



# Tuberculosis in wild animals in India

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## Abstract

India is renowned for its complex megadiverse ecosystems and abundant biodiversity. Bovine tuberculosis (bTB) often remains synonymous with *Mycobacterium bovis* infection in cattle. The domain of tuberculosis (TB) among wild animals, induced by members of the *Mycobacterium tuberculosis* complex organisms (MTBC), is often underexplored and underreported in India. Within this context, instances of wild animal tuberculosis (wTB) have manifested across both captive and free-roaming animals. The sources contributing to wTB in animals can be human, animal, or environmental factors, thus illuminating the complex transmission pathways. The diagnosis of wTB continues to pose a formidable challenge, a consequence of the expansive taxonomic diversity in both the host and the pathogen. Complications inherent in acquiring samples from wildlife, the absence of standardized diagnostic protocols, limited insights into infection prevalence, and resource constraints compound diagnosis. Amidst these, *adopting* the comprehensive One Health paradigm surfaces as an imperative, accentuating the interconnectedness bridging human, animal, and environmental health. Recognizing key stakeholders and fostering intersectoral collaboration to provide enhanced diagnostic techniques driven by skilled personnel and advanced infrastructure play pivotal roles in a comprehensive strategy. Additionally, leveraging vaccination efforts contributes to effective control. A national wTB surveillance program is a cornerstone, ensuring an integrated and holistic approach to disease management. Through this review, we delve into the current landscape of wTB in India, unveiling its multifaceted challenges, and further explore the multifarious strategies that the One Health approach proffers in this dynamic endeavor.

**Keywords** Tuberculosis · *Mycobacterium tuberculosis* complex · One health · Wild animal · Bovine tuberculosis

## Introduction

India is a megadiverse country with rich biodiversity and varied ecosystems and has long been home to an array of wildlife species (Ministry of Environment and Forests 2014). With rapid industrialization and urbanization, there is a lesser demarcation between wildlife and human habitats, leading to increased interaction and conflicts between humans and wild animals (IUCN 2022). While it is reported that around 60% of emerging infectious diseases are zoonotic in nature, it is documented that out of the 30 new pathogens that have been identified over the last thirty years,

75% of the diseases have an animal origin (WHO EMRO 2014). Tuberculosis (TB) is a well-recognized global public health issue affecting millions of people worldwide (WHO 2022). While TB is commonly associated with human populations, its presence in wildlife has emerged as a significant challenge.

Bovine tuberculosis (bTB) is often misconstrued as a chronic illness exclusive to cattle caused by *Mycobacterium bovis*. As per the WOAHA Terrestrial Manual 2022, the primary pathogens linked with TB disease in animals comprise *M. bovis*, *M. caprae*, *M. microti*, *M. orygis*, and *M. pinnipedii* (World Organisation for Animal Health 2022), while cases of TB disease due to *M. tuberculosis* have also been reported in animals (Palaniyandi et al. 2019; Veerasami et al. 2018). There have been several reports of TB caused by *Mycobacterium tuberculosis* complex organisms (MTBC) in animals such as deer, blackbucks, pigs, goats, badgers etc. (Refaya et al. 2020). For the purpose of this review, we define wild animal tuberculosis (wTB) as a complex disease

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caused by the members of MTBC in animals that are generally not reared for their products or meat. While numerous programs have been in action to control and eradicate tuberculosis globally in humans as well as cattle, the persistent wTB infection in wild animals remains to be explored and successful eradication/control programs are yet to be designed. Wild animal TB can have detrimental effects on individual animals and wildlife populations. The disease can result in reduced reproductive success, decreased population sizes, and changes in behavior and ecology. Wild animal TB can pose a threat to endangered species, disrupt ecosystem dynamics, and impact biodiversity conservation efforts (Dwyer et al. 2020).

There is also a risk of spillback where the disease could traverse from humans or domestic animals to animals in the wild, owing to the complex interplay between forest and human habitats (Mukherjee et al. 2018; Refaya et al. 2022; Ramanujam and Palaniyandi 2023). Though some wild animals have been recognized as reservoir hosts for wTB, like the European badgers (*Meles meles*) in the UK (Kelly et al. 2021), European wild boar (*Sus scrofa*), red deer (*Cervus elaphus*) in Spain (Santos et al. 2022), the African buffalo (*Syncerus caffer*) in Africa (Lakin et al. 2022), brushtail possums (*Trichosurus vulpecula*) in New Zealand (Warburton and Livingstone 2015), white-tailed deer (*Odocoileus virginianus*) in the USA (VerCauteren et al. 2018) etc., it has been additionally isolated from several other animals (Thoen et al. 2014; Thapa et al. 2017; Dwyer et al. 2020; Bernitz et al. 2021).

Wild animal TB transmission occurs through direct contact or ingestion of contaminated materials, such as respiratory secretions or infected carcasses and also from the environment (Barasona et al. 2017; Allen et al. 2021). In a country like India with a high TB burden in humans, wTB is underreported and often not checked for. Understanding the epidemiology, host range, and dynamics of wildlife TB is crucial for effective surveillance, management, and prevention strategies to safeguard both wildlife and human populations. This review aims to shed light on wTB in India and the need for a One Health approach to combat the disease. This approach is necessary not only to ensure that the animals in the wild do not become diseased but also to ensure that it does not spill back from the wild reservoir hosts to humans or livestock even after disease eradication.

## Wild animal tuberculosis in India

In India, wTB has been reported in both captive as well as free-ranging animals. However, owing to the challenges in diagnosis, the identified cases in free-ranging animals are quite low. Tuberculosis has been identified in numerous

animals in captivity, like cervids, bovids, pachyderms, birds, carnivores and non-human primates (Table 1).

As in the case of any infectious disease, the susceptibility of the host plays a significant role post-exposure to the infectious agent. Host susceptibility in turn depends on the immunological response elicited in the animal to clear the disease. The immunological response to TB infection can vary among different animal species. It may be influenced by various factors, including the genetic makeup of the host, the virulence of the TB strain, and the overall health of the animal (Dwyer et al. 2020). Some of these animals may be maintenance hosts, where the infection persists in the population even without an external source. In contrast, other animals may be dead-end hosts, where the infection would no longer persist once the source of infection has been removed (Madeira et al. 2017). It is of paramount importance to find out which animals are maintenance hosts in the Indian context in order to design proper disease control and eradication protocols.

It is evident from Table 1 that a wide array of animals is susceptible to TB infection, a substantial number of studies in India have reported wTB infection in Cervids and Bovids. This observation is noteworthy because, even though the reason for their increased susceptibility and persistence of infection in them still remains an enigma, cervids have been identified to be MTBC maintenance hosts in USA (white-tailed deer), Spain (red deer and roe deer (*Capreolus capreolus*)) while bovids have been identified in Zambia (lechwe (*Kobus lechwe*)) and the sub-Saharan Africa (African buffalo) (Hang'ombe et al. 2012; Santos et al. 2015; VerCauteren et al. 2018; Lakin et al. 2022). Contemporary information suggests that the frequency of emergence and reemergence of infectious diseases in wildlife reservoirs has risen, prompting new concerns about disease pathogenesis and epidemiology and heightening the urgency of identifying effective wildlife disease management strategies (Rhyan and Spraker 2010). Many of the animals reported here are protected under the Wildlife Protection Act 1972, like black buck, gazelle (*Gazelle bennettii*) and tiger (*Panthera tigris*) (Government of India 1972), and identification of wTB in these animals hampers efforts to ensure their conservation.

It is very interesting to note that the conventional animal pathogen, *M. bovis*, that has been the cause of wTB elsewhere in the world, has only been identified in a few of the reported studies so far. The predominant pathogen that causes TB in these animals, as reported, is *M. tuberculosis*, especially in captive animals that are constantly exposed to humans. Infection in wildlife has also been reported to be caused by *M. orygis*, the native oryx pathogen that was added to the list of MTBC species in 2012 (van Ingen et al. 2012). This pathogen has also been identified in captive wild animals such as blue bull (*Boselaphus tragocamelus*),

