

Tuberculosis in Wild Animals a Great Threat for Wildlife Conservation and Public Health- A Review

Muhammad Azhar^{1*}, Muhammad Hassan Saleem¹, Roheela Yasmeen², Ayesha Safdar³, Waqar Ahmad⁴, Muhammad Asif³, Awais Bokhari⁵, Irfan Aslam²

¹Department of Veterinary Medicine, University of Veterinary and Animal Science, Lahore- 54000 Pakistan

²Department of Biology, Lahore Garrison University, Lahore, Pakistan

³Department of Veterinary Surgery and Pet Sciences, University of Veterinary and Animal Science, Lahore- 54000 Pakistan

⁴Department of Theriogenology, University of Veterinary and Animal Science, Lahore- 54000 Pakistan

⁵Livestock and Dairy Development Department, Government of Punjab, Pakistan

*Email: azhar.vet11@gmail.com

Received: 16 November 2022 / Revised: 05 February 2023 / Accepted: 08 February 2023/ Published online: 15 February 2023.

How to cite: Azhar, M., et al. (2023). Tuberculosis in Wild Animals a Great Threat for Wildlife Conservation and Public Health- A Review, Journal of Wildlife and Biodiversity, 7(4), 1-10. DOI: <https://doi.org/10.5281/10.5281/zenodo.7783044>

Abstract

Tuberculosis (TB) is one of the rising problems in the whole world in wild animals. As we know, countries like India, Sri Lanka, Malaysia, Pakistan, etc. are considered the home of many endangered wildlife species like the Asian elephant, Rhinoceros, Markhor, Snow Leopard, and Bengal tiger. These countries carry 44% of the burden of TB in the form of human TB, livestock TB, and wildlife TB. The TB pathogen has a wide host range, therefore it is of great importance and need of the hour to work under one health concept to construct a composite TB control program in the medical as well as veterinary fields. Moreover, further research is yet to be made on this challenging arena. Globally, many studies have been conducted to discover the types of tuberculosis in wild animals. Recent studies have declared that *Mycobacterium tuberculosis* is not only the causative agent of tuberculosis in wild animals but other mycobacterium species are also a growing threat. It was demonstrated that a wide variety of mycobacterium strains are present in the whole region, particularly in developing countries pointing out its endemic distribution. Tuberculosis in the free-ranging Rhinoceros in South Africa, Nepal, and Sri Lanka is associated with the *M. bovis* and *M. orygis* that also signify the threats of TB in other wild animal species including chances of unknown maintenance host. Some other research has revealed that there is a rising challenge of TB in the elephants in different countries of the World. Being a major reservoir host of tuberculosis, wildlife has become a conservation and public health challenge all over the World.

Keywords: Endangered species, Public health, Tuberculosis

Introduction

Mycobacterium tuberculosis complex (MTBC) is a cluster of closely related pathogens that are genetically similar and cause tuberculosis in both humans as well as animals including wild animals (Gordon and Behr, 2015). The pathogens in the MTBC are human-adapted p, including *tuberculosis* and *M. africanum* (De Jong et al., 2010). Other species which cause TB in a wide variety of animals are *M. bovis*, *M. caprae*, *M. pinnipedii*, *M. microti*, *M. suricatte*, *M. orygi*, *M. mungi* (Chimpanzee bacillus) and Dassie bacillus (Brosch et al., 2002; Rodríguez et al., 2011). In most of the studies of tuberculosis in animals, *M. bovis* has been found causative agent which is considered an emerging pathogen of free-living wild animals and a serious threat to several protected wildlife species (Bengis et al., 2002). As per previous studies regarding different species of TB, the TB in “near to extinction” species like Ivory Coast inhabitant chimpanzee (Coscolla et al., 2013) is caused by Chimpanzee bacillus while in free-living Rhinoceros, it is caused by *M. orygi* (Thapa et al., 2016). The above-mentioned few examples of TB portray TB as an emerging disease in wild animals and a big hindrance in the conservation process.

