give rise to clinical signs more rapidly in individuals with lowered competence (Bercovier & Vincent, 2001). Stress as a result of translocations also slows down the glucocorticoid response, alters the cardiac output and behavioural coping ability, resulting in reproductive suppression, altered metabolism and reduced fight or flight response all of which may contribute to the failure of the translocation (Parker *et al.*, 2012). Stress is evident in translocations through a change in stress-response physiology which includes the secretion of glucocorticoids from the adrenal glands and the release of corticosterone, and the persistent series of stressors involved such as handling, transport and release can give rise to chronic stress (Dickens *et al*., 2009; Parker *et al.*, 2012). However, rhino translocation in Nepal has a high success record, with over 80 GOH rhinos being translocated between National Parks in Nepal since 1986 resulting in the births of over 27 calves, the decline in their numbers once translocated being as a result of poaching (Kafley *et al.*, 2015). Therefore there is a low likelihood that stressed translocated GOH rhino will suffer disease associated with *Mycobacterium orygis* infection and that the translocation will fail.

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Disease risk management for Mycobacterium orygis as a carrier hazard

Methods to reduce stress levels in translocated GOH rhino are important.

Risk estimation for Mycobacterium orygis as a carrier hazard

The likelihood of *Mycobacterium orygis* release is low, the likelihood of exposure and dissemination high and the likelihood of disease in GOH rhino and failure of the translocation low. The overall risk is low.

Level of uncertainty

As noted above in Table 1 knowledge of the natural history, biology and epidemiology of *Mycobacterium orygis* is limited. Our understanding of the pathogenesis of *Mycobacterium orygis* in stressed rhino is also poor and studies of the pathogenesis of the disease in translocated rhino would reduce the level of uncertainty of this DRA.

Disease risk communication

One of the authors, Amir Sadaula, from the National Trust for Nature Conservation provided detailed information for the disease risk analysis including the quarantine procedures during translocation, the tuberculous test used, the translocation pathway and the personnel involved in the translocation.

The completed report will be circulated to WWF Nepal, Nepal's Ministry of Forests and Soil Conservation, the Department of National Parks and Wildlife Conservation and the National Trust for Nature Conservation to enable them to use this disease risk analysis to assess future risks of disease during GOH rhino translocations and improve conservation outcomes.

Discussion

This report has described a disease risk analysis for the translocation of five GOH rhino from Chitwan National Park to Bardia National Park. The parasite analysed in this assessment was the source and carrier hazard *Mycobacterium orygis.* The overall risk from disease associated with *Mycobacterium orygis*, as either a source or carrier hazard, as a consequence of GOH rhino translocation, was evaluated as low. The known low susceptibility or high resistance of Rhinocerotidae to *Mycobacterium tuberculosis complex* had a

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strong influence on this evaluation. However, since mycobacteria are generalist parasites, *Mycobacterium orygis* is likely to establish at the destination, Bardia National Park, if introduced with translocated GOH rhino, because generalist parasites are more successful at establishing themselves in a novel environments than host specific parasites on account of their diverse range of susceptible hosts (McVey *et al*., 2013).

The criteria described by Rideout *et al*. (2017) to differentiate the risk from source hazards suggested that *Mycobacterium orygis* was of relatively high risk of leading to an outbreak of disease in the destination populations. If multiple source hazards are analysed concurrently during disease risk analysis this method may help to clarify differential risk from disease. The scenario tree provided a visual tool to decipher the release assessment.

A desirable method of reducing risk of a source hazard would include testing of GOH rhino for exposure to mycobacteria, quarantine while test results are awaited, followed by the translocation of GOH rhino apparently free of mycobacterial infection. The tests available are unreliable and the procedures would be stressful, and therefore while *Mycobacterium orygis* remains apparently widespread in the environment in Nepal, such prevention and control methods appear counterproductive. Until diagnostic methods improve, the best strategy for prevention of disease in rhino due to mycobacteria would appear to be to use best practice translocation to reduce stressors, while at the same time monitoring health and disease carefully. Health surveillance would best include testing any live GOH rhino, examined for other purposes, for mycobacterial infection, and considering mycobacterial disease in the differentials when carrying out pathological examination of GOH rhino found dead. Vaccinating rhino in Bardia National Park is not an option because a specific vaccine is not available. Given that GOH rhino have already been translocated to Bardia National Park with a source hazard, *Mycobacterium orygis*, diligent monitoring of the health of the GOH rhino population in Bardia would be advisable. This disease risk analysis would best be regularly updated using the results of surveillance. The risk of disease in rhino in Bardia National Park would likely be increased by interactions with neighbouring livestock and communities and further translocations of any ungulates into the Park for conservation purposes, and these management practices must be carefully considered.

Cases of *Mycobacterium* tuberculosis complex infection and disease in perissodactyls appear to be sporadic and may occur as a spillover event. For example, isolated reports of tuberculosis in wild rhino in South Africa at the same time as an outbreak of disease associated with *Mycobacterium bovis* in buffalo (Espie *et al*., 2009; *et al*., 2016). Cases in tapir are restricted to one documented report of *Mycobacterium pinnipedii* infection in a Malayan tapir (*Tapirus indicus*) (Moser *et al*., 2008) and less than ten of

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Mycobacterium bovis infection in captive tapir (Durr *et al*., 2000; Pavlick *et al*., 2002). Horses have been described as being highly resistant to *Mycobacterium* infections (O'Reilly & Daborn, 1995; Pavlik *et al.*, 2004; Pavlik *et al*., 2008) and *Mycobacterium avium* and to less of an extent *Mycobacterium bovis* have been isolated from sporadic cases (Keck *et al.*, 2010; Hamzah, 2013). The GOH rhino in Chitwan National Park is the only documented case of *Mycobacterium orygis* infection in perissodactyls suggesting that perissodactyls, including rhino, have a relatively low susceptibility to infection.

Completing disease risk analysis retrospectively rather then prior to the translocation offers many benefits including the ability to analyse the true incidence rates and risks rather than predicting the outcome, this also allows unanticipated outcomes to be measured and prevents selection bias, however disease risk analysis completed prior to translocation would allow for changes to be made to reduce risk. Ideally a disease risk analysis would be completed both pre and post translocation.

In conclusion this report describes the disease risk analysis conducted on Mycobacterium orygis as a source and carrier hazard during the translocation of five GOH rhino using a modified method of disease risk analysis for conservation translocations. Completing disease risk analyses for conservation translocations and studying the outcome for the health of the populations at the destination may lead to improved methods of DRA and an understanding of which translocations to avoid.

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