**Table 1** Cases of wild animal tuberculosis reported in India

S.no	Reference	Animal infected	Captive/ free-ranging	Species of MTBC	State	Remarks
1.	Sanyal 1892	Mona Monkey ( <i>Cerco- pitheous mona</i> ) Sumatran Rhinoceros ( <i>Rhinoceros sumatrensis</i> ) Hairy tapir ( <i>Tapirus roulini</i> ) The park housed more than 45 species of aquatic birds, includ- ing the mandarin duck, jacana, common snipe, Eider duck etc. The park housed more than 45 species of aquatic birds, includ- ing the various types of kaleege birds, silver pheasants, the Bor- nean firebacks, roufús tailed pheasant etc.	Captive	NA	West Bengal	Most captive Mona monkeys succumbed to tuberculosis, where lesions were found in the liver during post mortem analysis.  A case of TB was diagnosed in one rhinoceros during post mortem examination. Lesions were found in lung and lymph nodes.  One female tapir suffered from obstruction of bowels and died three days later. Lesions suggestive of TB were found in the lungs and liver during post mortem examination.  The aquatic birds share a common enclosure and it was noted during post mortem analysis that TB like lesions were present in the lungs and liver.  The birds were held in captive enclosures and TB lesions were commonly found in lungs and liver during post mortem examination.
2.	Liston and Soparkar 1922	Deer (general), llamas, antelopes,	Captive	NA	Maharashtra	TB epidemic was recorded in the Bombay Zoological Gardens in 1915, where the disease had spread to around 30 animals. The authors speculate the origin of the epidemic might be a llama imported from Germany.
3.	Chan- drasekharan and Krish- namurthi 1951	Macaca monkey	Pet	<i>M. tuberculosis</i>	Tamil Nadu	A pet monkey belonging to an army officer (captured from Myanmar (then Burma) was brought to Madras Veterinary College hospital for treatment of ulcerating open sinus wounds discharging thick creamy pus. The animal deteriorated and passed away 4 months after the initial admission. At the time of necropsy, the original wounds were still present and gross visible cheesy lesions typical of TB infection was found in the underlying musculature. Caseo-purulent lesions were also found in kidneys and liver. Lymph nodes were enlarged and caseating, and pin head lesions were found on the lungs. On staining the exudates from these lesions, several acid fast bacilli were found. They were further cultured on suitable media and histopathology showed well developed granulomas.  The exudate from the liver were inoculated into two guinea pigs and two rabbits, and the animals died between 12–18 weeks post infection, showing TB like lesions in various organs.  A notable mention in this paper is the mode of infections the authors suggest. Since there were no lesions present in the alimentary tract despite their presence in the lings and other internal organs, suggesting the route of infection is neither respiratory nor alimentary. The authors argue the infection might be introduced via the ulcerating sinus wounds.
4.	Rai and Chan- drasekharan 1958	Giraffe ( <i>Giraffa camelopardalis</i> )	Captive	<i>M. tuberculosis</i>		The 12 year old male giraffe suffered from diarrhea and succumbed to death following treatment. Post mortem examination revealed TB like lesions in the lungs. Tuberculosis was confirmed to be caused by <i>M. tuberculosis</i> using microscopy, culture and biochemical tests. The results of the <i>in-vivo</i> tests conducted in guinea pigs and rabbits also confirmed TB, since similar lesions were observed in lungs. Histopathological analyses revealed typical TB granulomas.
5.	Basak et al. 1976	Langur ( <i>Semnopithe- cus entellus</i> ), stumped monkey ( <i>Macaca arc- toides</i> ) and porcupine ( <i>Hystrix indica</i> )	Captive	<i>M. bovis</i>	West Bengal	Post mortem examination following natural death of two monkeys and one porcupine revealed tuberculosis like lesions in their lungs. They were confirmed as <i>M.bovis</i> using microscopy, culture and biochemical tests performed on the cultured isolates.

Table 1 (continued)

S.no	Reference	Animal infected	Captive/free-ranging	Species of MTBC	State	Remarks
6.	Rathore and Khhera 1979	Spotted deer ( <i>Axis axis</i> ) and barking deer ( <i>Muntiacus vaginalis</i> )	Captive	NA	NA	The paper identifies TB as the cause of death in 16 spotted deer and 1 barking deer based on post mortem examination alone. However, the source and nature of pathogen is unknown.
7.	Rathore and Khhera 1981	Black buck ( <i>Antelope cervicapra</i> )	Captive	NA	Maharashtra and Delhi	The paper describes the causes of mortality in captive members of the Bovidae family across 13 zoological parks in India. From around 220 animals that died due to various reasons between the years 1975–1979, TB was found in 5 black bucks (3 from Bombay (now Mumbai) and 2 from Delhi)
8.	Singh et al. 1986	Deer (general)	NA	NA	Bihar	About 38 deer were autopsied and among them 18 deer revealed tuberculous lesions in their lungs. Acid fast bacilli were present in these lesions, and histopathology revealed granulomas. The nature of deer and their cause of death were not mentioned.
9.	Uyadhyaya et al. 1986	Sambar deer ( <i>Rusa unicornis</i> ), hog deer ( <i>Axis porcinus</i> ) and spotted deer	Captive	<i>M. tuberculosis</i>	Assam	The deer were housed together in the same enclosure and died successively, showing emaciation. TB like lesions were found in the lungs and mediastinal lymph nodes were enlarged. Tuberculosis was confirmed to be caused by <i>M. tuberculosis</i> using microscopy and biochemical tests. The results of the <i>in-vivo</i> tests conducted in guinea pigs also confirmed TB. Histopathological analyses revealed typical TB granulomas.
10.	Shah et al. 1986	Spotted deer and black buck	Captive	<i>M. tuberculosis</i>	Gujarat	A 5 year old female spotted deer and 1 year old male black buck died after prolonged wasting and laboured breath. TB like lesions were found in the lungs. Tuberculosis was confirmed to be caused by <i>M. tuberculosis</i> using microscopy. Histopathological analyses revealed typical TB granulomas.
11.	Gangadharan et al. 1996	Elephant ( <i>Elephas maximus</i> )	Captive elephant, involved in temple procession	NA	Kerala	Lung samples were collected during post mortem examination of a captive elephant that participated in temple processions upon observing peculiar TB like lesions. The cause of death was a puncture wound in the abdominal region caused by another elephant during a festival temple procession. Tuberculosis was confirmed using microscopy and histopathology. The authors speculate the causative organism to be <i>M. elephantis</i> , owing to the peculiar appearance of the lesions. However, no confirmatory tests have been made.
12.	Valandikar and Raju 1996	Black rhinoceros ( <i>Diceros bicornis</i> )	Captive	NA	Karnataka	Two black rhinos (one female, 13 and one male 22) showed symptoms of TB disease, did not respond to treatment with rifampicin (450 mg/day), terramycin (500 mg/day) and died in the premises. Post mortem examination in both animals revealed TB like lesions. Further, microscopy and culture were used to confirm TB infection. It is of interest to note that the mycobacterial species identified from the male rhino was resistant to both rifampicin and isoniazid.
13.	Rao and Acharyo 1996	Spotted deer, barking deer, sambar deer, black bucks, four horned antelope ( <i>Tetracerus quadricornis</i> ), hog deer, gaur ( <i>Bos gaurus</i> ), wild pigs, primates, birds, wallaby	Captive	NA	Assam	Tuberculosis infection was confirmed using post mortem examination, microscopy and histopathology in 17 spotted deer, 9 barking deer, 6 sambar deer, 5 black bucks, 1 four horned antelope, 1 hog deer, 1 gaur, 15 wild pigs, 7 primates, 5 birds, and 1 wallaby between the years 1967 and 1990.
14.	Tanwar et al. 2001	Spotted deer	Captive	NA	Rajasthan	Tuberculosis infection was confirmed using post mortem examination, microscopy and histopathology in one spotted deer.
15.	Baviskar and Bhandarkar 2010	Barking deer	Captive	NA	Maharashtra	Post mortem examination performed upon death of a 4-month-old male barking deer revealed TB like lesions. Microscopy confirmed acid fast bacilli and histopathology confirmed typical TB granulomas.
16.	Rishikesavan et al. 2010	Leopard ( <i>Panthera pardus</i> )	Captive	NA	Karnataka	A 22 years old male leopard exhibited weakness, lethargy, inappetence, chronic weight loss, emaciation and respiratory distress. Post mortem examination performed upon death revealed TB like lesions. Microscopy confirmed acid fast bacilli and histopathology confirmed typical TB granulomas.

