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Chapter 5

Wildlife Tuberculosis: An Emerging Threat for Conservation in South Asia


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Abstract

Wildlife tuberculosis (TB) is becoming one of the emerging challenges for conservation globally. South Asian region is home to many endangered species like Asian elephants, rhinoceros, and Bengal tigers. Although it carries more than one-third of global burden of human TB, TB in livestock and wildlife has not been adequately studied. This chapter reviews the present knowledge and information about animal-adapted members of Mycobacterium tuberculosis complex and wildlife TB in South Asia. Recent studies of TB from different wild animals in Nepal and Bangladesh have found that M. orygis is an emerging threat of wildlife TB in the region. These studies have demonstrated wide diversity of M. orygis strains circulating in the region indicating its endemic distribution. M. orygis-associated TB was discovered from a free-ranging rhinoceros in Nepal and the finding could signify threat of TB in other wild animals, including a possibility of unknown maintenance host. Recent studies also revealed an emerging challenge caused by TB to elephants in different South Asian countries like Nepal, India, and Sri Lanka. Wildlife TB is becoming a conservation challenge in South Asia, but given the paucity of research in this area, it is overlooked and underexplored.

Keywords: wildlife tuberculosis, Mycobacterium tuberculosis complex, Mycobacterium orygis, Asian elephants, South Asia
1. Introduction

South Asia or the South Asian Association for Regional Cooperation (SAARC) region consists of eight countries, namely, Afghanistan, Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka. This region is one of the world’s hotspots for biodiversity as it includes 17 regions in the World Wildlife Fund (WWF)’s Global-200 biodiverse ecoregions [1], and 2 of 25 priority regions for biodiversity conservation [2]. It is also considered as a high-risk region for emerging infectious diseases that could originate in wildlife [3].

The *Mycobacterium tuberculosis* complex (MTBC) is a group of genetically closely related pathogens that can cause tuberculosis (TB) in humans and animals [4]. The MTBC includes the typical human-adapted pathogens *M. tuberculosis* and *M. africanum* [5] and those members reported to cause TB in a range of animals which are *M. bovis*, *M. caprae*, *M. pinnipedi*, *M. microti*, *M. orygis*, *M. suricattae*, Dassie bacillus, *M. mungi*, and so-called the Chimpanzee bacillus (Figure 1) [6–14]. *M. bovis*, the most studied animal TB pathogen, is now considered as an emerging pathogen of free-ranging wildlife and an emerging threat to several protected wildlife species [15]. Similarly, the threat of TB in endangered species, such as chimpanzees in the Ivory Coast [14] caused by the chimpanzee bacillus, and to free-ranging rhinoceroses in Nepal by *M. orygis* [16], are examples of the challenges that TB poses for wildlife conservation. Through this review, we will provide a global overview of animal-adapted members of the MTBC; highlight their importance to defining “One Health” connections between humans, animals, and wildlife; and draw attention to their emerging threat to wildlife conservation.

TB caused by *M. tuberculosis* is a high-priority disease in South Asia as it carries 34% of the global TB burden, with 3 million people infected and a mortality of 0.4 million people in 2013 [17]. However, TB caused by *M. bovis* is a neglected disease both in the livestock sector and in human health in the region. In human health, MTBC species differentiation is not a priority because it requires mycobacterial culture and subsequent use of specialized molecular tests for diagnosis [18]. There are only a few confirmatory reports of zoonotic TB caused by *M. bovis* in the region [19] and we are not aware of any livestock TB control programs. In addition, despite the limited number of publications that describe TB in different wildlife species, to the best of our knowledge, there are no reports of confirmed diagnosis of *M. bovis* infection from wildlife in the region. Thus, zoonotic TB caused by *M. bovis* and other members of the MTBC that may be present in livestock, wildlife, as well as humans is largely overlooked. In recent studies we have demonstrated the conservation challenge posed by *M. orygis* in Nepal [16, 20]. Complementing this finding, from our studies in Bangladesh [21], we have demonstrated a wide distribution of *M. orygis* in the region. Recent studies also point to an emerging challenge caused by TB to elephants, as Asian elephants in Nepal have been shown to be infected with *M. tuberculosis* [22], elephants in India were seroreactive to TB antigens [23], and TB was detected from a wild elephant in Sri Lanka [24]. Additionally, we will discuss some case reports that describe TB in other wildlife. Thus, the second component of this review will be to address the conservation challenge caused by TB in wildlife of South Asia, with particular emphasis on *M. orygis*-associated TB and the emerging threat of TB in elephants.
This study reviews the current knowledge and information about animal‐adapted members of the MTBC, the emerging threat of TB in wildlife, and the problem posed by TB to wildlife conservation in South Asia.