In South Asian countries, tuberculosis which is caused by *Mycobacterium tuberculosis* is a more potent disease with 44% load globally. But there are a few numbers of confirmed reports about zoonotic TB caused by the *Mycobacterium bovis* all over the World and almost nobody is well aware of the TB control programs in animals. Although there are a few numbers of research publications present about *M. bovis* as a TB causative agent in different wild species. The other members of MTBC may also be a cause of TB in domestic animals, wild animals as well as in humans which is overlooked. Some recent publications have demonstrated that *M. orygis* is a conservation challenge in wild animals all over the World (Thapa et al., 2015). Some recent studies have also pointed out TB as a rising challenge in elephants, as Asian elephants are infected with *M. tuberculosis* (Paudel et al., 2014). Some of the elephants were also found zero-reactive to the TB antigens (Abraham et al., 2008). Moreover, some TB cases have also been reported in African elephants (Perera et al., 2014). *Mycobacterium bovis* and rarely *Mycobacterium caprae* are also been reported as the causative agents of tuberculosis in a wide range of animals including cattle and wild ungulates like red deer and wild boar (Cunha et al., 2012)

The current review study was designed to enlighten and summarize the main causes of tuberculosis in wild animals and to develop a relationship between animal and human tuberculosis as a public health hazard.

Material and methods

Molecular methods like genome sequencing, deletion typing, and spoligotyping are effective methods for the genotyping of *Mycobacterium tuberculosis* in different organisms. Moreover, by increasing awareness for the conservation of wildlife, there is a need to pursue TB in wild conditions (Fitzgerald and Kaneene, 2013). As an outcome, many new species of *Mycobacterium tuberculosis* have been identified and these new species have helped us to understand *M. tuberculosis* and its reservoir host animals. A large number of reported data has confirmed wildlife as a reservoir host of TB (add at least 2 or 3 references). These findings have several limitations to undermine TB control programs in humans, domestic and wild animals. Moreover, the development of zoonotic transmission pathways of vulnerable diseases has also been disrupted.

Results and discussion

***Mycobacterium bovis* can transmit TB in humans**

Mycobacterium bovis is found to be the main cause of bovine TB but it can also infect humans, and domestic and wild animals (Fitzgerald and Kaneene, 2013). It is distributed in all geographical areas of the World as one of the major causes of zoonosis. In 1998, the World Health Organization (WHO) reported that 3.1% of human TB was caused by *Mycobacterium bovis* and the isolated sputum samples from African patients showed a 1-10% prevalence of *M. bovis* (Firdessa et al., 2013). This showed that animal TB is also an important issue for public health.

***Mycobacterium bovis* is a major causative agent of TB in wild animals of different regions**

M. bovis has been found in many wildlife hosts in many geographical locations. As in Great Britain, badgers living in the wild were found transmitting *M. bovis* into the cattle which is an example of wildlife involvement in spreading TB (Donnelly et al., 2003). White-tailed deer acted as a wildlife reservoir for *M. bovis* in Michigan and Minnesota, United States in another study (Carstensen and DonCarlos, 2011; O'Brien et al., 2011). Moreover in New Zealand, the brush-tailed possum was found a reservoir host for TB (Caley et al., 2001). In all the above examples wild animals are acting as a reservoir host for bovine TB which is posing a great hindrance in the complete eradication of bovine TB. The only option left is the complete culling of wildlife reservoirs, but such approaches are costly and ineffective being impracticable. In the United Kingdom, it was reported that culling the wild badgers increased the number of TB cases in the wild. In the Iberian Peninsula and Europe, wild ungulates like wild boar and red deer were acting

as maintenance hosts for *M. bovis* which were found to transmit the pathogen to other ungulates, wild carnivores, and livestock (Santoret al., 2012).

In Zambia, Lechwe was found a reservoir host of *M. bovis* participating in a wide spread of infection in other domestic animals. In the same region, other species like elephants, hippos, and lions were also found transmitting TB but there were no confirmed reports. In South Africa, *M. bovis* has its strongest impact on wild animals because it is present in African buffalos which then spread to over 13 other wild animal species including endangered species like lions, greater kudu, and cheetah (Michel et al., 2006). Some recent publications have also declared that TB caused by *M. bovis* in the wild rhinoceros is now an emerging threat (Milleret al., 2017).

In Europe, mycobacterium species like *M. caprae* were first isolated from the domestic goat but in the recent survey, it was isolated from wild boar, deer, and livestock too. In the Southern Hemisphere including Australia and Argentina, *M. pinnipedii* had been isolated from a seal (pinnipeds) which is a specific host to this TB member. In the UK and Europe, *M. microti* had been isolated from voles. The three most endemic and animal-adapted subspecies of MTBC in the South African sub-region are *M. suricattae*, *M. mungi*, and Dassie bacillus (Clarke et al., 2016). In Northern Botswana, *M. mungi* had been isolated from the banded mongoose (Alexander et al., 2002), and in recent studies, the transmission in the moose from the environment has also been defined (Alexander et al., 2016). In South Africa, the Dassie bacillus had been isolated from the rock hyrax.