**Table 1** (continued)

S.no	Reference	Animal infected	Captive/free-ranging	Species of MTBC	State	Remarks
17.	Verma-Kumar et al. 2012	Elephant	Captive	<i>M. tuberculosis</i>	Tamil Nadu, Kerala, Karnataka	From about 600 captive Asian elephants spread across 3 states, a random of 179 serum samples were collected from 179 animals. ELISA was carried out using for <i>M. tuberculosis</i> specific antigens ESAT-6, CFP10, PE_PGRS17 and PE_PGRS11, a diagnostic kit developed in house. For samples that were positive by ELISA, trunk washes were collected from respective animals and TB isolation was carried out with no success. However, upon natural death of one of the animals during the study period, <i>M. tuberculosis</i> was isolated from lung sample.
18.	Fefar et al. 2012	Sloth bear ( <i>Meursus ursinus</i> )	Captive	NA	Gujarat	Post mortem examination performed upon death of 23 year old female sloth bear revealed TB like lesions. Microscopy confirmed acid fast bacilli and histopathology confirmed typical TB granulomas.
19.	Gho-dasara Priya et al. 2014	Nilgai ( <i>Boselaphus tragocamelus</i> )	Captive	NA	Gujarat	Post mortem examination performed upon death of a nilgai revealed TB like lesions. Microscopy confirmed acid fast bacilli and histopathology confirmed typical TB granulomas.
20.	Sharma et al. 2014	Spotted deer, hog deer, nilgai,	Captive	NA	Uttar Pradesh	The study records TB as the cause of death in 19 spotted deer, 2 Nilgai and 1 spotted deer based on post mortem examination, microscopy and histopathology.
21.	Budhe et al. 2014	Deer (general)	Captive	NA	Maharashtra	Passive haemagglutinin test was performed on sera samples of 48 deer. The estimated prevalence of tuberculosis in the present study caused by <i>Mycobacterium tuberculosis</i> was 8.33% and that of <i>M. bovis</i> was 2.08% (no molecular test was done to ascertain this)
22.	Mukherjee et al. 2015	Black buck and gazelle ( <i>Gazelle bennettii</i> )	Captive	<i>M. tuberculosis</i>	Western India	Lung and pre-scapular lymph node tissue were collected from one gazelle and one black buck during post mortem analysis. <i>M. tuberculosis</i> was confirmed using nested PCR and commercial line probe assay genotyping kits (Hain Lifescience, Germany) that specifically identifies MT within the MTC group.
23.	Nigam et al. 2016	Tiger ( <i>Panthera tigris</i> )	Captive	NA	Uttar Pradesh	The report records two cases of TB in tigers, one case in Uttar Pradesh and one in Tamil Nadu
24.	Hedau and Kamdi 2016	Sloth bear	Captive	NA	Maharashtra	The postmortem examination on a captive 10-year-old male sloth bear from Maharaj Bag Zoo revealed a pale mucous membrane revealed presence of large numbers of caseative nodules of various sizes with enlarged and calcified mediastinal lymph nodes. The impression smears prepared from the caseative nodules of lung showed abundant acid-fast tubercular bacilli. Histopathological examination of lungs revealed typical TB granulomas. Molecular methods were not performed to identify species.
25.	Chandranak et al. 2017	Elephant	Free-ranging	<i>Mycobacterium tuberculosis</i>	Karnataka	Following natural death, lung samples were collected from a free roaming Asian elephant (male, ~ 65years). <i>Mycobacterium tuberculosis</i> was isolated from lungs, confirmed using histopathology, PCR and nucleotide sequencing of the PCR product.
26.	Zachariah et al. 2017	Elephant	Free-ranging	<i>Mycobacterium tuberculosis</i>	Kerala	A total of three free-ranging wild Asian elephants in Wayanad Wildlife Sanctuary were confirmed to be infected with <i>M. tuberculosis</i> , using PCR and whole genome sequencing.
27.	Konch et al. 2017	Giraffe	Captive	<i>M. bovis</i>	Assam	Upon finding TB like lesions during post mortem examination, tissue samples were collected from lungs, liver and lymph nodes and subjected to Mycobacterial culture isolation and histopathology. DNA extracted from cultured isolates were subject to PCR using primers for <i>M.bovis</i> specific <i>oxyR</i> and <i>prnCA</i> primers. Histopathological analysis revealed typical granulomas.
28.	Veerasami et al. 2018	Sloth Bears, Elephants, Felidae, Cervidae, African Buffaloes ( <i>Syncerus caffer</i> ), Wild dog and Wild Bear	Captive	NA	Karnataka	Sloth Bear sera samples were from Wildlife SOS India and 48 sera samples from other wild animals such as those mentioned were collected from captive animals housed in various zoological parks and temples in India. Veerasami et al., developed a Rapid TB antibody detection kit using recombinant fusion protein of ESAT-6::CFP-10 along with purified protein derivatives (PPDs) of <i>M.bovis</i> , validated the kit and used it to identify TB infection in the animals.
29.	Mukherjee et al. 2018	Black buck and gazelle	Captive	<i>M. tuberculosis</i>	Gujarat	Post mortem lung sample from one black buck and one gazelle were collected from Gujarat, India. <i>M. tuberculosis</i> was confirmed using culture and spoligotyping. Spoligotyping further revealed that these belonged to the EAI3_IND lineage, that was prevalent among the human beings in that area.

Table 1 (continued)

S.no	Reference	Animal infected	Captive/ free-ranging	Species of MTBC	State	Remarks
30.	Hota et al. 2020	Spotted deer	Captive	<i>M. tuberculosis</i>	Odisha	Lung tissue samples were collected following natural death from a captive spotted deer. <i>Mycobacterium tuberculosis</i> was isolated from lungs, confirmed using culture, Real time PCR and 16srRNA sequencing.
31.	Marihaik et al. 2022	Sloth bear	Captive	<i>M. tuberculosis</i>	Karnataka	The study included post mortem samples from four sloth bears (comprising two males and two females, ~14–16 years of age), which were rescued from circus companies and relocated to the Bear Rescue Center (BRC), Karnataka, India. <i>Mycobacterium tuberculosis</i> was isolated from lungs, confirmed using histopathology, PCR and nucleotide sequencing of the PCR product.
32.	Sharma et al. 2022	Blue bull, Black buck, African buffalo, Gazelle, Spotted deer, Sambar deer, Sloth bear, Rhesus monkey, langur, Elephant.		<i>M. tuberculosis</i> , <i>M. bovis</i> , <i>M. orygis</i>	Across India	A total of 89 animal tissue samples from 23 different zoological parks/botanical gardens/reserve parks were tested for TB infection. A total of 32 (35.95%) cases were confirmed as TB, comprising of 12 carnivores, 9 bovinds, 6 cervids, 4 non-human primates, and a pachyderm. The TB lesions in the lungs, liver, and lymph nodes varied from the large-sized caseous nodules filled with dry cheesy material in bovinds and cervids to variable-sized cavitations containing liquefied caseum in carnivores' lungs. The lungs, livers, and spleens of non-human primates exhibited small to medium-sized nodules. Molecular detection by PCR revealed <i>M. tuberculosis</i> (12 carnivores, 2 non-human primates and 01 pachyderm), <i>M. bovis</i> (2 cervids and 1 bovid) and <i>M. orygis</i> (2 cervids and 1 bovid). Histopathological analyses revealed granulomas in various stages, grouped according to the extent of necrosis, the presence of mineralization, giant cells, and fibrous encapsulation. <i>M. orygis</i> was isolated post mortem from lung and lymph node samples of free-ranging wild ungulates, specifically two black bucks and one spotted deer. It was confirmed using culture, microscopy, PCR, spoligotyping and whole genome sequencing.
33.	Refāya et al. 2022	Black buck, spotted deer	Free-ranging	<i>M. orygis</i>	Tamil Nadu	
34.	Sharma et al. 2023	Spotted deer, Indian Bison	Free-ranging	<i>M. orygis</i>	Gujarat	Upon natural death, lung, liver and lymph node samples were collected from two adult free-ranging spotted deer (1 male and 1 female) and one emaciated adult male bison. <i>Mycobacterium orygis</i> was confirmed using culture, histopathology, PCR and WGS.

spotted deer (*Axis axis*) and rhinoceros (*Rhinoceros unicornis*) in Nepal and Bangladesh (Thapa et al. 2016; Rahim et al. 2017). *Mycobacterium orygis* has also been isolated from humans in India (Duffy et al. 2020). Considering the changing landscape of bTB infection, Vitale hypothesizes that *M. bovis* became a pathogen of *Bos taurus* in Europe and the Americas, whereas *M. orygis* became a pathogen of *Bos indicus* in south Asia, urging the need for proper delineation of MTBC species that have been isolated from animals (Vitale 2020).

## Sources of disease spread and persistence of infection in wildlife

Several factors are responsible for the presence of MTBC in wildlife. Wildlife TB in animals can be attributed to human, animal, and environmental sources. However, in many cases, a combination of sources is responsible for infection as well as disease persistence of wTB in wildlife (Fig. 1).

### Human sources

In India, reports of wTB until now have largely been from captive animals. There may be two reasons for this: the animals in captivity are constantly exposed to the human population, from where they are infected with the disease, or even if the disease is a pre-existing condition from some other source, the animals in captivity are periodically subject to health check-ups and diagnosed for health conditions if they are ill or show symptoms for any health condition. There have been evidences of transmission of TB infection in cattle from human sources as well as in non-human primates, elephants and psittacine birds in the wild as well as captive animals reported globally (Montali et al. 2001; Oceppek et al. 2005; Romero et al. 2011; Marieke et al. 2016; Miller et al. 2019; Lombard et al. 2021).

India accounted for 34% of TB deaths globally in humans in 2020 and 26% of the global TB prevalence, highest burden recorded (WHO 2021). The National TB prevalence conducted in humans in India, records a prevalence of 312 per 1,00,000 population (2021a). Since TB in humans is endemic in India, though the source of infection in many captive animals listed in Table 1 is unknown, these animals are constantly in contact with animal handlers, zoo keepers and the general public, the chance of infection from humans is relatively high.

Humans as a source of wTB has been reported in studies in India, while molecular conclusive evidence is available only for a few studies. Mukherjee et al. in 2018 isolated *M. tuberculosis* from a gazelle (*Gazelle bennettii*) in Gujarat, India that shared a similar spoligotype to the human TB

strains circulating in the area and referred it to be a spillback mechanism from human to animal. (Mukherjee et al. 2018). Similarly, *M. tuberculosis* that has been isolated from sloth bears (*Melursus ursinus*) rescued from circus groups in Karnataka that share a 100% homology to human strains, indicating the possible source of transmission since they were constantly exposed to humans while in the circus (Chandra-naik et al. 2022). An elephant encyclopedia, managed by Dan Koehl, an elephant consultant, records almost 21 wTB deaths in captive elephants (*Elephas maximus*) in India, that resided in temples and/ or zoological parks (Koehl 2023).