2. Animal‐adapted members of MTBC

With the advent of a suite of molecular methods for genotyping of the MTBC, such as spoligotyping, deletion typing, and genome sequencing, in combination with increasing veterinary awareness for wildlife conservation, we now have the opportunity to scientifically pursue TB in wildlife. As a result, many new members of MTBC have been identified and the findings have clearly contributed to our understanding of the MTBC and their wildlife reservoirs (Table 1). Increasingly wildlife are recognized as reservoirs of these MTBC organisms and
these findings pose a threat to wildlife conservation, undermine livestock TB control programs, and provide another avenue for zoonotic transmission.

*M. bovis* is the main cause of bovine TB but also infects many other domestic animals, wildlife, and also humans [15, 25]. It is globally distributed and one of the major causes of zoonosis in the geographic area where it is endemic. The WHO reported in 1998 that 3.1% of human TB cases were caused by *M. bovis* and 0–10% of sputum isolates from African patients could be from *M. bovis* [26]. A relatively recent study has crudely estimated 7 zoonotic TB cases/100,000 population/year in Africa [18].

*M. bovis* has successfully spread to several wildlife hosts and various geographical locations. The most well-known examples of wildlife involvement in the transmission of *M. bovis* infection are badgers in Great Britain which are involved in transmitting *M. bovis* to cattle [27], with a similar situation also evident in Ireland [28]. White-tailed deer in Michigan and Minnesota in the United States are an important wildlife reservoir of *M. bovis* [29, 30], and the brush-tailed possum is New Zealand being another well-studied reservoir [31]. In all of the above examples, the wildlife reservoirs of bovine TB have greatly hindered the success of bovine TB eradication programs. While culling of these wildlife reservoirs has been carried out as a control option, such approaches are expensive and sometimes ineffective; indeed in one particular instance from the United Kingdom it was reported that badger culling increased TB in cattle [27]. Similarly, wild ungulates such as wild boar and red deer are maintenance hosts of *M. bovis* in the Iberian Peninsula, Europe and transmit *M. bovis* to other ungulates.

<table>
<thead>
<tr>
<th>MTBC species</th>
<th>Reservoir host</th>
<th>Geographical area</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. bovis</em></td>
<td>European badger</td>
<td>UK and Ireland</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td>Brush-tailed possum</td>
<td>New Zealand</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>White-tailed deer</td>
<td>North America</td>
<td>[29, 30]</td>
</tr>
<tr>
<td></td>
<td>Wild boar</td>
<td>Spain</td>
<td>[68]</td>
</tr>
<tr>
<td></td>
<td>African buffalo</td>
<td>South Africa</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>Lechwe</td>
<td>Zambia</td>
<td>[33]</td>
</tr>
<tr>
<td><em>M. caprae</em></td>
<td>Wild boar</td>
<td>Spain</td>
<td>[7]</td>
</tr>
<tr>
<td><em>M. pinnipedii</em></td>
<td>Seal, sea lion</td>
<td>Australia, Argentina</td>
<td>[8]</td>
</tr>
<tr>
<td><em>M. microti</em></td>
<td>Voles</td>
<td>UK</td>
<td>[9]</td>
</tr>
<tr>
<td><em>M. orygis</em></td>
<td>Antelopes; deer, antelope</td>
<td>Middle east, South Asia</td>
<td>[10, 16, 20]</td>
</tr>
<tr>
<td></td>
<td>Meerkats</td>
<td>South Africa</td>
<td>[11, 39]</td>
</tr>
<tr>
<td><em>M. suricattae</em></td>
<td>Rock hyraxes</td>
<td>Southern Africa</td>
<td>[12, 36]</td>
</tr>
<tr>
<td>Dassie bacillus</td>
<td>Chimpanzee</td>
<td>Ivory Coast</td>
<td>[14]</td>
</tr>
</tbody>
</table>

