

Tuberculosis in Rhinoceros: An Underrecognized Threat?

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Summary

Historical evidence of tuberculosis (TB) affecting primarily captive rhinoceroses dates back almost two centuries. Although the causative *Mycobacterium tuberculosis* complex (MTC) species has not been determined in many cases, especially for those that occurred before bacterial culture techniques were available, the spectrum of documented reports illustrates the importance of TB as cause of morbidity and mortality in different rhinoceros species across continents. In more recent years, sporadic suspected or confirmed cases of TB caused by *Mycobacterium bovis* (*M. bovis*) have been reported in semi-free or free-ranging rhinoceroses in South Africa. However, the true risk TB may pose to the health and conservation of rhinoceros populations in the country's large conservation areas where *M. bovis* is endemic, which is unknown. Underlying the current knowledge gap is the lack of diagnostic tools available to detect infection in living animals. As documented in other wildlife species, TB could establish itself in a rhinoceros population but remain unrecognized for decades with detrimental implications for wildlife conservation at large and should such animals be moved to uninfected areas or facilities. This paper reviews the current state of knowledge regarding TB in rhinoceros including critical gaps that need to be addressed to effectively assess the threat that this disease may present to rhinoceros.

Keywords: Ceratotherium simum; Dicerorhinus sumatrensis; Diceros bicornis; mycobacteriosis; rhinoceros; Rhinoceros unicornis; tuberculosis

Introduction

Tuberculosis (TB) affects many free-ranging and captive wildlife species and presents a potential threat to conservation efforts. Detection of infection often occurs only after development of chronic, debilitating disease when clinical signs become apparent (Bengis, 1999). Susceptibility to infection, pathogenesis and impact on the affected population differs widely between species, although relatively little is known for most wildlife species (Michel et al., 2006; Pesciaroli et al., 2014). *Mycobacterium bovis* (*M. bovis*) and *Mycobacterium tuberculosis* (*M. tuberculosis*) have been shown to cause morbidity and mortality in rhinoceros species (Stetter et al., 1995; Rookmaaker et al., 1998; Espie et al., 2009). Rhinoceros are iconic species that have been exported to zoos worldwide and translocated for conservation programmes. However, large knowledge gaps exist in understanding susceptibility, transmission, diagnosis, development of disease and management of infected animals. Although TB has not been shown to be an immediate threat to free-ranging rhinoceros populations, the potential impact is currently unknown. Kruger National Park (KNP) and iMfolozi-Hluhluwe Game Reserves (HiP), KwaZulu Natal, South Africa, have large numbers of both white (*Ceratotherium simum*) and black (*Diceros bicornis*) rhinoceros which share range and resources with *M. bovis*-infected buffalo populations, which are important maintenance hosts for this disease (Michel et al., 2006; Fitzgerald and Kaneene, 2013). A fatal case of *M. bovis* infection in a recently translocated black rhinoceros on a private game ranch in South Africa (M. Otto, pers. comm. 2014) emphasizes the importance of developing accurate methods of detection suitable for surveillance and early diagnosis.

TB in rhinoceros may have other consequences associated with zoonotic risks and regulatory constraints as a notifiable disease. In the event of *M. bovis* being diagnosed in a rhinoceros, regulatory interventions could result in quarantine of a zoo, park, or game ranch and restrictions placed on further movements of rhinoceros. In response to the international demand for TB diagnostic tests in wildlife, tools such as the STAT-PAK assay (Lyashchenko et al., 2008; Duncan et al., 2009) and rhinoceros-specific interferon-gamma (IFN-gamma) assay have been recently developed (Morar et al., 2013). However, these tests need to be validated in known infected rhinoceros before they can be used reliably for diagnostic purposes and risk assessments. At the same time, knowledge of the pathogenesis of this multistage disease in rhinoceros is a prerequisite for understanding the risk of contracting, developing disease and transmitting the infection. Ultimately, gaining knowledge on a few elementary disease determinants constitutes the key to successfully managing a slow, progressive disease such as TB without over- or under-estimating the necessity for control measures. This study reviews the current state of knowledge of TB both in captive and free-ranging rhinoceros and identifies critical gaps required to address the threat that this disease presents to rhinoceros.

Historical Case Reports of TB in Rhinoceros

Although there is very limited information for the presence of infection in free-ranging rhinoceros due to logistical and technical constraints, TB in captive rhinoceros held in zoological collections dates back to the late 1800s (Table 1). The popularity of the different

rhinoceros species for exhibition led to their distribution worldwide, which often required long transport in close contact with humans.