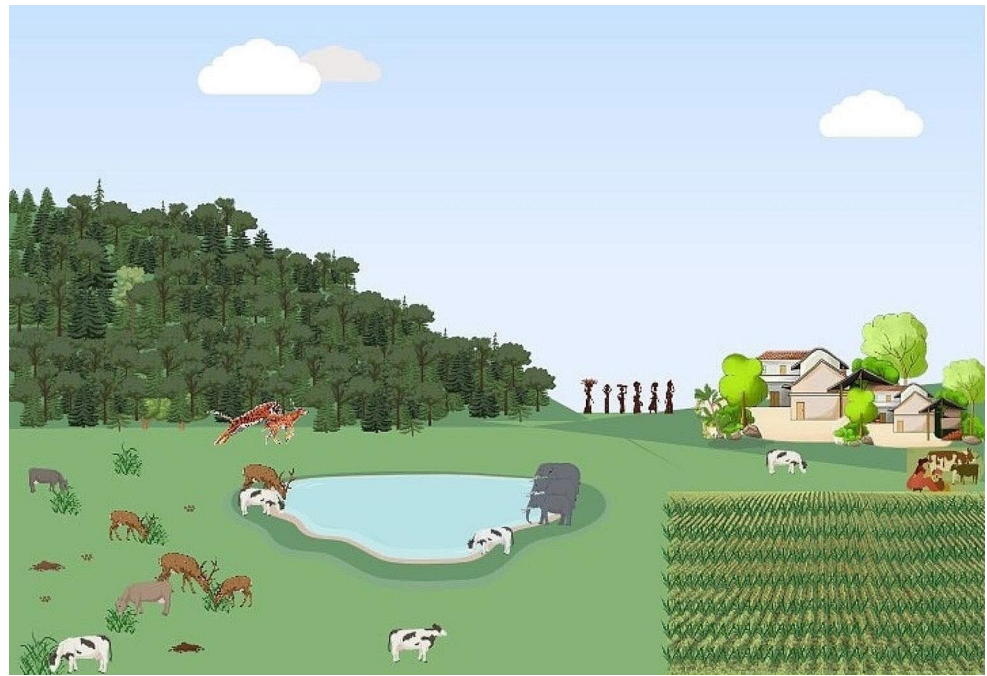
In the case of infections in animals in the wild, humans can be a notable carrier, since India has around 1.73 lakh villages located nearby forests in India. They depend on products from the forest for their livelihood and often forage inside the forest for edible flowers, fruits, firewood etc. (Nayak et al. 2012). The prevalence of TB infection in tribal populations in India, according to a systematic review, is 703 per 100,000 population. The review accounts for many indigenous tribes of India who live in or near forests (Thomas et al. 2015). Transmission of infection from such humans should also be considered.

There is growing interest among tourists in India with forest-related activities, like trekking, safaris, cabin stays in forests, etc. (Nandanwar 2020). This has created a nexus of vendors, guides, resort staff, travel agencies, chauffeurs etc. to engage in tourism-related activities that involve travel to deep wildlife covers that were earlier not widely explored by human population (The Times of India 2021). Though hunting is illegal in India, hunting, and poaching animal parts as well as animals have been on the rise owing to the lucrative market and the ill-gotten profits (Rana and Kumar 2023). These might lead to the exposure of animals that reside in these parts to wTB and may significantly contribute to the spread of the disease.

### Animal sources

One of the major sources of disease spread documented for wTB worldwide has been animals. The spread of wTB at the wildlife-livestock interface has been extensively documented and their repercussions analyzed across the globe (Hang'ombe et al. 2012; Malama et al. 2013; Mohamed 2020; Sichewo et al. 2020). In the Indian context, there is an undisputable risk of infection spread at the wildlife-livestock interface since there is considerable infringement of human activities into conserved forest spaces (in the context of national parks, biosphere reserves etc.) that results in the sharing of natural resources. This is mainly due to human activities like shifting cultivations, land encroachments, construction of hydroelectric projects, conversion of forest areas into plantations etc. These activities have in turn

**Fig. 1** Possible sources of tuberculosis transmission in a typical fringe village in India. Humans depend on the forest for its many products and hence keep visiting it. Cattle and wild animals co-graze and drink water from the same sources. Shed faecal material can be commonly seen on the pastures. There is often predation by carnivorous species in these villages as well



increased the potential interaction of wildlife with livestock which would otherwise be impossible (Oommen 2021).

This changing landscape forces animals to visit the infringement spaces in search of food, water and in some cases, even shelter (Nayak et al. 2012). While studying the human-wildlife interactions in 4 wildlife sanctuaries in Rajasthan (Jaisamand Wildlife Sanctuary, Kumbhalgarh & Todgarh Raoli Wildlife Sanctuary, Phulwari ki Nal Wildlife Sanctuary and Sitamata Wildlife Sanctuary), Karanath et al. 2017 reported that nearly three out of four households in the area report damage to their crops by 20 species of wild animals, the maximum damage done by nilgai (*Boselaphus tragocamelus*), jackal (*Canis aureus*) and wild boar (*Sus scrofa*). Interestingly, while the wild boar has been recognized as a wTB reservoir in Europe, no studies have been performed in India to assess the presence of wTB in the said animal (Karanath et al. 2017). In a similar study conducted earlier in 1998 in and around the villages in Sariska tiger reserve in Rajasthan, Nilgai and wild boar were responsible for the destruction of more than half the crops in the area (Sekhar 1998). Sometimes, herbivores graze in pastures alongside the farming cattle. Considering that the prevalence of bTB in India is 7.3%, and its prevalence in human population, the chance of disease spread by traditional aerosol inhalation is very high (Srinivasan et al. 2018). Transmission from cattle to different species of wildlife has been reported in several countries worldwide, like South Africa, Ethiopia, Uganda, Nigeria, Sudan, Ghana, New Zealand, Australia, USA, Canada etc. (Thoen et al. 2014). Reports of wTB transmission among wildlife have also been reported in South Africa (Roos et al. 2023).

In addition to the infection caused by *Mycobacterium avium* complex in birds, they are also susceptible to infection by *M. tuberculosis*. The isolation has been reported in amazon parrots and a green tailed macaw (*Ara chloropterus*) in New York City, USA (Montali et al. 2001). A case of human to bird transmission has also been identified in an African Grey parrot (*Psittacus erithacus erithacus*) that was imported from Zaire to Germany, using culture and molecular diagnostic methods. It was noted that the owner of the bird had successfully completed anti-tuberculosis treatment 2.5 years before the parrot was diagnosed. *Mycobacterium tuberculosis* strain isolated from the bird and the owner showed identical spoligotyping pattern (Schmidt et al. 2008). A case of *M. tuberculosis* was also reported in a green-tailed macaw in Switzerland, while the source of the disease was not reported (Steinmetz et al. 2006). Since birds easily traverse from the fringe villages into the forest covers, and are an essential part of the forest ecosystem, it is possible that they could also be carriers of the disease.

Another route of wTB transmission in animals is the alimentary route that would affect carnivores that prey on infected animals. Several free-ranging lions (*Panthera leo*) are reported to be affected with wTB in the Kruger National Park in South Africa, because they prey on infected African buffaloes, which are known reservoirs of wTB infection in South Africa (Gumbo et al. 2022). In addition to this, free-ranging African leopards (*Panthera pardus*) and African wild dogs (*Lycaon pictus*) have been diagnosed with tuberculosis caused by *M. bovis* (Bernitz et al. 2021). *Mycobacterium bovis* was also isolated from African wild dogs in various locations, including Kruger National Park

(KNP), Hluhluwe–iMfolozi Park (HiP), and a private facility in Hoedspruit. The authors noted the lesions in tissues of the gastrointestinal system (such as mesenteric lymph nodes, liver, and intestines), as well as in other tissues which led them to speculate that these animals might have been infected through the oral route (Meiring et al. 2021). In an earlier study conducted in Kruger National Park, *M. bovis* isolated from lions, cheetahs (*Acinonyx jubatus*) and leopards were similar to those identified in the African buffalo, as confirmed by restriction fragment length polymorphism (Renwick et al. 2007). In Spain, *M. bovis* was isolated from an Iberian lynx (*Lynx pardinus*), and the possible route of infection as suggested by the authors could be the fact that they prey on red deer in the area, that are known wTB reservoirs (Briones et al. 2000). In Michigan, USA, *M. bovis* was isolated from the lymph nodes of coyotes (*Canis latrans*), raccoons (*Procyon lotor*), red fox (*Vulpes vulpes*), and black bear (*Ursus americanus*). The authors of this study speculate that the nature of these animals to prey on the white-tailed deer, a known wTB reservoir host in the area, may be the source of infection (Bruning-Fann et al. 2001). Since carnivorous predators prey on the weakest member of the herd, the risk of transmission of infection via the alimentary route is further amplified. Another alternative route of infection in predators may be considered respiratory, that occurs during the terminal asphyxiation of their prey, when they puncture their airway (Renwick et al. 2007).