1Antelope and oryx from Middle East countries were considered to be a source of transmission.

2Deer and antelope in South Asia may be a reservoir host.

3Only one case, may not be a reservoir host.

Table 1. *Mycobacterium tuberculosis* complex (MTBC) species and their wildlife reservoirs.
carnivores, and livestock [32]. Lechwe, an antelope in Zambia, is another reservoir host of *M. bovis* that contributes to the spread of the infection to livestock [33]. Furthermore, Zambia has other flagship wildlife species such as elephants, lions, and hippos, to name a few, but the situation of TB in these species has yet to be ascertained. The strongest impact of *M. bovis* for wildlife conservation is probably in South Africa where the bacillus is maintained within African buffalo and has spilled over into 13 different wild animals including protected species like the lion, cheetah, and greater kudu [34]. Recently, TB caused by *M. bovis* in free-ranging rhinoceros in Southern Africa has been considered as an under recognized threat [35].

The MTBC species *M. caprae* was first isolated from domestic goats in Europe but has now been isolated from deer, wild boar, and livestock [7]. *M. pinnipedii* is a seal (pinnipeds)-specific member of the MTBC that has mostly been identified in animals from the Southern Hemisphere such as Australia and Argentina [8]. *M. microti* is a rodent MTBC mostly reported from voles in the UK and Europe [9]. There are three animal-adapted MTBC subspecies that are endemic to the Southern Africa subregion, namely, *M. mungi*, *M. suricattae*, and the Dassie bacillus [36]. *M. mungi* has been isolated from the banded mongoose from northern Botswana [13, 37], and its unique environmental mode of intra-mongoose transmission pathway has recently been elucidated [38]; however, this species has not yet been reported from other animals. The Dassie bacillus has been mostly isolated from rock hyraxes in Southern Africa [12]. *M. suricattae* is mostly reported from meerkats [11] and has a unique genetic feature in that it lacks the direct repeat locus and hence has no spoligotype pattern [39]. Interestingly, *M. mungi*, *M. suricattae*, and the Dassie bacillus have partial deletions of the RD1 locus, a locus that has been shown to be important for virulence of *M. tuberculosis* and *M. bovis* in various animal models (Figure 1).

Historically, it was believed that human TB evolved from bovine TB as a zoonosis. The hypothesis for that belief was based on the characteristic of a wide host range for *M. bovis* and a narrow host range limited to humans of *M. tuberculosis* [6]. However, from recent studies of whole genome and deletion analysis of *M. tuberculosis*, *M. bovis*, and other MTBCs, it has been shown that genome of *M. bovis* and other MTBCs including *M. orygis* has undergone numerous deletions relative to *M. tuberculosis* (Figure 1) indicating that *M. tuberculosis* predated the other MTBCs [6]. These successive losses of genetic material are postulated to have led to the appearance of different MTBCs that have become successful pathogens in certain hosts.

3. *M. orygis*, an emerging MTBC and a conservation threat in South Asia

*M. orygis*, also described as the oryx bacillus or the antelope clade, is a novel member and a subspecies of the MTBC with a unique phylogenetic position (Figure 1) that causes TB in animals and humans [10].