Table 1. Summary of TB cases in rhinoceros

Date	Location	Rhinoceros species	MTC species	Key finding
1879	Zoo – England	Sumatran rhinoceros	Unknown	Died; TB in lung, spleen
1892	Zoo – Poland	Black rhinoceros	Unknown	Died of TB
Circa 1900	Zoo – India	Sumatran rhinoceros	Unknown	Died; TB in lung, liver
1910	Zoo – Austria	Sumatran rhinoceros	Unknown	Died; TB in lung
1941	Zoo – England	Indian rhinoceros	Unknown	Died of TB
1944	Zoo – Germany	Black rhinoceros	Unknown	Died of TB
1956	Zoo – South Africa	Black rhinoceros	Unknown	Died of TB
1957	Zoo – Germany	Black rhinoceros	Unknown	Died of TB
1961	Zoo – Japan	Black rhinoceros	Unknown	Died of TB
1969	Zoo – Czech Republic	Black rhinoceros	Unknown	Died of TB
1970	Free-ranging – South Africa	Black rhinoceros	<i>M. bovis</i>	
1978	Zoo – USA	Black rhinoceros	<i>M. bovis</i>	Ante-mortem diagnosis
1979	Zoo – USA	Black rhinoceros	<i>M. bovis</i>	Ante-mortem diagnosis
1984	Zoo – USA	Black rhinoceros	Unknown	Died of TB
Early 1990s	Free-ranging – South Africa	Black rhinoceros	Unknown	Died; TB lesions
1990	Zoo – USA	Black rhinoceros	<i>M. tuberculosis</i>	
1991	Zoo – USA	White rhinoceros	<i>M. bovis</i>	Died; pulmonary TB
1992	Zoo – India	Black rhinoceros	<i>M. tuberculosis</i>	Died; pulmonary TB
1994	Zoo – India	Black rhinoceros	<i>M. tuberculosis</i>	Died; pulmonary TB
1994	Zoo – USA	Black rhinoceros	<i>M. tuberculosis</i>	Treated; died
2001	Zoo – USA	Black rhinoceros	<i>M. tuberculosis</i>	Treated; euthanized; negative at necropsy
2002	Zoo – Sweden	White rhinoceros	<i>M. tuberculosis</i>	Euthanized; ZN+ lung granulomas
2007	Zoo – South Africa	Black rhinoceros	<i>M. bovis</i>	Euthanized; incidental lesions
2013	Zoo – USA	Black rhinoceros	<i>M. tuberculosis</i>	Died; pulmonary TB
2014	Private reserve – South Africa	Black rhinoceros	<i>M. bovis</i>	Died; pulmonary TB

Sumatran rhinoceros (*Dicerorhinus sumatrensis*) were periodically displayed in European and Asian zoos around the turn of the 20th century (Rookmaaker et al., 1998). A pair of Sumatran rhinoceros was transported from Malaysia to the Alipore Zoological Gardens, Calcutta, India, and the female died within a year of arrival with TB of the lungs and liver. Pulmonary TB was reported as the cause of death in another female Sumatran rhinoceros housed at the Schönbrunner Tiergarten (Vienna, Austria). The Austrian consul purchased the animal from the Botanical Gardens in Singapore and transported her to Europe where she lived from 1900 to 1910.

The London Zoo has had two recorded historical cases of TB in rhinoceros (Rookmaaker et al., 1998). A male Sumatran rhinoceros died shortly after arrival at the zoo in April 1879 with tuberculous lesions in the lungs and spleen. A young male Indian rhinoceros (*Rhinoceros unicornis*) was transported from Calcutta by ship and arrived at the zoo in 1924. The animal reportedly died of TB in 1941.

There are several recorded historical cases in which TB was identified as the cause of death in black rhinoceros housed in zoos (Rookmaaker et al., 1998). Interestingly, all five animals of known gender were males. The earliest report of TB was in a black rhinoceros that died at Miejski Ogród Zoologiczny (Wrocław, Poland) in 1892. Prior to arriving at the zoo in 1888, the animal had travelled with Hagenbeck's Nubian Africa show for 10 years. A male black rhinoceros was imported from East Africa to the Zoologischer Garten (Dresden, Germany) in 1928 and died in 1944 from TB. Another male black rhinoceros died in 1957 from TB at Zoologischer Garten Köln (Cologne, Germany) after being resident for 4 years. More recently, an imported male black rhinoceros succumbed to TB at the Zoologická Zahrada (Prague, Czech Republic) in 1969 after living at the zoo for 15 years.