In India, there are several instances of predators interacting with potentially infected prey. In Uttarakhand, in the area surrounding the Corbett National Park, tigers (*Panthera tigris*) and leopards were responsible for 8365 incidents of livestock predation in 356 villages and Gujjar settlements between 2006 and 2015 (Bargali and Ahmed 2018). A study was conducted in the villages surrounding sixteen wildlife sanctuaries in Tamil Nadu. Forty-eight percent ( $n=694$ ) of households reported losing livestock because of large carnivores. They recorded 866 incidents of livestock predation involving leopards, dholes (*Cuon alpinus*), and tigers. 78% of these farmers experienced multiple incidents of livestock predation (Ramesh et al. 2020). During a 14-year period (1990–2003) in the Binsar Wildlife Sanctuary in Uttarakhand, leopards murdered 1,763 domestic animals, 90% of which were cattle. Leopards killed 1 person and injured 9 others in the reserve. (Kala and Kothari 2013). As already cited above, the prevalence of bTB in cattle in India is very high. The possibility of transmission via the alimentary route needs to be considered and adequately addressed.

Apart from animals in the wild coming into human habitations, there are several cases of animals raised around humans being released back into the wild. Around 300 spotted deer and sambar deer (*Rusa unicolor*) were released from the Abhayaranya Eco-tourism center in the forest

cover in the Malayattoor division following the increasing deer population. These animals have been exposed to humans for a long time. Given that many cases of wTB have been reported in deer, the introduction of these animals into the forest may introduce wTB infection there, if not already present (Sreemol 2022). Similarly, the Darjeeling State Zoo has released two pairs of red pandas (*Ailurus fulgens*) into the Singalila national park (Chhetri 2022) and around 30 wolves (*Canis lupus pallipes*) that were bred in captivity at the Sakkarabaug zoo in Gujarat were released into the forest covers at Rann of Kutch and Banaskantha (Kaushik 2022). In Delhi, there is a longstanding problem of overpopulation of stray Rhesus monkeys (*Macaca mulatta*) that cause menace to the people. They often steal food and use the common water pipes to drink water. These monkeys have often been captured and released in the Asola Biodiversity Park by the authorities of the forest department, yet the problem continues (Dixit 2023). Since the physiology of non-human primates is very similar to that of humans, they pose a high risk of becoming reservoir hosts (Sharma et al. 2022). The exposure of these animals to TB-infected humans may make them ideal carriers of TB disease into the forests.

## Environmental sources

The persistence of MTBC species in the environment has been documented as early as 1887, when Hoffmann discovered flies in the residence of a recently deceased TB patient. Hoffmann meticulously gathered these flies, dissected them, and microscopically identified tubercle bacilli in their intestines and feces. In 1904, Lord conducted an experiment, wherein distinct groups of flies were fed varying substances— one consumed meat, sugar, and water (control group), another consumed sputum sourced from infected TB patients, and a third group consumed sputum from uninfected individuals. Among the flies ingesting TB patient sputum, *M. tuberculosis* was detected in both the feces and intestines of all flies. In stark contrast, control flies exhibited no presence of *M. tuberculosis* in either excrement or intestines. Subsequently, the excrement from the flies fed TB patient sputum was left to sit for different time spans (1, 8, 15, 28, and 55 days). The excrement from each duration was then administered to guinea pigs. Interestingly, guinea pigs exposed to excrement aged 1, 8, and 15 days all developed TB, while those exposed to feces aged beyond 15 days remained healthy. Several other earlier studies have also successfully reported presence of MTBC in river water, soil (exposed to sunlight and otherwise), dust in the environment around TB patients, in their belongings etc. in the period between 1880 and 1920 and went on to prove the infectivity of the environment by inoculating guinea pigs with suspected infectious material. Mycobacterium

*tuberculosis* complex organisms have also been identified in soil and water in high TB burden countries (Lord 1904; Martinez et al. 2019; Ramanujam and Palaniyandi 2023).

Mycobacterial shedding by infected animals tends to promote indirect transmission through contamination of the environment, while oronasal shedding, in addition to environmental contamination, allows also an easier direct transmission by aerosol. Mycobacteria were initially soil dwellers and have adapted themselves to infect and survive in a variety of hosts (Cambier et al. 2014). It was experimentally shown by Ghodbane et al., that not only *M. tuberculosis*, *M. bovis* and *M. canettii* can survive in the soil for about 12 months, but they remain virulent and are capable of causing infection in mice (Ghodbane et al. 2014). It can hence be speculated that the presence of mycobacteria in water and soil samples from the animal's environment can not only be a passive indicator of wTB, but also help us understand the spread of TB at both the intra and interspecies level.

Mycobacteria are often shed into the environment of the infected animal through oronasal, bronchial-alveolar, fecal and/or urinary excretions. The shedding of MTBC via oronasal, fecal, and urinary routes has been reported in various studies worldwide. In Spain, red deer are often referred to as super shedders for this reason (Lugton et al. 1998; Santos et al. 2015; Dorn-In et al. 2020). The randomized badger culling trial (RBCT) and 1970s British research have reported tuberculous lesions in the renal system of badgers. Badgers may periodically discharge *M. bovis* cells in sputum, feces, and urine after infection. They also dig latrines in their territorial grounds. Exposure of cattle to infected badger urine and feces is a plausible route of transmission (King et al. 2015). A study in France demonstrated that indirect transmission was responsible for transmission of wTB more than direct interactions between badgers, red deer and wild boar (Payne et al. 2017). Environmental transmission of this sort was experimentally proven by Palmer et al. in 2004, where white-tailed deer acquired infection by exchanging pens with experimentally infected cross-bred calves (oral route) with *M. bovis*, whereas naïve calves were able to contract the infection by eating uneaten food from the pens of infected deer (Palmer et al. 2004). The free-living amoeba that live in the soil are also experimentally proven to host *M. bovis*, both in the trophozoite and the cyst form, and aid in their long-term survival in the soil (Sanchez-Hidalgo et al. 2017).

Another aspect of consideration is the infection of MTBC via the percutaneous route. The MTBC present in the environment can infect an open wound, or when animals injure themselves on vegetation contaminated with MTBC. An example of this would be the peculiar case of the greater kudu (*Tragelaphus strepsiceros*) in the South African multi-host system. Abscessation and fistulation of cranial lymph

nodes, most frequently the parotid lymph nodes, is a common sign of clinical *M. bovis* infection in kudu. This characteristic of the pathology caused by *M. bovis* is integral to the suspected epidemiology. It is believed that infectious exudates from draining fistulae contaminate thorns and foliage, such as those of *Acacia* spp. trees, which are the kudu's primary source of food. Other kudus are exposed to *M. bovis* through consumption of contaminated browse or percutaneous thorn injuries (Renwick et al. 2007). Similarly, lesions were found on the skin and salivary glands of field voles (*Microtus agrestis*), the characteristic hosts of *M. microti*, where authors suggest that saliva/ sputum and exudates from the skin may contaminate the environment from which infection may spread to other voles. This is also substantiated by the fact that lesions are found in the salivary glands, suggestive of the route of infection being oral (Kipar et al. 2014).

## Diagnosis of wild animal tuberculosis

Wild animal TB in India has been detected as early as 1892 (Sanyal 1892) in several captive animals in the Culcutta (now Kolkata) zoo in West Bengal. Several cases have been reported from then on (Table 1). However, due to the limitation of their time period or the availability of infrastructure, modern molecular biology techniques were not used in many cases to confirm infection or perform speciation. However, diagnosis in wildlife is difficult due to the wide taxonomic diversity, the difficulties inherent to collection of samples from wildlife (such as capture and restraint), the frequent absence of gold standard diagnostic techniques, the lack of knowledge regarding the actual infection status, the difficulty in interpreting and conducting experimental studies, as well as the limited financial resources (Ryser-Degiorgis 2013).

According to Thomas et al. 2021; the diagnostic tests available for wTB in animals globally can be split into three major categories, namely, (a) the identification of TB-like lesions during post-mortem examination and their subsequent histopathological findings, (b) identification of microorganisms in various samples collected from the animals (microscopy, culture methods followed by biochemical tests, molecular methods such as PCR) and (c) immunological assays, both cell-mediated (tuberculin skin test, interferon-gamma release assay etc.) and antibody-mediated assays (enzyme linked immunosorbent assay (ELISA), multiantigen print immune assay (MAPIA). Each of these methods has its own merits and limitations, and often, a combination of these methods is used to diagnose wTB (Thomas et al. 2021).

There are significant challenges in diagnosing wTB in India, especially in free-ranging animals. The primary challenge is the identification of diseased animals. Many countries like USA and Spain depend on the samples from hunter harvested deer to check for wTB infection (Santos et al. 2015; VerCauteren et al. 2018). In countries like England, feral badgers are captured using steel straps and tests are administered to them while in New Zealand, feral brushtail possums and ferrets are captured and tested (Warburton and Livingstone 2015; Buzdugan et al. 2016). In Hluhluwe–iMfolozi Park South Africa, African buffaloes are captured and wTB tests are administered (Clarke et al. 2022). In India, no such measures are currently employed to identify the animals at the risk of wTB infection and diagnose TB in them.