Before the use of molecular genotyping tools for differentiation of the members of the MTBC, *M. orygis* could have been misidentified as *M. tuberculosis* if isolated from infected humans, or *M. bovis* if isolated from infected animals. It was first reported in antelopes
(oryx and waterbuck) at a zoo in the Netherlands, where the authors reported it originally as *M. bovis* but discussed its unusual feature of having a high copy number of the IS6110 insertion sequence. In the same study, a human MTBC isolate having a similar IS6110 pattern was identified, and both the human and animal isolates had an epidemiological link [40]. The clear molecular genetic distinction of *M. orygis* from other members of the MTBC was reported in 2005 [41]. While the subspecies name of this MTBC member was proposed as *orygis* to convey the fact that it was first characterized from oryx [10] or as an antelope clade to convey the group of animals from which it was most frequently isolated [6, 41–43], recently it has been isolated from many other animals and humans (Table 2). Thus, the isolation of *M. orygis* from a wide range of host species and the widespread geographical locations from where it has been isolated challenge previous concepts of it being an exclusively antelope clade and its host range and geographical distribution.

We reported the isolation of *M. orygis* from wild animals (a spotted deer and a blue bull) from a captive facility in Nepal [20]. We were aware of TB infections in the wild animals in this captive facility, and had assumed it to be caused by *M. bovis*; however, after molecular characterization, the TB isolates were confirmed to be *M. orygis*. Later in 2015, another *M. orygis* was isolated from a free-ranging greater one-horned rhinoceros in Chitwan National Park (CNP); the clinical history and necropsy of this animal suggested that it died from TB [16]. All three of these animals (spotted deer, blue bull, and rhinoceros) had extensive TB granulomatous lesions on the lungs, well encapsulated and filled with caseous necrotic material. The TB

<table>
<thead>
<tr>
<th>Host (number of isolates)</th>
<th>Geographical location</th>
<th>Wild/captive/domestic</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antelope (2)</td>
<td>Netherlands</td>
<td>Captive</td>
<td>[10]</td>
</tr>
<tr>
<td>Water buck (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oryx (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antelope (1)</td>
<td>South Africa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deer (1)</td>
<td>United Kingdom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (10)</td>
<td>South Asia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human (1)</td>
<td>South East Asia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African buffalo (1)</td>
<td>South Africa</td>
<td>Captive</td>
<td>[69]</td>
</tr>
<tr>
<td>Cattle (1)</td>
<td>New Zealand</td>
<td>Domestic</td>
<td>[51]</td>
</tr>
<tr>
<td>Human (1)</td>
<td>New Zealand/Indian Immigrant</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Blue bull (1)</td>
<td>Nepal</td>
<td>Captive</td>
<td>[20]</td>
</tr>
<tr>
<td>Spotted deer (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinoceros (1)</td>
<td>Wild</td>
<td></td>
<td>[16]</td>
</tr>
<tr>
<td>Monkey (2)</td>
<td>Bangladesh</td>
<td>Captive/wild captured</td>
<td>[21]</td>
</tr>
<tr>
<td>Cattle (18)</td>
<td></td>
<td>Domestic</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Reported cases of *Mycobacterium orygis* from different host species and geographical location.
lesions of the rhinoceros were limited to the lungs, whereas in the case of spotted deer and blue bull there were extrapulmonary lesions on lymph nodes, the gastrointestinal tract, and liver (Figure 2) [16, 20]. When cultured, all three *M. orygis* isolates yielded smooth and moist colonies unlike rough and dry colonies from *M. tuberculosis* (Figure 3). Although all three *M. orygis* isolates from these animals had the same spoligotyping pattern (SIT587), further molecular characterization by multilocus variable number of tandem repeat analysis (MLVA) revealed that both the deer and blue bull isolate had the same MLVA type while the rhinoceros isolate was a different type differing at one MLVA locus [16]. When we isolated *M. orygis* from the deer and blue bull from a captive wild animal facility in an earlier study [20], we had postulated that the origin of this TB might be from other animals in the CNP; the new finding of a slightly different strain of *M. orygis* from a free-ranging rhinoceros in CNP supports our original hypothesis [16].

