*JZWM* 2021 52(4):1257-1262

[**ASSESSMENT OF MULTIANTIGEN PRINT IMMUNOASSAY AND RAPID LATERAL-FLOW TEST FOR THE DETECTION OF *MYCOBACTERIUM BOVIS* INFECTION IN MALAYAN TAPIR (*TAPIRUS INDICUS*)**](https://doi.org/10.1638/2021-0054)

Chaney SB, McAloose D, Greenwald R, Lyashchenko KP, Calle PP

**ABSTRACT:** A multiantigen print immunoassay (MAPIA) and rapid test (RT) developed and validated for detection of mycobacterial antibodies in elephants (*Elephas maximus* and *Loxodonta africana*) was assessed in Malayan tapir (*Tapirus indicus*). Retrospective analysis of banked serum from one *Mycobacterium bovis* infected and seven presumably uninfected tapir was performed by MAPIA and RT. A sample collected 2 mon prior to the death of a culture-confirmed *M. bovis*-infected tapir served as a positive control. Seroreactivity of this sample was demonstrated via both MAPIA and RT testing. Seven uninfected animals, including four without postmortem evidence of mycobacterial disease and three that remain healthy, were negative controls; none demonstrated seroreactivity to key antigens with either test. These results suggest that MAPIA and RT have potential utility for rapid detection of *M. bovis* infection in Malayan tapir.

**Key Points:**

* Malayan tapir endangered and susceptible to mycobacterial diseases
	+ Historically tested with intradermal PDD skin testing and bacterial culture for screening
* Serological test for mycobacterium in elephants developed using multiantigen print immunoassay (MAPIA) and a lateral flow rapid test (TR)
* Objective: evaluate MAPIA and RT in retrospective serum samples of tapir with confirmed *Mycobacterium bovis* and seven presumably uninfected tapir
	+ Key antigens detected were ESAT-6, CFP10, MPB83 and fusion proteins E6/P10 and 16/83
	+ Tested positive on RT and MAPIA
	+ Widespread reactivity to MBCF antigen
* Negative controls did not react with antigens of either test
* Antigens used in MAPIA are from *M. tuberculosis* and *M. bovis*, making this a potential screening tool to identify immunodominant antigens during infection
	+ May also be used to detect *M. pinnipedii* infection in tapir

**Related Articles:**

Jurczynski K, Lyashchenko KP, Gomis D, Moser I, Greenwald R, Moisson P. Pinniped tuberculosis in Malayan tapirs (*Tapirus indicus*) and its transmission to other terrestrial mammals. *J Zoo Wildl Med*. 2011;42(2):222-227

*JWD* 2022 58(2):309-321

[**Characterizing tuberculosis progression in wild meerkats (*Suricata suricatta*) from fecal samples and clinical signs**](https://doi.org/10.7589/jwd-d-21-00063)

Donadio J, Risely A, Müller-Klein N, et al

**ABSTRACT:** Tuberculosis (TB) is an increasing threat to wildlife, yet tracking its spread is challenging because infections often appear to be asymptomatic, and diagnostic tools such as blood tests can be invasive and resource intensive. Our understanding of TB biology in wildlife is therefore limited to a small number of well-studied species. Testing of fecal samples using PCR is a noninvasive method that has been used to detect *Mycobacterium bovis* shedding amongst badgers, yet its utility more broadly for TB monitoring in wildlife is unclear. We combined observation data of clinical signs with PCR testing of 388 fecal samples to characterize longitudinal dynamics of TB progression in 66 wild meerkats (*Suricata suricatta*) socially exposed to *Mycobacterium suricattae* between 2000 and 2018. Our specific objectives were 1) to test whether meerkat fecal samples can be used to monitor TB; 2) to characterize TB progression between three infection states (PCR-negative exposed, PCR-positive asymptomatic, and PCR positive with clinical signs); and 3) estimate individual heterogeneity in TB susceptibility, defined here as the time between TB exposure and detection, and survival after TB detection. We found that the TB detection probability once meerkats developed clinical signs was 13% (95% confidence interval 3–46%). Nevertheless, with an adapted test protocol of 10 PCR replicates per sample we detected hidden TB infections in 59% of meerkats before the onset of clinical signs. Meerkats became PCR positive approximately 14 mo after initial exposure, developed clinical signs approximately 1 yr after becoming PCR positive, and died within 5 mo of developing clinical signs. Individual variation in disease progression was high, with meerkats developing clinical signs from immediately after exposure to 3.4 yr later. Overall, our study generates novel insights into wildlife TB progression, and may help guide adapted management strategies for TB-susceptible wildlife populations.