In the Indian context, the process of wTB diagnosis in wildlife is not only an important first step in the management and control of diseases, but it is also crucial in the assessment of surveillance techniques, pathophysiology, epidemiology, and transmission research, as well as the evaluation of the effectiveness of vaccination trials, once respective policies are in place.

### Existing ways to combat wTB in other countries - few examples

Wildlife tuberculosis is a recognized threat in many countries. Some countries have developed strategies to control the disease, aiming at its eradication.

In New Zealand, a similar policy to ‘test and slaughter’ is in effect to control the prevalence of wTB in brushtail possums. Possums are captured using steel traps, and the tuberculin skin test or the interferon gamma release assay is administered. If the test is positive, the animals are culled. This is predominantly carried out in the livestock-wildlife interface areas, where traps are easily laid. Apart from this, specific strategies to reduce the population of brushtail possums are in place. Poisoned bait, typically poisoned with the metabolic toxin sodium fluoroacetate (1080), is scattered in the forest grounds, and this is fatal when ingested by the possums. The death of non-target species has been significantly reduced, while sporadic cases have been reported in birds. The baits have also been sown via the aerial route to cover more grounds easily. These measures have proven to control the wTB reported in possums, however, complete eradication has not been achieved (Warburton and Livingstone 2015; Nugent et al. 2018).

Control practices of wTB in white-tailed deer in the USA involve a combination of strategies aimed at reducing disease prevalence, including systematic surveillance programs that encompass regular testing of both deer and cattle

populations. In areas where TB is prevalent, targeted culling of infected or high-risk deer has been implemented to minimize disease reservoirs. In Michigan, there exists a multi-phased approach to deer management combining efforts such as culling, hunting regulations, usage of exclusionary fences, strategically locating feed and water for cattle in places that are not accessible to the deer, use of livestock protection dogs to repel away deer. These strategies strive to balance conservation concerns with disease control imperatives (Walter et al. 2012; VerCauteren et al. 2018).

In South Africa, Kruger National Park and Hluhluwe–iMfolozi Game Reserve have implemented a comprehensive array of measures to combat wTB even though a specified national wTB eradication program is not present. The focus on TB control is evident in the surveillance and regular testing of African buffalo populations. They are captured and tested for wTB, followed by culling (in case of wTB positive animal) or release. Selective culling of buffalo in advanced stages of TB serves as a proactive strategy to minimize disease transmission and reduce buffalo’s disease burden. This has been actively in use in the Hluhluwe–iMfolozi Game Reserve, where the park authorities are trying to keep the burden of wTB prevalence in African buffaloes to 10% (Davey 2023). A similar approach is used for black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) as well. Captured animals that exhibit symptoms or animals that have been tested positive are held in separate ‘bomas’ (pens). The establishment of quarantine zones for suspected TB cases acts as a preventive measure, isolating infected individuals to avert transmission. The abattoir at Skukuza serves as a transit point for hunted animals, wherein a primary meat inspection is conducted to identify carcasses that are infected (Dwyer et al. 2020; Brits 2022).

The Madikwe Game Reserve has devised a comprehensive surveillance strategy and buffalo salvage plan with the objective of establishing a buffalo breeding herd that is free from diseases. Sometimes, buffaloes are transmitted from one park to another to promote hunting (Hlokwe et al. 2016). The movement of African buffaloes is based on the Buffalo Veterinary Procedural Notice, that requires Single Intradermal Comparative Tuberculin Test (SICTT) performed on buffaloes before movement. To ensure individual identification and traceability, all buffaloes are microchipped before testing. Ear tags are often utilized in combination with microchips to facilitate the process of identification. Pre-movement testing of buffalo entails testing for the presence of a variety of pathogens, namely *Mycobacterium bovis*, *Brucella abortus*, Foot and Mouth disease, and Corridor disease (Davey 2023).

In England and Wales, wTB in badgers is considered a great challenge to reduce bTB in cattle, especially in border

towns, since there is reported transmission between badgers and cattle, especially in towns that share borders with wildlife covers (Swift et al. 2021). The complex interactions between badgers, cattle, and the environment have led to implementing management strategies to mitigate disease spread. The Randomized Badger Control Trials (RBCT) encompassed a comprehensive series of scientific investigations carried out in the United Kingdom between 1998 and 2007. The RBCT underscored the intricacy of managing bTB within the framework of its control in wildlife reservoirs and the significance of robust scientific investigation in designing disease control methods grounded in evidence. The primary objective of RBCT was to evaluate and generate dependable empirical data regarding the potential efficacy of badger culling as a means of reducing bTB in cattle. Badger culling was either proactive (badgers were culled in designated areas) or reactive (badgers were culled in response to bTB outbreaks in cattle). Badgers are often trapped and shot or shot at night by trained marksmen, taking care to be humane about the process. The results of the study revealed that the implementation of proactive culling measures resulted in a slight decrease in bTB occurrence among cattle in the designated culling zones. However, the effectiveness of reactive culling measures in reducing bTB incidence was not as evident. Furthermore, the investigation examined the phenomenon of ‘perturbation effects’, wherein the act of culling resulted in the dispersion of badgers, potentially leading to further dissemination of the disease. The conclusions of the RBCT have had a significant impact on the formulation of badger culling policies in the United Kingdom. This influence has sparked extensive discussions and debates over the efficacy of such programs as well as the ethical considerations associated with them, with the government scrubbing off the policy and implementing it again in response to public opinion as well as scientific evidence (Independent Scientific Group on Cattle Tb 2007; Cassidy 2019; Van Tonder et al. 2021; Langton et al. 2022).

Several European countries, including Spain, France, Germany, and the United Kingdom, have implemented culling of wild boar as a component of their comprehensive disease control initiatives. In the context of Spain, where bTB poses a substantial obstacle, eliminating infected or high-risk wild boars and red deer has been implemented in conjunction with eradication initiatives. This often includes open hunting seasons in the case of red deer and hunting, combined with trapping in the case of wild boar. Fences are being constructed in border towns to reduce the interaction of wildlife with livestock (Boadella et al. 2012; Che’Amat et al. 2016; Herrero-García et al. 2023). In a similar vein, France has introduced culling procedures to address the transfer of tuberculosis from wild boars to domestic animals (Payne et al. 2017; Réveillaud et al. 2018). In addition to

culling, these countries often employ surveillance, research, and collaboration among various stakeholders to reduce wTB prevalence.

## One health approach

The importance of the One Health approach lies in its recognition that the health of humans, animals, and the environment are interconnected and mutually influential. By viewing these elements as an integrated whole, the One Health approach acknowledges that disease threats and environmental challenges cannot be effectively addressed in isolation. Instead, it emphasizes the necessity of collaborative and interdisciplinary efforts across medical, veterinary, environmental, and other relevant disciplines. This holistic perspective is crucial in combating zoonotic diseases, endangering human and animal populations (OHHLEP et al. 2022). One Health approach will be an effective way to combat wTB, especially since all the three components, human animal and environment are involved in disease transmission and maintenance, and hence there is an inherent need to address the control and elimination of disease from all three fronts. This requires decision-making and action from several stakeholders, both simultaneously and sequentially (Fig. 2).

## Improved diagnostic methods

The diagnosis of wTB serves as the pivotal first step in the comprehensive endeavor to eradicate the disease and is often considered the weakest aspect of TB care and control in humans (Pai et al. 2023). Accurate diagnosis is the cornerstone of informed decision-making. Identifying wTB-infected wild animal populations provides essential data on the disease prevalence, distribution, and dynamics within the ecosystem and lays the groundwork for designing evidence-based interventions. Moreover, poor diagnostics may lead to failure of wTB eradication since many infected animals might be misclassified as disease-free and vice versa.

As explained in “[Diagnosis of wild animal tuberculosis](#)” section, diagnosis wTB in wild animals is challenging, because the samples on which diagnostic tests can be performed are difficult to obtain. Some countries rely on carcasses presented by hunters to perform gross physical examinations for TB-like lesions in animals. While hunting in India is prohibited, ‘found dead’ surveys, like the ones employed for badgers in England and Wales can be employed. Typically, carcasses of badgers that are killed by road traffic accidents, or by natural means are examined for TB-like lesions and tissue samples from the thoracic regions are tested for wTB disease. This method could act

as a passive surveillance method for the detection of wTB in a particular region (Sandoval Barron et al. 2018; Schroeder et al. 2020; Swift et al. 2021). Similarly, in India, when animals are found dead in bioserves, national parks, zoological parks, lung and lymph node samples can be tested for presence of wTB disease (Refaya et al. 2022).