Emerging strains of tuberculosis as a threat to wildlife conservation and public health

Oryx bacillus from the Antelope clade named *M. orygis* is a popular member and subspecies of the MTBC having a unique phylogenetic position and causing TB in animals as well as humans. The *M. orygis* may be misdiagnosed with *M. tuberculosis* or *M. bovis* while isolating it from infected humans or animals respectively when we do not use molecular genotyping differentiation. In the Netherlands, Antelopes (Oryx and Water buck) were reported to have *M. bovis* infection because of having an unusually high number of IS6110 insertion sequences. In that particular study, a similar IS6110 pattern was also identified from a human MTBC which showed that animals and human beings have some epidemiological link (Van Soolingen et al., 1994). In 2005, the genetic distinction of *M. orygis* from other species of mycobacterium was reported. The name *M. orygis*, as subspecies of the MTBCs, was proposed based on either its first isolation from the Oryx or to show the group of animals from which it can be isolated (Smith et al., 2006). From all the above studies it may be concluded that *M. orygis* has been found in humans along with many animal

species. All the studies were evident for *M. orygis* identification from a variety of hosts and wide distribution of it in different geographical locations.

In Nepal, *M. orygis* had been isolated from wild animals (blue bull and spotted deer) in captive breeding conditions. In those conditions, TB infection was reported in wild animals, which was supposed to be caused by *M. bovis*. But further molecular investigations showed that it was caused by *M. orygis* rather than *M. bovis*. In 2015, *M. orygis* was isolated from a greater one-horned rhinoceros in Chitwan National Park (CNP); the history, clinical signs, and necropsy findings showed that the rhinoceros died of *M. orygis*. All of the above animals included in the studies (blue bull, spotted deer, and rhinoceros) had granulomatous lesions on the lungs that were filled and encapsulated with caseous necrotic material specifically found in case of TB (Thapa et al., 2015). In Rhinoceros, the TB lesions were confined to the lungs whereas, in spotted deer and blue bull, lesions were not confined rather also found in extra pulmonary areas like lymph nodes, GIT, and liver. Upon culturing all the samples, characteristic smooth and moist colonies of *M. orygis* yielded rather dry colonies as of *M. tuberculosis*. In earlier studies, when *M. orygis* was isolated from spotted deer and blue bull in the captive facility, it was postulated that the origin of that TB was the animals other than spotted deer and blue bull which were housed at that facility (Thapa et al., 2016). Similarly, in meerkats, *M. suricatte* had been reported only and it lacks a direct repeat locus with one unique genetic feature from other MTBC species which is why it has no spoligotype pattern. Some of the studies conducted in recent years showed that *M. suricatte*, *M. mungi*, and Dassie bacillus have partial deletions of RD1 locus, a virulence locus for *M. tuberculosis* and *M. bovis*, in various animals (Alexander et al., 2016).

TB in wild animals acts as a challenge in the conservation process

Diseases like TB have devastating and chronic effects on the conservation process of endangered species which are already suffering from conditions like habitat destruction and poaching. It is a great matter of concern for the survival of wild animals in the upcoming times. The United Nations Educational, Scientific and Cultural Organization (UNESCO) declared the Central National Park a World Heritage Site because of its enriched biodiversity, which mimics the homes of the globally protected animals such as Asian elephants, Bengal tigers, and Rhinoceros. The outcomes of the study in the park signify the threat of TB for other animals that are present in that territory. It had been postulated that there were unknown maintenance hosts of TB in and around the park.

In India 16 necropsy samples were found positive out of 25 TB suspected cases. Further studies showed that the infection was because of other mycobacterium species (Sharma et al., 2007).

Endemic distribution of TB

The *M. orygis* had been isolated from 18 cattle and 2 monkeys in Bangladesh. All the cattle were from a dairy farm and the monkeys were from the zoo in Dhaka. During postmortem, TB lesions were found in the lungs of cattle as well as in monkeys. All of the results of isolates showed a similar spoligotyping pattern. The exact origin of these outbreaks was not identified on the cattle farm but the large clusters indicated the widespread prevalence of TB in domestic as well as wild animals. Some other strains have also been identified in that particular area. According to all findings, TB has been found to be retained in the cattle population as well as in the unknown reservoir host that is indicating the endemic presence of TB (Coscolla et al., 2013).