The greater one-horned rhinoceros is the largest species of rhinoceros that is listed in Appendix I (most endangered) of the Convention on International Trade in Endangered Species (CITES), categorized as vulnerable by the International Union for Conservation of Nature (IUCN) Red List, and listed as a protected species by the Government of Nepal [44–46]. The present day free-ranging population of rhinoceroses in Nepal and India has increased from only 600 individuals in 1975 to 3555 individuals by mid-2015 [47]. In Nepal, the population of rhinoceroses is 645 individuals, of which 605 individuals live in CNP in a relatively narrow area of riverine grassland [48, 49]. From a conservation point of view, having a chronic and devastating disease like TB in this vulnerable and isolated population, that is already threatened from habitat destruction and poaching, is a matter of great concern for the animal’s long-term survival. Also, CNP is listed by the United Nations Educational, Scientific and Cultural Organization (UNESCO) as a World Heritage Site because of its rich biodiversity, being home to globally protected animals such as Bengal tigers, Asian elephants, and greater one-horned

![Figure 2. Description of tuberculosis lesions obtained during postmortem of a dead spotted deer. (A) Extrapulmonary. (B) Lung. Extrapulmonary tuberculosis lesions were of various sizes and capsulated with extensive liquefaction. Pulmonary tuberculosis lesions were of varying sizes from a single focal granuloma of 1-2 cm to extensive granulomatic lesion affecting a larger area of lung tissue (figure obtained with permission from Thapa et al. 2015 [20]).](image)
rhinoceroses. The finding of TB in rhinoceroses in the park could also signify TB as a threat in other animals, including a strong possibility of unknown maintenance hosts of *M. orygis* in and around the national park [16]. CNP also shares an international border with the Valmiki National Park of India, and many CNP wildlife species, including rhinoceroses, routinely migrate between Nepal and India over this border. So, we cannot rule out the possibility of *M. orygis* infection being prevalent in wild animals in India.

In a study in India, 16 postmortem tissue samples from 25 suspected TB cases of spotted deer from a zoo were positive for IS6110 PCR, indicating the presence of infection with members of the MTBC [50]. Since IS6110 is present in all the MTBC species it cannot be used to differentiate the various members of the MTBC. Hence, as was the case in Nepal, the causative agent of TB in those deer from India could have been *M. orygis*. Therefore, *M. orygis*-associated TB in wild animals of South Asia may be a conservation threat and it should be further explored to ascertain its real impact on conservation of wild and protected animal species.

4. *M. orygis* may be endemically distributed in South Asia

In a recent study we isolated 20 *M. orygis* isolates from 18 cattle and two monkeys in Bangladesh [21]. All the cattle belonged to a farm and the two monkeys were from a zoo in Dhaka. TB lesions were identified in the lungs of the cattle and monkeys during postmortem examination. All of the cattle and monkey isolates shared the same spoligotyping pattern (SIT587) and the MLVA analysis divided the isolates into 3 clusters where the biggest cluster comprised 15 of the cattle isolates and the 2 monkey isolates. When compared with the larger cluster, one cattle isolate had a difference at one MLVA locus, whereas two cattle isolates had

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**Figure 3.** Comparative culture morphology of *M. orygis* and *M. tuberculosis* isolates. Culture of *M. orygis* isolated from the deer isolate (A), the blue bull isolate (B), and a comparative culture of a *M. tuberculosis* isolate from human (C). All the cultures are grown in Löwenstein-Jensen medium in the same laboratory but at different times (figure obtained with permission from Thapa et al. 2015 [20]).
differences at three loci [21]. Despite not being able to trace the origin of the cattle on the farm, the finding of a large cluster with 15 cattle isolates and two monkey isolates indicated the widespread prevalence of this *M. orygis* strain in that area in both domestic and wild animals. Furthermore, the presence of other strains with one or three MLVA loci difference to the main cluster suggested a heterogeneous population of strains in that area. These finding suggest that *M. orygis* in that region of Bangladesh may be maintained in the cattle population, although we cannot rule out the possibility of an unknown reservoir host that would ultimately indicate the endemic presence of *M. orygis* in that area of Bangladesh.