Concurrently, the imperative for development of point-of-care (POC) tests in lower-middle-income countries (LMICs) like India, is pressing, driven by the need to enhance accessibility and accuracy in resource-constrained settings. These tests have the potential to revolutionize diagnostics, offering rapid and on-site results for wTB. In places where access to a central testing facility is limited, these tests can significantly reduce delays in diagnosis, curtail the need for complex sample transportation involving cold storage to preserve specimen integrity. The affordability and user-friendliness of POC tests make them suitable for healthcare providers with limited training (Hobbs et al. 2021). Current WHO approved POC tests for TB infection in humans include line probe assays (GenoType MTB-DRplus, GenoType MTBDRsl, NTM + MDRTB; Hains life-sciences, Nipro), cartridge based nucleic acid amplification tests (GeneXpert MTB/RIF and GeneXpertUltra; Cepheid), chip based nucleic acid amplification tests (Truenat MTB and Truenat MTBplus; Molbio), tuberculosis loop-mediated isothermal amplification (Loopamp MTBC detection kit; Eiken Chemical Company Ltd) (Hong et al. 2022).

Research in POC diagnostics must bring out assays and kits fulfill the WHO-defined ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users) criteria (Hong et al. 2022). They should be capable of identifying wTB in a wide variety of host species and also be able to diagnose wTB caused by different MTBC species, not just *M. bovis*. Since ‘catch and release’ and ‘trap and test’ methods can seldom be employed for free-ranging wild animals, these assays should not rely on nasal swabs and blood samples, but must be able to identify the presence of disease in non-conventional samples, such as urine, feces etc, even in paucibacillary conditions. For instance, in Spain, diagnosis of *M. bovis* in hunted wild boar was done using dried blood spots (Santos et al. 2018). The developed kits should also be able to differentiate vaccinated animals from diseased animals, in the event an administrable vaccine is discovered. It is also essential that research and development of these diagnostic assays happen in India, and their manufacturing be diversified, so that the outcome assay kits are affordable and the dependency on high-priced kits from developed countries can be reduced (Pai et al. 2023). In India, a Rapid TB antibody detection kit using recombinant fusion protein of ESAT-6::CFP-10 and purified protein derivatives (PPDs) of *M. bovis* in the form of an immunochromatographic strip test

has been developed, marking the first step towards development of wTB diagnosis (Veerasami et al. 2018).

### **Adequately trained workforce and improved infrastructure**

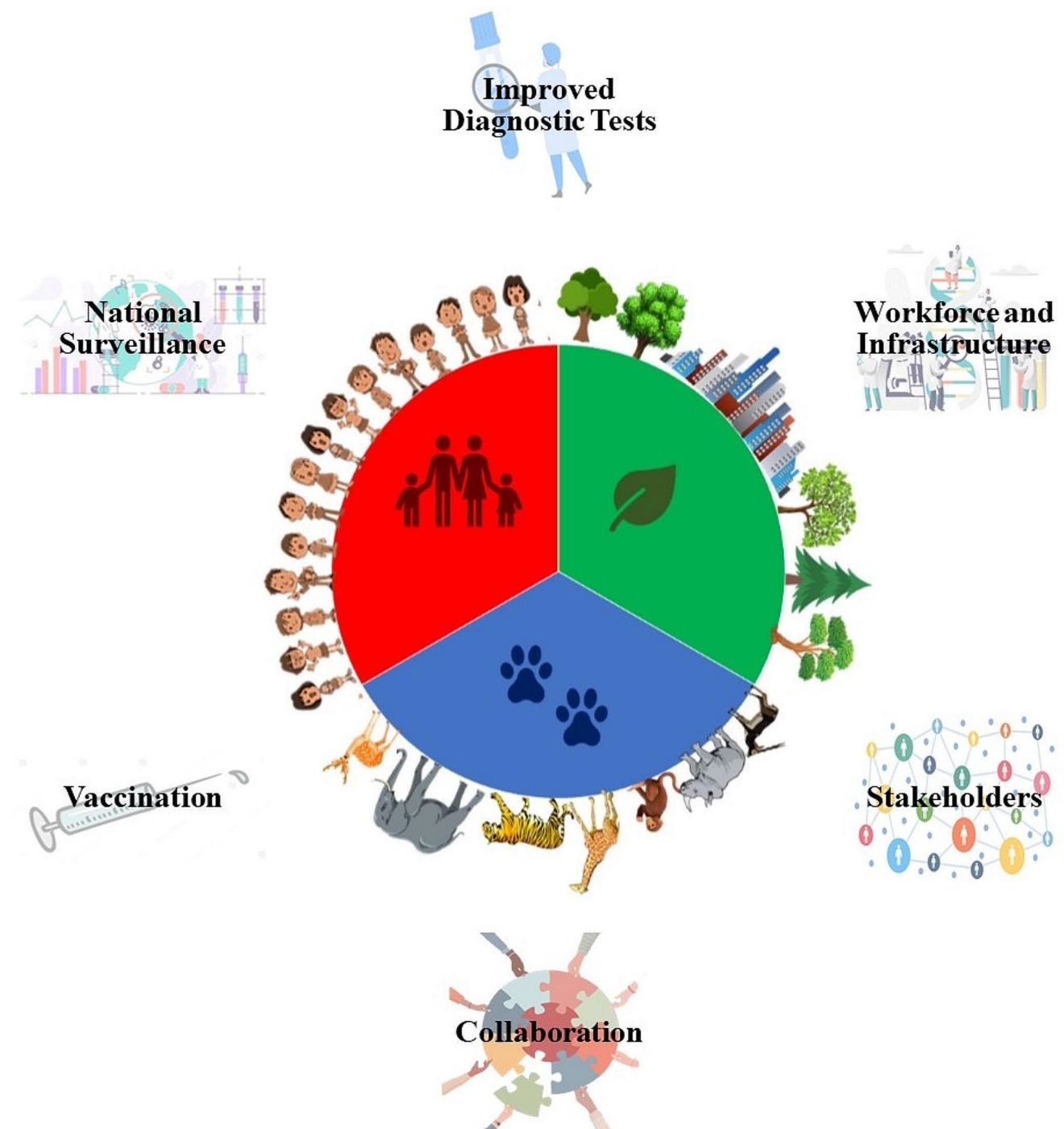
Trained personnel are critical and essential in the eradication of wTB. The presence of trained workforce not only strengthens the technical components of wTB eradication but also serves as a driving force behind effective, long-term, and ethical outcomes in the goal of disease control and ecosystem health. A systematic review in India identifies an overburdened workforce, unqualified practitioners, inadequately trained workforce and inadequate infrastructure as some of the system-side limiting factors in the TB care cascade for human TB patients and a similar corollary can also be drawn for wTB as well (Yasobant et al. 2021).

The multifaceted nature of wTB necessitates using skilled workers with specialized knowledge and competence at all levels of the control program from various disciplines, such as veterinarians, ecologists, technicians, pathologists, and researchers. Trained professionals are required for accurate disease surveillance, as they are capable of recognizing infected wildlife populations, track disease progress, and conduct targeted interventions. They can easily be trained to perform POC and gold standard tests. Their capacity to perform reliable diagnostic tests, analyze results, and manage data ensures that disease assessments are accurate. They are essential for effective management measures such as culling or vaccination campaigns, where they can assure humane and ethical methods, thereby minimizing animal stress and adherence to conservation standards. Moreover, wTB eradication requires skilled personnel to involve local communities, stakeholders, and decision-makers in education and awareness programs. With their extensive knowledge, skilled personnel fosters collaborative efforts, enabling the One Health approach.

Improved infrastructure encompasses research facilities, diagnostic laboratories and transportation systems. State-of-the-art laboratories equipped with adequate equipment and instruments accelerate accurate disease detection and promote research for effective wTB control. India has four National Reference Laboratories (NRLs), one Intermediate Reference Laboratory (IRL) in each large state, and almost 13,000 designated microscopy centres (DMCs) (WHO 2010). Laboratories with BSL-3 containment facilities need to be erected in rural fringe areas, where human TB and wTB testing can be carried out simultaneously. As a welcome move, Indian Council for Medical Research (ICMR) in India has launched a mobile BSL-3 facility (Ministry of Health and Family Welfare 2022). Such mobile facilities can serve as substitutes for laboratories in remote places,

where samples have to be collected in deep forest covers. Efficient transportation systems facilitate the swift transfer of samples, data, and personnel, ensuring timely response and coordinated efforts. The National Strategic Plan for Tuberculosis Elimination (2017–2025) acknowledges that transportation is a significant challenge for human TB

eradication in India, while strategizing to allocate sufficient funds for it (Ministry of Health and Family Welfare 2017). Transportation units, equipped with cold storage units for transportation of samples and/or diagnostic kits and vaccines are required for effective wTB management.



**Fig. 2** Comprehensive One Health approach strategies for wTB control and eradication

## Identification of stakeholders

The interconnected nature of the disease's transmission pathways demands a united effort among diverse stakeholders whose expertise is crucial to understanding the intricate dynamics of wTB and effectively mitigating its impact. By recognizing the shared risks and responsibilities, stakeholders can collectively design interventions considering the broader ecological context, disease reservoirs, and human-animal interactions.