There are also cases of TB in captive black rhinoceros in other parts of the world. In 1961, a male black rhinoceros died at the Tennoji Zoo in Osaka, Japan (Rookmaaker et al., 1998). An adult female and male black rhinoceros died after developing clinical illness due to pulmonary *M. tuberculosis* infection at the Mysore Zoo (India) in 1992 and 1994 (Valandikar and Raju, 1996).

Zoos in the United States have intermittently reported cases of both *M. tuberculosis* and *M. bovis* in black rhinoceros. Two black rhinoceros were diagnosed in 1978–1979 at the National Zoo based on ante-mortem isolation of *M. bovis* (Mann et al., 1981). Other sporadic cases of *M. tuberculosis* in black rhinoceros have been identified at four different zoos, occurring in 1984, 1990, 1994 and most recently in 2013 (Barbiers, 1994; Oh et al., 2002; Miller et al., 2015; D. Janssen, pers. comm. 2014). The two rhinoceros that died in 1984 and 2013 had been housed at the same facility prior to the death of one individual in 1984. An additional black rhinoceros that died in 2001, which was housed at the same zoo as a known TB case and had a positive ante-mortem culture for *M. tuberculosis* in 1998, also had serological and histopathological evidence of infection although culture was negative at necropsy, possibly due to prior treatment with anti-mycobacterial drugs (Oh et al., 2002; Duncan et al., 2009).

Although the majority of TB cases have been in black rhinoceros, *M. bovis* was isolated from a sick white rhinoceros at the Audubon Zoo in 1991 (Stetter et al., 1995). As the zoo did not have any previous history of TB and the animal was imported, it was speculated that the disease had been acquired in the wild (Dalovisio et al., 1992). *M. tuberculosis* was isolated from a tracheal lavage in an asymptomatic white rhinoceros in a Swedish zoo that had been housed in the same building as infected elephants; however, the authors speculated that it could have been environmental contamination (Lewerin et al., 2005).

Mycobacteriosis has been identified in wild-born black rhinoceros in South Africa. A black rhinoceros succumbed to suspect TB in 1956 at the National Zoological Gardens in Pretoria, South Africa (Hofmeyr, 1956). Another geriatric bull black rhinoceros translocated from the

Mkhuze Game Reserve, KwaZulu Natal to the National Zoological Gardens was euthanized in 2007 due to deteriorating health (weight loss, chronic intermittent diarrhoea) (Espie et al., 2009). Two small encapsulated lesions associated with *M. bovis* were identified as an incidental post-mortem finding in this individual. *M. bovis* has also been isolated from a free-ranging black rhinoceros in Hluhluwe–iMfolozi Park (HiP), KwaZulu Natal in 1970 (Keep and Basson, 1973). Lesions compatible with mycobacterial infection were found in another black rhinoceros from HiP that died in the early 1990s, although culture was not performed (Morar et al., 2013). More recently, an adult bull black rhinoceros died at a private game farm with granulomatous pneumonia from which *M. bovis* was cultured (M. Otto, pers. comm. 2014).

Based on this limited information, the majority of recorded TB cases have occurred in browsing species of rhinoceros, with an obvious paucity of cases in white rhinoceros. This may be related to the differences in housing or management of these species in zoological collections, other risk factors such as exposure to infected animals or people, or species-specific susceptibility. It is clear from these cases that TB in rhinoceros is not a new condition and has been recognized as a disease threat for over 150 years (Table 1).

Clinicopathologic Manifestation of TB in Rhinoceros

TB in rhinoceros, caused by either *M. tuberculosis* or *M. bovis*, presents as a chronic progressive respiratory infection that may only be clinically evident once the animal has developed advanced disease (Dalovisio et al., 1992; Valandikar and Raju, 1996). Clinical changes may include weight loss, nasal discharge, coughing, sneezing, diarrhoea, lethargy, inappetence, and terminal dyspnoea or tachypnoea. However, asymptomatic shedding of *M. tuberculosis* has also been detected in a captive black rhinoceros (Duncan et al., 2009). Limited reports show evidence of immunological responses (reactivity to intradermal tuberculin test, antibodies in serological assays) in rhinoceros that have been exposed to other known TB cases, although necropsy results have not shown evidence of disease (Mann et al., 1981; Stetter et al., 1995; Duncan et al., 2009). Two of three white rhinoceros that had been housed in the same building as *M. tuberculosis*-infected elephants had rare pulmonary granulomas consistent with mycobacteriosis (Karlstam et al., 2015). The presence of three identified granulomas in the 18-year-old female white rhinoceros was associated with a previous isolate of *M. tuberculosis* from a tracheal culture and increasing serologic response to mycobacterial antigens. These observations suggest that rhinoceros may be able to harbour asymptomatic mycobacterial infections.