**Key Points:**

* Diagnosis of TB in live wild animals is notoriously challenging (no gold standard)
* Meerkats are highly social mammals inhabiting arid regions of southern Africa
	+ Periodically experience TB outbreaks caused by *Mycobacterium suricattae*
	+ Individuals roving between social groups provide TB transmission opportunities
	+ TB outbreaks within social groups may last many years, with pups born during outbreaks being socially exposed from birth
	+ Typical clinical signs of TB in meerkats are submandibular swellings, inguinal and cervical lumps, emaciation and lethargy, and eventual death
* Fecal PCR revealed hidden TB infections on average 1 yr prior to external clinical signs
	+ Estimated infection period may be doubled compared with clinical sign data
* Approx. 1/4 of TB-exposed meerkats never became PCR+ or developed clinical signs
	+ Significant pool of individuals within the population that have very low susceptibility
* Average time between exposure and a meerkat becoming PCR+ is about 1 year
* TB progression is highly variable across individuals at every stage of TB progression
	+ Long infection period (particularly long asymptomatic period) is likely to have consequences for TB maintenance, social structures, survival, and reproduction
	+ Asymptomatic but TB-infectious meerkats could function as undetected super spreaders
* Study also found high heterogeneity in susceptibility and survival after TB-infection in meerkats
* *Mycobacterium* detection in feces indicates active shedding but cannot identify latent infections
	+ Detection probability for animals with clinical signs was low (13%)
	+ In 13.6% of individuals clinical signs were observed yet fecal samples were never PCR+
	+ Possibility of false-negative results even in presumably infectious and shedding animals

**TLDR:** TB progression in meerkats is characterized by an extended asymptomatic period followed by a shorter period with exhibited clinical signs, with high heterogeneity, potentially promoting TB persistence within even asymptomatic populations. PCR-based TB detection from fecal samples has potential as a diagnostic tool in wildlife.

**Related Articles:**

Thomas J, Balseiro A, Gortázar C, Risalde MA. 2021. Diagnosis of tuberculosis in wildlife: A systematic review. *Vet Res* 52:31.

**TREATMENT OF MYCOBACTERIOSIS CAUSED BY MYCOBACTERIUM AVIUM SSP. HOMINISSUIS IN A GROUP OF CAPTIVE LOWLAND TAPIRS ( TAPIRUS TERRESTRIS).** JZWM 2021. Sandra Marcordes, Imke Lueders, Lisa Grund, Alexander Sliwa, W. Nikolaus Kuehn-Velten, Doris Hillemann, Florian P. Maurer, Stefanie A. Barth.

Abstract: Tapirs are a taxonomic group with a high susceptibility to mycobacterial diseases. However, successful therapy has only been documented sporadically. Here treatment of mycobacteriosis diagnosed in three, one male and two female, lowland tapirs (Tapirus terrestris) in a zoo in Germany is reported. Two of the animals showed chronic mild respiratory signs, and conventional therapy did not improve the condition. Culture of broncho-alveolar lavage (BAL) samples was positive for Mycobacterium avium ssp. hominissuis. Upon airway endoscopy, bronchial edema and increased mucus production were visible. Initially, all three infected tapirs received oral antimycobacterial therapy consisting of 5 mg/kg body weight isoniazid, 10 mg/kg rifampicin, and 10 mg/kg clarithromycin q24h. Based on therapeutic drug level monitoring, the doses of rifampicin were adjusted to 12 and 15 mg/kg in the females and the male, respectively. The treatment with all three drugs was continued for 11 mon. Six months into treatment, the clinical condition resolved, and repeated BAL samples of all three tapirs tested negative for mycobacteria by culture. Here the approach for a treatment protocol with minimal side effects suitable to control infections with nontuberculous mycobacteria in lowland tapirs is reported.

Background:

* *Mycobacterium avium-intracellulare* complex (MAC) = 12 species of mycobacterium
	+ Opportunistic pathogens of humans/animals; disease dependent on immunocompetency
	+ Acquired primarily from environment (soil, water, dust, feed)
		1. Vs. *M. tuberculosis* complex (MTC) mainly acquired from direct host contact
* Tapirs can be affected by M. tuberculosis, M. bovis, M. pinnipedii, M. avium
	+ Typically nonspecific signs, weight loss, progressive respiratory
* Few reports of myco treatment in tapirs against MTC; no reports of tx for MAC species

Key Points:

* Goal of study: report successful treatment of MAC species in lowland tapir
* Cases: 1.2 lowland tapirs; 2 females had mild resp signs (cough) resistant to empiric therapies
* Sampling: bronchoscopy and BAL used to obtain samples for culture and sensitivity, abnormal scope findings in tapirs 2 and 3 (mucopurulent secretions, tracheitis, edema)
	+ MAH detected in BAL samples of 1 and 2 but not 3
	+ **BAL and culture on all three revealed Mycobacterium avium ssp. hominissuis**
* BW normal for all 3 tapirs
	+ *Typically can see leukocytosis, anemia, low albumin, high calcium*
* Due to zoonotic potential and chronic clinical signs, elected treatment
* Treatment based on MICs included 5mg/kg isoniazid, 10mg/kg rifampicin, 10mg/kg clarithromycin + milk thistle (dt increasing LE) for **11 MONTHS**
	+ Monitored serum concentrations → increased rifampin to 12-15mg/kg; did not detect clarithro but continued anyway; isoniazid levels adequate
* After 3 months, clinical signs resolved, after 6 months repeat endoscopy showed resolved abnormalities and BAL samples all cultured negative
* Side effects: feces and urine were tinged red, AST GLDH and GGT increased throughout treatment and increased serum iron (not reported in antimycobacterial tx in other species)
	+ LE values and serum iron improved after discontinuation of treatment