From our studies in Nepal and Bangladesh, we have identified five MLVA types, two types in Nepal and three types in Bangladesh, in different wild and domestic animals. In Nepal, we speculated that there may be an unknown reservoir host of *M. orygis*, whereas in Bangladesh we suggest the possibility that cattle were a reservoir host. The genetic variation of *M. orygis* strains in different animals and geographical locations supports the view of its endemic distribution in South Asia. Adding further support to this latter hypothesis, 10 *M. orygis* isolates from South Asian human patients have been reported [10]. Similarly, *M. orygis* was also isolated from an Indian immigrant in New Zealand, with the probable origin of infection suggested to be in the patient’s native country where there was opportunity of contact with local animals [51].

To the best of our knowledge, there are no confirmed diagnoses of *M. bovis* from human and cattle in Bangladesh although there are several studies of bovine TB in Bangladesh based on serological diagnosis [52]. Some of the cases of bovine TB could be from *M. orygis*. Similarly, wildlife TB in the South Asian region is largely understudied and probably underreported; even if TB lesions are observed during postmortem examination, confirmatory testing to identify species is generally not performed. Hence, these recent reports of *M. orygis* from South Asia present an important research question as to the origin and distribution of animal TB in South Asia; there could be a possibility that *M. orygis* may have predated *M. bovis* as a cause of TB in animals in South Asia, if the latter was introduced and rapidly expanded along with importation of European cattle. This assumption is supported by the discovery of different strains of *M. orygis* in wild animals in Nepal, the wide distribution of *M. orygis* strains in Bangladesh, and the potential links of *M. orygis* isolates to India. Thus, these findings support the One Health significance of *M. orygis* through the human-animal-environment connection as well as its endemic distribution in South Asia.

5. Elephant TB in South Asia

For centuries, elephants have been revered in Asia where they are the part of the region’s culture and religion. They are seen as the guardians of forest, play a critical role to maintain the forest ecosystem, and are lately an indispensable asset for conservation work in Asia. Unfortunately, because of shrinking protected habitats and extensive poaching their numbers have declined and elephants are now classified as an endangered species [53]. As in other South Asian countries, elephants have sociocultural and economic value in Nepal [54]. However, elephants in Asia now face a potentially grave threat from TB.
TB in elephants is a reemerging disease caused primarily by *M. tuberculosis*, a human form of TB; however, infection with *M. bovis* has also been infrequently reported. Intensive study on elephant TB only received attention after 1996 when two circus elephants died of TB in the United States. Recently, TB in elephants is increasingly being detected in their host range countries and in zoological collections around the world [55].

TB screening in Asian elephants (*Elephas maximus*) has been carried out in South Asian countries including Nepal, India, and Sri Lanka, countries that also have a high TB prevalence in the human population. The tradition of keeping and training elephants in South Asia is long standing and continues to support various religious and tourism-related activities, as well as forestry and conservation management. This practice provides ample opportunity for transmission of TB from humans to elephants or vice versa.

There are about 200 captive elephants in Nepal that are used by government authorities for patrolling of protected areas, by the private sector for eco-tourism, and in some cases for wildlife research projects [56]. TB was first reported in captive elephants in Nepal in CNP in 2002 [57] and from 2002 to 2014, more than 10 elephants died of TB in Nepal. A comprehensive study was conducted in Nepal for screening of TB in 115 captive elephants; the results show that 15 of 115 (13%) elephants were reactive on the Elephant TB Stat Pak® assay [58]. The Elephant TB Stat Pak® is a licensed serological test developed by ChemBio Diagnostics, Inc., Medford, NY, USA, that uses a cocktail of several selected *M. tuberculosis* and/or *M. bovis* antigens (ESAT-6, CFP-10, and MPB83) to detect TB antibodies in elephants [59]. However, the antigens used are common for the MTBCs so they cannot differentiate MTBC subspecies including *M. orygis*. Also, the *M. tuberculosis* strain isolated from three of the cases in Nepal was identified as the same strain found in Nepalese human TB patients [22].