Pathological findings associated with TB in rhinoceros include lymphadenopathy, lack of fat stores, and cavitary or nodular lung lesions (Mann et al., 1981). Gross lesions may vary from a single granuloma to severe pyogranulomatous pneumonia affecting multiple lung lobes (Stetter et al., 1995; Espie et al., 2009; Karlstam et al., 2015; M. Otto pers. comm. 2014). Nodules are firm, tan to cream coloured with central caseation or mineralization, and may coalesce in chronic disease (Fig. 1). Tannish-white foci may also be detected in the liver. Microscopically, lesions typically consist of necro-granulomas associated with varying degrees of fibrosis and multinucleated giant cells, epithelioid macrophages and lymphocytic infiltrate. Acid-fast bacilli may or may not be detected in the granulomas. Lesions have also been found in other tissues including lymph nodes, liver and colon in individual cases.



Figure 1. Lung lesions in black rhinoceros infected with *Mycobacterium tuberculosis*.

Epidemiology of TB in Rhinoceros

As most cases of TB in rhinoceros affect primarily the lungs, the route of transmission is believed to be through aerosolized mycobacteria (Stetter et al., 1995; Oh et al., 2002). Pathologic changes suggest that individual animals may be infected for months to years prior to developing overt disease. However, epidemiological investigations of rhinoceros cases have not provided adequate information to determine route and duration of infection. Temporo-spatial evidence suggests that interspecies transmission can occur in zoos where there are other infected animals, particularly elephants that may be housed in shared buildings or adjacent exhibits (Oh et al., 2002; Lewerin et al., 2005). In a few cases, rhinoceros that have resided at the same facility will develop infection (Valandikar and Raju, 1996; Duncan et al., 2009; Miller et al., 2015). An elephant and black rhinoceros, that were housed approximately 90 m apart, were shown to share the same strain of *M. tuberculosis* (Oh et al., 2002); however, mode of transmission was not determined. Genotyping of mycobacterial isolates has not been performed consistently in enough rhinoceros TB cases to demonstrate the most likely source of infection.

There is little information regarding susceptibility of rhinoceros to mycobacterial infections. Extrapolation from historical cases suggests that browser species, particularly black rhinoceros, may be more likely to acquire and develop TB than the grazing rhinoceros. However, mycobacterial infection has been diagnosed in all species except the Javan rhinoceros (Rookmaaker et al., 1998). Intensive management of rhinoceros appears to be a risk factor for infection. This may be due to the influence of density, stress, nutrition or presence of concurrent diseases. Captivity may also increase exposure to infected humans or other animals. For example, captivity appears to increase susceptibility to mycobacteriosis in llamas (Barlow et al., 1999).

It has been speculated that captive rhinoceros may be at greater risk of acquiring TB through close contact with human visitors or caretakers (Valandikar and Raju, 1996; Lewerin et al., 2005), although the potential exists for transmission in both directions. Accredited zoos in the United States now require a TB screening/surveillance programme be implemented for appropriate staff and animal collection (AZA 2010). There is a lack of studies examining risk factors for zooanthropogenic TB in zoological collections. There are published reports of retrospective investigation of zoo employees that have had contact with infected animals. In one zoo, seven of 23 potential contacts with the *M. tuberculosis*-infected rhinoceros showed skin test conversion, with six exposed during hosing of the rhinoceros barn and one present at the necropsy (Dalovisio et al., 1992). There was one additional contact that had a previous history of positive skin test; however, further examination and test results were normal. As none of the seven animal handlers had any other known exposure to TB, it appears that contact with the infected rhinoceros was the likely source of exposure.

The possibility exists that rhinoceros may be exposed to TB through infected livestock in areas where there is a wildlife-livestock interface, such as private game ranches or reserves where they share habitat with livestock. Although perissodactyls are generally considered relatively resistant to TB (Pesciaroli et al., 2014), sporadic cases occur in horses, especially in areas where they co-inhabit pastures with infected cattle (Keck et al., 2010). Ingestion is considered the primary route of TB transmission in horses (Pesciaroli et al., 2014). Similarly, rhinoceros are found in areas with endemic bovine TB, especially in buffalo such as Kruger National Park and Hluhluwe–iMfolozi Park, South Africa (Michel et al., 2006). Spillover from buffalo to lion, kudu, baboon and other species has been documented. Shedding by these animals may result in potential exposure of resident rhinoceros populations to TB, although there have been no formal studies investigating these risks to date. However, anecdotal evidence suggests that black rhinoceros can become infected when maintained in proximity to other infected animals (Keep and Basson, 1973; M. Otto, pers. comm. 2014). Due to the lack of reliable diagnostic tests for the detection of infection, many questions regarding the epidemiology and pathogenesis of TB in rhinoceros remain.