Possible stakeholders in wTB control include governmental bodies responsible for wildlife -Ministry of Environment, Forests and Climate Change; livestock management- Ministry of Fisheries, Animal Husbandry & Dairying; health departments- Ministry of Health and Family Welfare, academic and research institutions, veterinary professionals, ecologists, local communities, indigenous groups and non-profit organizations (Yasobant et al. 2020). Other international agencies include the WHO, the World Organisation for Animal Health (WOAH) and World Wildlife Fund. Each stakeholder brings a unique perspective, expertise, and resources to the table, forming a dynamic network that collaboratively contributes to wTB control efforts.

Local communities and indigenous forest inhabitants are invaluable stakeholders in wTB control since they interact closely with the environment, wildlife, and livestock. Their traditional knowledge and deep understanding of local ecosystems can provide critical insights into disease dynamics and animal behavior. Engaging these communities in wTB control initiatives fosters culturally sensitive interventions, promotes compliance with control measures, and enhances disease surveillance. Their active participation contributes to holistic One Health strategies, acknowledging the interconnectedness of human, animal, and environmental health, ultimately fostering more effective and sustainable wTB control efforts (WHO 2020; Taaffe et al. 2023).

## Collaboration

Collaboration stands as a linchpin in the versatile and complex task of controlling wTB. Only a collaborative approach can adequately address the complex interplay between the three domains of the One Health approach. Collaboration serves as a conduit through which knowledge, resources, and expertise are pooled, transcending disciplinary boundaries and fostering comprehensive strategies. It can bring together experts from fields such as veterinary science, wildlife ecology, public health, microbiology, policy, and community engagement and enables the harmonization of efforts and resources from government agencies, non-governmental organizations, academic institutions, research organizations, and local communities (Yasobant et al. 2019).

Though the importance of collaboration is very well known, it has its own challenges when practiced for wTB control. Effective collaboration of Ministry of Health and Family Welfare (human front), Ministry of Fisheries, Animal Husbandry & Dairying (veterinary front) and the Ministry of Environment, Forests and Climate Change (forest conservation, including protection of wild animals) is seldom observed due to their differing goals and power dynamics. There also exists a disparate allocation of resources and ineffective stratification of health care duties among the state and central health departments that makes collaboration even more complex (Asaaga et al. 2021). However, there is also a need to ensure effective third-party collaborations other than the ones between government agencies. These collaborations may bring novel initiatives and aid in their implementation, while offering some flexibility and bridging the gap between the stake holders.

Twelve key factors have been identified to aid in effective One Health collaborations at the individual, organizational and network-related levels. At the individual level, two factors have been identified- education and training of the collaborators that ensures they understand the need for collaboration and prior relationship between the collaborators that ensures smooth proceedings. The factors at the organizational level include organizational structure of the collaborating institutes, work culture, human resources and proper communication within the organization. Network-related factors include network structures, relationships, leadership, management, available & accessible resources and the political environment (Errecaborde et al. 2019). Collaborative networks facilitate the sharing of data, information, and best practices, allowing for evidence-based decision-making. Collaborative efforts can comprehensively tackle wTB's complex transmission pathways and effectively mitigate its impact on wildlife, livestock, and human populations.

## Vaccination

A wTB vaccine is the most feasible, sustainable, long-term control solution to effectively manage and eventually eliminate the disease. While conventional methods like culling and separation of wildlife and domestic animals are often impractical, costly, and may have unintended ecological consequences, a well-designed wTB vaccine offers a proactive approach, capable of breaking the cycle of infection and transmission among various hosts (Balseiro et al. 2020). Moreover, the vaccine's impact extends beyond disease control, contributing to conservation efforts by safeguarding susceptible wildlife species from infection.

Wildlife tuberculosis vaccine development is intricately challenging. The primary obstacle lies in discovering a vaccine that effectively induces robust and lasting immunity

across a wide variety of wildlife species, each with unique immune responses different food habits, feeding patterns, different places in the food chain, habitats across different temperate zones in India etc. Additionally, ensuring the safety of the vaccine is paramount, as alterations to ecosystems resulting from disease dynamics could have unforeseen ecological consequences. The variability of mycobacterial strains poses another challenge, where wTB in animals is not caused by a single microorganism, but the entire spectrum of MTBC species (Srinivasan et al. 2021; Ramanujam and Palaniyandi 2023). The need for field trials to validate the vaccine's effectiveness in natural settings further complicates the development process, necessitating long-term monitoring of wildlife populations and their interactions (Conlan et al. 2018). Apart from the challenges that ensue a vaccine development for use in animals, the most significant and concerning challenge is the delivery of vaccines to animals in the forest. The route of vaccine delivery for forest-dwelling animals presents formidable challenges inherent to their remote and often inaccessible habitats. The diverse behaviors, movements, and sensitivities of these animals necessitate innovative approaches to ensure vaccine effectiveness.

Oral baits have been used to deliver wTB vaccines across the globe, both experimentally and in the field (Palphramand et al. 2017; Perrett et al. 2018). These baits consist of edible carriers containing the BCG vaccine strategically designed to attract target species. Once consumed, the vaccine stimulates an immune response, providing protection against the disease. This method offers several advantages, such as non-invasive delivery without capturing animals, making it suitable for remote or sensitive habitats. A lipid-based BCG has been employed as an oral vaccination for brushtail possums and European badgers, though white-tailed deer found such formulations unpalatable (vaccination was still achievable). In Spain, a successful method involved creating baits by combining feed with paraffin, sucrose, and cinnamon-truffle powder to effectively administer BCG to wild boar (Palmer et al. 2014; Gormley et al. 2017; Palmer and Thacker 2018). Oral baits can be deployed on a large scale through aircraft or ground distribution, efficiently covering expansive areas. This was used in New Zealand to vaccinate brushtail possums, where 3 baits per hectare (ha) of BCG vaccine in a lipid matrix was sown over 136 ha of land. Follow-up studies over the year showed an 81% reduction in the prevalence of MTBC in the area (Nugent et al. 2016).

Other novel vaccine delivery methods include the use of self-disseminating vaccines using replicative virus-based vectors with immunity-conferring genes against MTBC. In such cases, the self-disseminating capacity of the virus could be used to our advantage, where vaccination of a few animals would be enough to vaccinate an entire herd,

owing to the use of replicating viral vectors (Murphy et al. 2016). Polymer bullets filled with vaccines were used with less success for control of brucellosis in elk in USA, but the strategy might prove useful for wTB vaccination (Rhyan and Spraker 2010). Another approach is using plant-based vaccines, which involves incorporating selected immunogenic pathogen antigens into plant cells, to be released in the animal's gut, creating a recombinant edible vaccine. Plant-based vaccines are economically viable, for large-scale production and mitigates issues related to taste and acceptance. When antigenic proteins of the vaccine are produced within plants, they become encapsulated within plant cells. This has been successfully achieved in carrots (Permyakova et al. 2015).

### **Need for a national wildlife tuberculosis surveillance program**

India needs a comprehensive national surveillance program for wTB to provide a structured framework to systematically monitor, assess, and manage WTB in the country's diverse wildlife populations. All the strategies discussed above could be brought under the umbrella of a national wTB surveillance program. Since there is a lack of a surveillance program, the true burden of wTB in India is still unknown, which leads to lower funds dedicated to wTB eradication, which again hinders the constitution of a national surveillance program. This cycle has to be broken for effective wTB eradication (Ramanujam and Palaniyandi 2023).

The pivotal part of such a program is to define clear achievable objectives and engage all the necessary stakeholders towards that goal, empowering collaborative research and capacity-building initiatives, facilitating the training of personnel and the development of standardized protocols. This will also necessitate open enhanced communication channels, laying the groundwork for a common reporting system to record any wTB case in animals. Such enhanced communication networks foster real-time information exchange among researchers, veterinarians, wildlife managers, and public health officials, vital for informed decision-making. Its existence in itself serves as a public awareness campaign, essential for garnering community support, compliance, and responsible behavior. By amplifying the reach and impact of wTB control measures, a national wTB surveillance program catalyzes the synergy between science, policy, and practice, bolstering the comprehensive and integrated approach necessary for successful wTB control, while also advancing broader global health goals and ensuring the preservation of wildlife, livestock, and human well-being.

## Conclusion

In the context of India's intricate ecosystems and burgeoning human-wildlife interactions, addressing wTB emerges as a pressing need. The challenges inherent in wTB diagnosis, spanning diverse wildlife species and logistical limitations, necessitate innovative approaches that marry scientific rigor with practical feasibility. As human encroachment deepens into natural habitats, robust surveillance, accurate diagnosis, and targeted intervention are essential to safeguard both wildlife and human populations. A comprehensive understanding of the complexity and sources of the disease remain to be seen and successful control and eradication programs remain to be developed. This requires a holistic One Health approach. Collaborative endeavors, integrating multidisciplinary expertise from conservationists, veterinarians, researchers, and policy makers, are pivotal to crafting control and eradication strategies that mitigate disease transmission, preserving the delicate equilibrium of India's ecosystems. Effective measures must be embraced to curtail the dissemination of wTB among diverse wildlife species and potential spillover to humans.

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## Declarations

**Competing interests** The authors declare no competing interests.

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