Five different types of TB species two in Nepal and three in Bangladesh have been identified in a study of different domestic and wild animals. In Nepal, it has been reported that there was an unknown reservoir host whereas, in Bangladesh, cattle were identified as a possible reservoir host of TB. The presence of genetic variation in the species of TB in different animals and their geographical distribution supports its endemic distribution globally (Bangis et al., 2002). Until now, there has been no confirmed case of *M. bovis* from humans to cattle. However there were several types of research conducted on bovine TB, which were based on the serological diagnosis. Bovine TB could be caused by *M. orygis*. Globally, TB in wild animals is largely under study and unreported; even the TB lesions were clear during postmortem examination but to identify the species, no confirmatory test was performed. As per studies, it can be assumed that *M. orygis* may be preceded by *M. bovis* causing TB in the whole world. And recent reports have declared the new emerging strains of TB in the whole World but still, we have to explore the origin and their geographical distribution. All of the above studies will be directed toward “One Health” significance against the animal, environment, and human connection as well as the endemic distribution of TB (Fitzgerald and Kaneene, 2013).

TB in elephants

Elephants have been living in Asia and Africa for many centuries and they are important species from religious and cultural points of view. Unfortunately, elephants are on the list of endangered animals because of the shrinkage of their habitat and poaching. Moreover, elephants are also facing a potential threat of TB in their conservation process (Mikota et al., 2015). TB in elephants is a re-emerging disease caused primarily by *M. tuberculosis*, a human form of TB. However, infection with *M. bovis* had also been infrequently reported. Attention for intensive study on elephant TB was gained after 1996 when two circus elephants died of TB in the United States. Recently, TB in

elephants is progressively being detected in their host range countries and zoological collections around the World (Mikota et al., 2015).

In Asian elephants, TB screening has been carried out because of its great prevalence in the human population. In that screening, among 300 captive elephants, 15% were positive for elephant TB assay. Most of the elephants were those who were in the temple or tourist places. Similarly, two elephants were found positive for TB in India. In Sri Lanka, wild elephants were also found positive for TB. The proper origin or source of infection has not been identified. TB has been identified in captive and wild animals in countries of Africa and Asia which indicates a great threat of TB transmission from infected elephants to other wild elephants as well as to the persons working with these elephants (Mikota et al., 2015).

TB in badgers

In badgers, the first case of bovine TB (*M. bovis*) was reported in Cabaneros National Park in Spain (Sobrino et al., 2008). In 1997, a similar pathogen to *M. bovis* was also isolated from the badger lymph nodes in Northern Spain (Garcia Marin, personal communication). A total of 23% of badgers were found seropositive in Donana National Park (Martín-Atance et al., 2006). The prevalence of TB in badgers in France was estimated at 7.2%. Similarly, in another study, it was found that 7.4% of the badgers were positive for *M. avium* complex in the UK (Balseiro et al., 2011). In Scotland, *M. avium* para tuberculosis was found inhabitant in mesenteric lymph nodes and intestines of badgers (Beard et al., 2001). The prevalence and geographical distribution of *M. bovis* and other mycobacterium species in the badgers is an indication of their status as endangered species and they can also spread the infection to other species, especially humans which are having close contact with badgers.

TB in lions

Similar to other wild animal species, lions are also susceptible to tuberculosis as 30% of their population has declined in the past 20 years. However, that aspect of tuberculosis has neither been critically evaluated nor its potential effects on the lion population. However, postulates have been made that the infected prey can be a source of the spread of tuberculosis in the lions but these postulates still need to be proven. Due to the scarcity of research data, we still don't know the host status and epidemiology of tuberculosis in lions. Some research has been made to diagnose tuberculosis in lions but these diagnostic tests cannot differentiate the presence of infection and stage of disease in lions. We don't even know the effect of disease on the reproduction and geographical distribution of lions (Viljoen et al., 2015).

Other cases of tuberculosis in wildlife

Some studies have reported the presence of TB in spotted deer in India. Similarly, a study was conducted in Pakistan in which TB was reported in the Spotted deer, Chinkara gazelle, and Blackbuck (Shahid et al., 2012). Some incidents of TB have also been reported in the Languor and Rhesus monkeys in Australia (Parmaret al., 2013; Wilbur et al., 2012), and these transmissions of *M. tuberculosis* were associated with human contact as they have a large population of feral monkeys living in the human communities. It is believed that in the future, awareness campaigns of this disease in wildlife will be increased by minimizing the risk factors from the wildlife conservation point of view.