Challenges and Status of Methods for Detecting TB in Rhinoceros

Disease detection techniques are implemented for surveillance (to establish disease status in a population), monitoring (detecting epidemiologic trends) or individual diagnosis (to determine presence or absence of infection or disease). However, one of the crucial issues is lack of availability of diagnostic assays for application to wildlife disease. Accurate, reliable, logistically feasible techniques for establishing presence or absence of disease are required to inform decisions on prevention, management and control in these species. Lack of tools may lead to under-recognition of disease threats until the established pathogen has reached the threshold for detection by passive surveillance.

There are no currently available tests to confirm the diagnosis of TB in rhinoceros, other than the gold standard method of mycobacterial isolation and identification from tissue or bodily secretions. Although most often detected post-mortem, techniques for obtaining

culture samples ante-mortem have been used in rhinoceros. These include nasal swabs, trachea-bronchial or gastric lavage, and faecal collection. *M. bovis* has been isolated from a percutaneous lung biopsy in a captive black rhinoceros (Mann et al., 1981). Euthanasia of this individual was performed 6 months later after treatment with oral isoniazid. Nodular lesions with suppurative centres and AFB were found throughout the lungs, consistent with mycobacteriosis, although cultures were negative. The challenge of determining TB status in rhinoceros ante-mortem has repeatedly been demonstrated in TB-exposed rhinoceros that were suspected to be infected and had serial tracheo-bronchial or gastric lavage samples that were culture negative (Stetter et al., 1995; Lewerin et al., 2005; Duncan et al., 2009). Mycobacterial culture is known to be relatively insensitive and previous cases of TB in large mammals have shown that this may lead to an inaccurate classification of an animal as negative (Mikota et al., 2001; Lewerin et al., 2005; Lyashchenko et al., 2006; Karlstam et al., 2015). Therefore, additional ante-mortem diagnostic tools have been investigated to determine disease status in these species.

TB testing of rhinoceros is not commonly performed except when animals are suspected of exposure or disease. A retrospective survey of North American zoos showed that 65% of institutions did not perform routine TB testing of rhinoceros (Godfrey et al., 1990). In animals that had intradermal tuberculin tests, sites of injection were determined; caudal fold was most common (34.2%), with eyelid, vulva and base of ear less commonly used sites (21.05%, 21.0% and 18.4%, respectively). The majority of results (43/53) were interpreted as negative with equivocal results in seven animals, although criteria have not been standardized.

Biopsy of intradermal injection sites in a culture-confirmed *M. bovis*-infected black rhinoceros showed marked delayed-type hypersensitivity (DTH) to mammalian old tuberculin (MOT), moderate response to bovine purified protein derivative (PPD) and only mild DTH to avian PPD (Mann et al., 1981). A female rhinoceros that had been exposed to this individual, and her calf, also became reactive to intradermal MOT with moderate DTH 2 years later. Both animals were started on a 6-month course of isoniazid treatment after which they tested negative on subsequent intradermal tuberculin tests. The female died of unrelated causes and did not have evidence of granulomatous disease on necropsy.

In another zoo with a confirmed *M. bovis* case in a white rhinoceros, the infected individual had a negative skin test approximately 2 ½ years prior to euthanasia, although a small firm swelling was detected 3 months later at the bovine PPD injection site (Stetter et al., 1995). Three other adult white rhinoceros housed with the infected animal were tuberculin-tested and although two were considered reactors to bovine PPD, none showed any evidence of infection on post-mortem examination.

The results reported for intradermal tuberculin tests in captive rhinoceros reflect what has been observed when free-ranging rhinoceros are tested. Due to the lack of an available confirmatory test, most reactions are considered non-specific or cross-reactions to environmental mycobacteria (R. Bengis, pers. comm.).