In 2007, a Nepal Elephant Healthcare and TB Surveillance Program was initiated to conduct regular screening of captive elephants. Similarly, in 2011, the Nepal Elephant Tuberculosis Control and Management Action Plan (2011–2015) was endorsed by the Government of Nepal providing detailed guidelines for the management of TB including the diagnosis and treatment of TB in elephants of Nepal [60]. This was the first elephant TB control action plan implemented in an Asian elephant range country.

A study conducted in India has shown that 15% of over 300 captive elephants tested were reactive on the Elephant TB Stat-Pak® assay. The highest seroreactivity was among temple elephants; these elephants had the greatest contact with humans of the three management groups studied [23]. *M. tuberculosis* was also isolated from two wild Asian elephants in India [61]. Similarly, *M. tuberculosis* was recently isolated from a wild elephant for the first time in Sri Lanka; however, the source of infection from possible human contact or potential wildlife reservoirs could not be traced for this elephant [24]. This finding is very important as it reflects the potential of TB transmission within wildlife populations in Sri Lanka.

Although, *M. tuberculosis* is usually identified from elephants, we cannot rule out the possibility of *M. bovis* or even *M. orygis* infection in elephants as all three MTBCs are endemic in South
Asia. TB is increasingly being identified in captive elephants in South Asian range countries and this directly poses a great risk of TB transmission from infected elephants to wild bull elephants that frequently visit captive herds for mating, to other endangered mammalian species, as well as to the people working with them. The regular screening of elephants for TB will help in prevention of this disease in elephants and susceptible hosts which will undoubtedly help in the conservation of this endangered species.

6. Other cases of TB in wildlife in South Asia

There are few reports of wildlife TB from South Asian countries. One study from a zoological collection in India reported TB in spotted deer [50], while a similar study in Pakistan reported TB in spotted deer and two antelopes, namely, chinkara gazella and black buck [62]. A few studies have reported TB from langur in India and rhesus monkey in Nepal [63, 64], and these may be associated with *M. tuberculosis* transmission from contact with humans as in South Asia as there are many feral monkeys residing in communities. However, TB in wildlife in South Asia has not been adequately studied to fully understand the impact. We hope that in the future an increased awareness of diseases in wildlife and better access to advanced diagnostic tests and technologies that allow for precise MTBC species identification will refocus efforts on the study of TB in wildlife and help to minimize or eliminate risks to species of conservation importance.

7. Conclusion

In summary, in this review we have provided an overview of animal-adapted members of the MTBC and indicated the role of wildlife as a reservoir host. In the future, the study of wildlife TB will not only be important for conservation efforts but also for finding potential novel subspecies of the MTBC. We also discussed in detail reports of *M. orygis*-associated TB in wildlife of South Asia, highlighting recent studies from Nepal and Bangladesh. The finding of *M. orygis* in a free-ranging rhinoceros, an endangered species, with evidence for the wide distribution of *M. orygis* attests to its threat for wildlife conservation in the region. The evidence of cattle as a potential reservoir host of *M. orygis* in South Asia warrants further analysis and increased surveillance in livestock so as to mitigate the risk to public health. Our review of elephant TB from different countries demonstrates another threat to the conservation of indigenous wildlife in the region. The cultural and socioeconomic values of elephants, in addition to their conservation importance, support the need for continued surveillance and appropriate management and response to this disease threat. Given the paucity of research in this area, with a low number of publications, we think it evident that wildlife TB is overlooked and underexplored. The topics discussed here and the paucity of scientific studies signify a greater underlying problem. We sincerely recommend that further studies be pursued as a matter of urgency to explore the threat of TB to wildlife in South Asia.
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