Conclusion

It was concluded tuberculosis is a big hindrance in the wildlife conservation process. There is a need to research wildlife species to highlight TB problems and to isolate certain novel mycobacterium species. Moreover, due to the scarcity of research data, it is believed that wildlife TB has been overlooked and this grey area must be explored. In the future, it is recommended that TB in wild animal species should be studied as a matter of urgency.

Reference

- Abraham D, Cheeran J, Sukumar R, Mikota S, Rao S, Ganguly S and Varma S (2008). Health assessment of captive Asian elephants in India with special reference to tuberculosis. Project Elephant. Ministry of Environment and Forests. Government of India.
- Alexander KA, Pleydell E, Williams MC, Lane EP, Nyange JF and Michel AL (2002). Mycobacterium tuberculosis: an emerging disease of free-ranging wildlife. *Emerg. infect. dis.* 8(6): 598.
- Alexander KA, Sanderson CE, Larsen MH, Robbe-Austerman S, Williams MC and Palmer MV (2016). Emerging tuberculosis pathogen hijacks social communication behaviour in the group-living banded mongoose (*Mungos mungo*). *MBio.* 7(3).
- Balseiro A, Rodriguez O, Gonzalez-Quiros P, Merediz I, Sevilla IA, Dave D and Bezos J (2011). Infection of Eurasian badgers (*Meles meles*) with Mycobacterium bovis and Mycobacterium avium complex in Spain. *T. Vet. J.* 190(2): e21-e25.
- Beard P, Daniels M, Henderson D, Pirie A, Rudge K, Buxton D and McKendrick I (2001). Paratuberculosis infection of nonruminant wildlife in Scotland. *J. clin. Microbiol.* 39(4): 1517-1521.
- Bengis R, Schmitt S and Obrien D (2002). Tuberculosis in free-ranging wildlife: detection, diagnosis and management. *OIE Rev. Sci. Tech.* 21(2): 317-334.
- Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K and Kremer K (2002). A new evolutionary scenario for the Mycobacterium tuberculosis complex. *Proc. Natl. Acad. Sci.* 99(6): 3684-3689.
- Caley P, Hone J and Cowan P (2001). The relationship between prevalence of Mycobacterium bovis infection in feral ferrets and possum abundance. *N Z Vet J.* 49(5): 195-200.

- Carstensen M and DonCarlos MW (2011). Preventing the establishment of a wildlife disease reservoir: a case study of bovine tuberculosis in wild deer in Minnesota, USA. *Vet. Med. Int.* 2011.
- Clarke C, Van Helden P, Miller M and Parsons S (2016). Animal-adapted members of the *Mycobacterium tuberculosis* complex endemic to the southern African subregion. *J. S. Afr. Vet. Assoc.* 87(1): 1-7.
- Coscolla M, Lewin A, Metzger S, Maetz-Rennsing K, Calvignac-Spencer S, Nitsche A and Parkhill J (2013). Novel *Mycobacterium tuberculosis* complex isolated from a wild chimpanzee. *Emerging Infect. Dis.* 19(6): 969.
- De Jong BC, Antonio M and Gagneux S (2010). *Mycobacterium africanum*—review of an important cause of human tuberculosis in West Africa. *PLoS. Negl. Trop. Dis.* 4(9): e744.
- Donnelly CA, Woodroffe R, Cox D, Bourne J, Gettinby G, Le Fevre AM and Morrison WI (2003). Impact of localized badger culling on tuberculosis incidence in British cattle. *Nature.* 426(6968): 834-837.
- Fitzgerald S and Kaneene J (2013). Wildlife reservoirs of bovine tuberculosis worldwide: hosts, pathology, surveillance, and control. *Vet. Pathol.* 50(3): 488-499.
- Firdessa, R., Berg, S., Hailu, E., Schelling, E., Gumi, B., Erenso, G., ... & Aseffa, A. (2013). Mycobacterial lineages causing pulmonary and extrapulmonary tuberculosis, Ethiopia. *Emerging infectious diseases*, 19(3), 460.
- Gordon SV and Behr MA (2015). Comparative Mycobacteriology of the *Mycobacterium tuberculosis* complex. *Tuberculosis, leprosy and mycobacterial diseases of man and animals: the many hosts of mycobacteria*, Wallingford: CABI, 17-29.
- Martin-Atance P, Leon-Vizcaino L, Palomares F, Revilla E, Gonzalez-Candela M, Calzada J and Delibes M (2006). Antibodies to *Mycobacterium bovis* in wild carnivores from Donana National Park (Spain). *J. Wildl. Dis.* 42(3): 704-708.
- Michel AL, Bengis RG, Keet D, Hofmeyr M, De Klerk L, Cross P and Buss P (2006). Wildlife tuberculosis in South African conservation areas: implications and challenges. *Vet. microbiol.* 112(2-4): 91-100.
- Mikota SK, Lyashchenko KP, Lowenstine L, Agnew D and Maslow JN (2015). 14 Mycobacterial Infections in Elephants. *Tuberculosis, Leprosy and Mycobacterial Diseases of Man and Animals: The Many Hosts of Mycobacteria.* 259.
- Miller M, Michel A, Van Helden P and Buss P (2017). Tuberculosis in rhinoceros: an underrecognized threat? *Transbound. Emerg. Dis.* 64(4): 1071-1078.
- O'Brien DJ, Schmitt SM, Fitzgerald SD and Berry DE (2011). Management of bovine tuberculosis in Michigan wildlife: current status and near term prospects. *Vet. microbiol.* 151(1-2): 179-187.
- Parmar S, Jani R, Kapadiya F and Sutariya D (2013). Status of tuberculosis in the free living hanuman langur (*Presbytis entellus*) of Gujarat state. *Indian Vet. J.* 90: 74-75.
- Paudel S, Mikota SK, Nakajima C, Gairhe KP, Maharjan B, Thapa J and Tsubota T (2014). Molecular characterization of *Mycobacterium tuberculosis* isolates from elephants of Nepal. *Tuberc.* 94(3): 287-292.
- Perera B, Salgado M, Gunawardena G, Smith N and Jinadasa H (2014). The first confirmed case of fatal tuberculosis in a wild Sri Lankan elephant. *Gajah.* 41: 28-31.
- Rodriguez S, Bezos J, Romero B, de Juan L, Alvarez J, Castellanos E and Saez-Llorente JL (2011). *Mycobacterium caprae* infection in livestock and wildlife, Spain. *Emerging infect. dis.* 17(3): 532.