In vitro immunoassays that detect antigen-specific humoral and cell-mediated immune responses have proven to be useful for detecting mycobacterial infection in a variety of

wildlife species (Cousins and Florisson, 2005). Serology provides a method for rapid screening of wildlife (Lyashchenko et al., 2008) and may have potential in rhinoceros diagnostics (Moser et al., 2008; Duncan et al., 2009). Presence of serum antibodies to *M. bovis*, detected by ELISA, corresponded to positive intradermal tuberculin reactions in four black rhinoceros (two of which were confirmed infected with *M. bovis*) and an Indian rhinoceros at the National Zoological Park in the United States (Mann et al., 1981). Antibody responses to ESAT-6, CFP10 and MPB83 antigens were detected in two *M. tuberculosis*-infected and one suspect black rhinoceros using multi-antigen print immunoassay (MAPIA) and ElephantTB STAT-PAK (Duncan et al., 2009). Serum antibodies gradually declined when the infected animals were treated with anti-tubercular therapeutics, suggesting potential value of serology for treatment monitoring.

Although intradermal tuberculin tests in rhinoceros have not proven to be reliable for TB diagnosis on their own, they may boost memory responses which can improve detection by other methods. Humoral responses to MPB70 were increased 3 weeks post-tuberculin skin test in two white rhinoceros that had been imported from HiP (Karlstam et al., 2015; K. P. Lyashchenko pers. comm.). In another zoo, seroconversion to ESAT-6 and CFP-10 (as detected by ElephantTB STAT-PAK, MAPIA and DPP VetTB) occurred 2 ½ weeks after intradermal tuberculin testing in a *M. tuberculosis*-infected black rhinoceros (Miller et al., 2015). This individual remained seropositive for an additional 12 years until it succumbed to pulmonary TB.

In vitro assays for the detection of cell-mediated immune responses are also being investigated for use in rhinoceros. Lymphocyte proliferative responses to mitogens and antigens have been compared between the four species of captive rhinoceros, with evidence that black rhinoceros may have less vigorous immune responses (Vance et al., 2004). To develop *in vitro* cytokine assays, the interferon-gamma (IFN- γ) gene has been cloned and sequenced (Morar et al., 2007). Antibodies were produced to the recombinant rhinoceros IFN- γ protein, then an enzyme-linked immunoassay was tested, using these antibodies, to detect IFN- γ in supernatants from mitogen-stimulated rhinoceros whole blood cultures (Morar et al., 2013). Evaluation of other cytokines and cytokine gene expression as a measure of immunological activation may result in recognition of other biomarkers for TB in these species.

Threat of TB to Conservation of Rhinoceros

Rhinoceros populations are under threat due to escalated poaching, habitat destruction and potentially under-recognized hazards such as infectious diseases. Zoonotic pathogens and those originating from livestock, such as *M. tuberculosis* and *M. bovis*, may pose a significant health risk to captive, semi-intensively managed and free-ranging rhinoceros. Presence of notifiable diseases in rhinoceros within a given reserve or facility may hinder conservation efforts by imposed quarantine and movement restrictions. The chronic nature of TB may result in establishment of infection that may not be recognized for decades, as has been documented with TB in multiple wildlife populations worldwide (Payeur et al., 2002; Michel et al., 2006; Fitzgerald and Kaneene, 2013; Hlokwe et al., 2014). Therefore, it is crucial to develop effective tools and devise methods of investigating the TB status in these species,

especially early detection of spillover into rhinoceros so that the source can be determined and controlled before infection becomes established in the population.

Summary

Based on published historical and current cases, tuberculosis can affect African and Asian rhinoceros, with an apparent predilection for the browsing species. Large knowledge gaps exist regarding our understanding of the transmission, risk factors impacting susceptibility, development of infection and progression of disease, prevalence in different species and populations, management and control of TB in rhinoceros. Investigation of the potential risks that TB poses for rhinoceros requires reliable tools to diagnose individuals and screen populations. However, there is a paucity of available techniques for detection of mycobacterial infection and disease in rhinoceros. Further studies are critically needed to address this impending threat to the conservation of rhinoceros and other wildlife.

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References

Association of Zoos and Aquariums, 2010: Accreditation Standards and Related Policies. Available at www.aza.org (accessed February 28, 2015).

Barbiers, R., 1994: *Mycobacterium tuberculosis* in a black rhinoceros (*Diceros bicornis*). *Proc. Am. Assoc. Zoo. Vet. Annu. Meet.* 1994, 171–172.

Barlow, A. M., K. A. Mitchell, and K. H. Visram, 1999: Bovine tuberculosis in llama (*Lama glama*) in the UK. *Vet. Rec.* 145, 639–640.