- Santos N, Correia-Neves M, Almeida V and Gortazar C (2012). Wildlife tuberculosis: a systematic review of the epidemiology in the Iberian Peninsula. *Epidemiol. Insights.* 273.
- Shahid A, Tariq JM, Nisar KM and Cagiola M (2012). Prevalence of bovine tuberculosis in zoo animals in Pakistan.
- Sharma S, Mallick G, Verma R and Ray S (2007). Polymerase chain reaction (PCR) amplification of IS6110 sequences to detect *Mycobacterium tuberculosis* complex from formalin-fixed paraffin-embedded tissues of deer (*Axis axis*). *Vet. res. commun.* 31(1): 17-21.
- Smith NH, Kremer K, Inwald J, Dal J, Driscoll JR, Gordon SV and Smith JM (2006). Ecotypes of the *Mycobacterium tuberculosis* complex. *J. Theor. Biol.* 239(2): 220-225.
- Sobrinho R, Martin-Hernando M, Vicente J, Aurtenetxe O, Garrido J and Gortazar C (2008). Bovine tuberculosis in a badger (*Meles meles*) in Spain: British Medical Journal Publishing Group.
- Thapa J, Nakajima C, Maharjan B, Poudel A and Suzuki Y (2015). Molecular characterization of *Mycobacterium orygis* isolates from wild animals of Nepal. *Jap. J. Vet. Res.* 63(3): 151-158.
- Thapa J, Paudel S, Sadaula A, Shah Y, Maharjan B, Kaufman GE and Suzuki Y (2016). *Mycobacterium orygis*-associated tuberculosis in free-ranging rhinoceros, Nepal, 2015. *Emerging infect. dis.* 22(3): 570.
- Van Soolingen D, De Haas P, Haagsma J, Eger T, Hermans P, Ritacco V and Van Embden J (1994). Use of various genetic markers in the differentiation of *Mycobacterium bovis* strains from animals and humans and for studying the epidemiology of bovine tuberculosis. *J. Clin. Microbiol.* 32(10): 2425-2433.
- Viljoen IM, Van Helden PD and Millar RP (2015). *Mycobacterium bovis* infection in the lion (*Panthera leo*): Current knowledge, conundrums and research challenges. *Vet. Microbiol.* 177(3-4): 252-260.
- Wilbur AK, Engel GA, Rompis A, Putra IA, LEE BPH, Aggimarangsee N and Schillaci MA (2012). From the Mouths of Monkeys: Detection of *Mycobacterium tuberculosis* Complex DNA From Buccal Swabs of Synanthropic Macaques. *Am. J. Primatol.* 74(7): 676-686.