Bengis, R. G., 1999: Tuberculosis in free-ranging mammals. In: Fowler, M. E., and R. E. Miller (eds), *Zoo and Wild Animal Medicine Current Therapy 4*, 4th edn. pp. 101–114. WB Saunders Co., Philadelphia.

Cousins, D. V., and N. Florisson, 2005: A review of tests available for use in the diagnosis of tuberculosis in nonbovine species. *Rev. Sci. Tech. Off. Int. Epiz.* 24, 1039–1059.

Dalovisio, J. R., M. Stetter, and S. Mikota-Wells, 1992: Rhinoceros' rhinorrhea: cause of an outbreak of infection due to airborne *Mycobacterium bovis* in zookeepers. *Clin. Infect. Dis.* 15, 598–600.

Duncan, A. E., K. Lyashchenko, R. Greenwald, M. Miller, and R. Ball, 2009: Application of elephant TB STAT-PAK assay and MAPIA (multi-antigen print immunoassay) for detection of tuberculosis and monitoring of treatment in black rhinoceros (*Diceros bicornis*). *J. Zoo Wildl. Med.* 40, 781–785.

Espie, I. W., T. M. Hlokwe, N. C. Gey van Pittius, E. Lane, A. S. W. Tordiffe, A. L. Michel, A. Muller, A. Kotze, and P. D. van Helden, 2009: Pulmonary infection due to *Mycobacterium bovis* in a black rhinoceros (*Diceros bicornis minor*) in South Africa. *J. Wildl. Dis.* 45, 1187–1193.

Fitzgerald, S. D., and J. B. Kaneene, 2013: Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance, and control. *Vet. Pathol.* 50, 488–499.

Godfrey, R. W., B. L. Dresser, and B. J. Campbell, 1990: Tuberculosis testing of captive rhinoceros. *Proc. Am. Assoc. Zoo Vet. Annu. Meet.* 1990, 312.

Hlokwe, T. M., P. van Helden, and A. L. Michel, 2014: Evidence of increasing intra and inter-species transmission of *Mycobacterium bovis* in South Africa: are we losing the battle? *Prev. Vet. Med.* 115, 10–17.

Hofmeyr, C. B. F., 1956: Two hundred and eighty autopsies at the National Zoological Gardens, Pretoria. *J. S. Afr. Vet. Assoc.* 27, 263–282.

Karlstam, E., E. Agren, T. Moller, G. Ramis, G. Bolske, B. Roken, K. Lyashchenko, and D. Gavier-Widen, 2015: *Mycobacterium tuberculosis* infection in captive white rhinoceros (*Ceratotherium simum simum*). *J. Comp. Pathol.* 152, 54.

Keck, N., H. Dutruel, F. Smvei, M. Nodet, and M. L. Boschioli, 2010: Tuberculosis due to *Mycobacterium bovis* in a Camargue horse. *Vet. Rec.* 166, 499–500.

Keep, M. E., and P. A. Basson, 1973: Mycobacteriosis in a black rhinoceros (*Diceros bicornis Linnaeus* 1758). *J. S. Afr. Vet. Assoc.* 44, 285–286.

Lewerin, S. S., S. L. Olsson, K. Eld, B. Roken, S. Ghebremichael, T. Koivula, G. Kallenius, and G. Bolske, 2005: Outbreak of *Mycobacterium tuberculosis* infection among captive Asian elephants in a Swedish zoo. *Vet. Rec.* 156, 171–175.

Lyashchenko, K. P., R. Greenwald, J. Esfandiari, J. H. Olsen, R. Ball, G. Dumonceaux, F. Dunker, C. Buckley, M. Richard, S. Murray, J. B. Payeur, P. Andersen, J. M. Pollock, S. Mikota, M. Miller, D. Sofranko, and W. R. Waters, 2006: Tuberculosis in elephants; antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. *Clin. Vaccines Immunol.* 13, 722–732.

Lyashchenko, K. P., R. Greenwald, J. Esfandiari, M. A. Chambers, J. Vicente, C. Gortazar, N. Santos, M. Correia-Neves, B. M. Buddle, R. Jackson, D. J. O'Brien, S. Schmitt, M. V. Palmer, R. J. Delahay, and W. R. Waters, 2008: Animal-side serologic assay for rapid detection of *Mycobacterium bovis* infection in multiple species of free-ranging wildlife. *Vet. Microbiol.* 132, 283–292.

Mann, P. C., M. Bush, D. L. Janssen, E. S. Frank, and R. Montali, 1981: Clinicopathologic correlations of tuberculosis in large zoo mammals. *J. Am. Vet. Med. Assoc.* 179, 1123–1129.

Michel, A. L., R. G. Bengis, D. F. Keet, M. Hofmeyr, L. M. deKlerk, P. C. Cross, A. E. Jolles, D. Cooper, I. J. Whyte, P. Buss, and J. Godfroid, 2006: Wildlife tuberculosis in South African conservation areas: implications and challenges. *Vet. Microbiol.* 112, 91–100.

Mikota, S. K., L. Peddie, J. Peddie, R. Isaza, F. Dunker, G. West, W. Lindsay, R. S. Larsen, M. D. Salman, D. Chatterjee, J. Payeur, D. Whipple, C. Thoen, D. S. Davis, C. Sedgwick, R. J. Montali, M. Ziccardi, and J. Maslow, 2001: Epidemiology and diagnosis of *Mycobacterium tuberculosis* in captive Asian elephants (*Elephas maximus*). *J. Zoo Wildl. Med.* 32, 1–16.

Miller, M. A., R. Greenwald, and K. Lyashchenko, 2015: Potential for serodiagnosis of tuberculosis in black rhinoceros (*Diceros bicornis*). *J. Zoo Wildl. Med.* 46, 100–104.

Morar, D., E. Tijhaar, A. Negrea, J. Hendriks, D. van Haarlem, J. Godfroid, A. L. Michel, and V. P. M. G. Rutten, 2007: Cloning, sequencing and expression of white rhinoceros (*Ceratotherium simum*) interferon-gamma (IFN- γ) and the production of rhinoceros IFN- γ specific antibodies. *Vet. Immunol. Immunopathol.* 115, 146–154.

Morar, D., J. Schreuder, M. Meny, P. J. S. van Kooten, E. Tijhaar, A. L. Michel, and V. P. M. G. Rutten, 2013: Towards establishing a rhinoceros-specific interferon-gamma (IFN- γ) assay for diagnosis of tuberculosis. *Transbound. Emerg. Dis.* 60 (Suppl. 1), 60–66.

Moser, I., W. M. Prodingler, H. Hotzel, R. Greenwald, K. P. Lyashchenko, D. Bakker, D. Gomis, T. Seidler, C. Ellenberger, U. Hetzel, K. Wuennemann, and P. Moisson, 2008: *Mycobacterium pinnipedii*: transmission from South American sea lion (*Otaria byronia*) to Bactrian camel (*Camelus bactrianus bactrianus*) and Malayan tapirs (*Tapirus indicus*). *Vet. Microbiol.* 127, 399–406.

Oh, P., R. Granich, J. Scott, B. Sun, M. Joseph, C. Stringfield, S. Thisdell, J. Staley, D. Workman-Malcolm, L. Borenstein, E. Lehnkering, P. Ryan, J. Soukup, A. Nitta, and J. Flood, 2002: Human exposure following *Mycobacterium tuberculosis* infection of multiple animal species in a metropolitan zoo. *Emerg. Infect. Dis.* 8, 1290–1293.

Payeur, J. B., S. Church, L. Mosher, B. Robinson-Dunn, S. Schmitt, and D. Whipple, 2002: Bovine tuberculosis in Michigan wildlife. *Ann. N.Y. Acad. Sci.* 969, 259–261.

Pesciaroli, M., J. Alvarez, M. B. Boniotti, M. Cagiola, V. Di Marco, C. Marianelli, M. Pacciarini, and P. Pasquali, 2014: Tuberculosis in domestic animal species. *Res. Vet. Sci.* 97, S78–S85.

Rookmaaker, L. C., M. L. Jones, H. G. Klos, and R. J. Reynolds III, 1998: The Rhinoceros in Captivity: a List of 2439 Rhinoceroses Kept from Roman Times to 1994. Kugler Publications, SPB Academic Publishing, Amsterdam.

Stetter, M. D., S. K. Mikota, A. F. Gutter, E. R. Monterroso, J. R. Dalovisio, C. Degraw, and T. Farley, 1995: Epizootic of *Mycobacterium bovis* in a zoological park. *J. Am. Vet. Med. Assoc.* 207, 1618–1621.

Valandikar, S. C., and R. Raju, 1996: Pulmonary tuberculosis in black rhinoceros (*Diceros bicornis*) in Mysore Zoo. *Zoos Print* 11, 16–17.

Vance, C. K., S. Kennedy-Stoskopf, A. R. Obringer, and T. L. Roth, 2004: Comparative studies of mitogen- and antigen-induced lymphocyte proliferation in four captive rhinoceros species. *J. Zoo Wildl. Med.* 35, 435–446.