**TLDR: First report of successful MAC treatment in Tapir with triple drug therapy; recurrence common due to reinfection or incomplete eradication → follow up screening is recommended.**

**CYTOKINE-RELEASE ASSAY FOR THE DETECTION OF MYCOBACTERIUM BOVIS INFECTION IN CHEETAH (ACINONYX JUBATUS).** JZWM 2021.Rachiel Gumbo, Elin Crockett, Wynand J. Goosen, Robin M. Warren, Paul D. van Helden, Michele A. Miller, Tanya J. Kerr.

Abstract: The lack of species-specific assays for the diagnosis of infectious diseases, such as bovine tuberculosis, poses a threat to the management of wildlife populations, especially for vulnerable species such as cheetah (Acinonyx jubatus). The aim of this study was to identify and develop a cell-mediated immunological cytokine-release assay that could distinguish between Mycobacterium bovis–infected and uninfected cheetahs using commercially available feline cytokine ELISA and domestic cat (Felis catus) recombinant proteins. Antibodies against domestic cat cytokines, tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and interferon gamma (IFN-γ), were screened for cross-reactivity with plasma cytokines from cheetah whole blood stimulated using QuantiFERON®-TB Gold Plus (QFT) tubes. Evidence of cytokine production in response to QFT mitogen stimulation was observed in all four ELISA assays. However only the Mabtech Cat IFN-γ ELISABasic kit could distinguish between M. bovis–infected (n = 1) and uninfected (n = 1) cheetahs and was therefore selected for further evaluation. A preliminary cheetah specific cutoff value (11 pg/ml) for detecting M. bovis infection using the Mabtech Cat IFN-γ release assay was calculated using a M. bovis uninfected cheetah cohort. Although this study only included one confirmed M. bovis culture-positive and one M. bovis culture-negative cheetah, the Mabtech Cat IFN-γ release assay demonstrated its potential for diagnostic application in this species.

Background:

* *Mycobacterium bovis* = bTB = causative agent of bovine tuberculosis
	+ Wild felids can become infected from consuming infected prey or direct contact
	+ Long-term consequences of bTB in cheetahs is unknown; can suffer M&M
	+ Risk of transmission associated with translocations (performed for fragmented pops)
* Tuberculin skin test (only widely available antemortem bTB test for wild felids; impractical in free-ranging animals due to 72h follow up and second immobilization
* Blood based test that only requires single capture sampling = better alternative
	+ Ab serology in lions not reliable
	+ Detection of antigen specific cell mediated immune responses have been used to ID biomarkers for diagnosis of bTB in other species
		- IFN-gamma, tumor necrosis factor (TNF-a) and IL12 are important cytokines in response to M. tuberculosis in humans/mice
		- IFN-gamma, IL10, IL12, IL23 in M. bovis infected domestic cats →dx potential

Methods/goals: (1) identify a feline cytokine ELISA kit that can detect cheetah cytokines, and (2) develop a cytokine release assay to distinguish between bTB cheetahs and non-bTB cheetahs

* Blood (n=47) and tissue (n=2) opportunistically sampled from free ranging and captive cheetahs
* Cheetahs classified as either bTB infected or uninfected based on hx of exposure + one of the following: positive ID test, positive Ab test, positive culture or PCR from tissue

Key Points:

* High cytokine response (TNF-a, IL1β, IFN-y) was produced in response to mitogen stimulation of cheetah plasma
* Evaluated multiple ELISAs to detect cytokines → all assays detected these three cytokines in Tb mitogen stimulated cheetah plasma
	+ Only one ELISA (Mabtech Cat IFN-y) was able to potentially distinguish between culture confirmed infected (n=1; 750 pg/ml) and non-infected (n=1; 0 pg/ml) cheetah → this kit then used on larger cohort of non infected cheetah to determine normal assay
	+ Antigen specific TNF-a and IL 1β concentrations were not differentiated between M. bovis infected cheetah and uninfected cheetah, but higher cytokines when plasma was spiked with mitogen stimulation → suggests immune activation of these cytokines which should be explored more as possible biomarkers of other conditions in cheetahs
* Used the Mabtech Cat IFN-y to screen plasma from 47 cheetahs (including the 1 infected and noninfected) → little to no antigen specific reactivity in most cheetahs except for the infected one (~750pg/ml) and one other random one presumed to be noninfected (~170pg/ml)
* Determined the cut off for uninfected cheetahs using Mabtech Cat IFN-y = 11pg/ml
	+ Most cheetahs aside from two randoms + the infected one were below that cut off
* Could not validate as only had one culture positive and one culture negative confirmed animals

**TLDR: Mabtech Cat IFN-y ELISA with QFT mitogen stimulation platform may be useful in identifying potentially bTB infected cheetahs, with normal cheetahs typically falling below the 11pg/ml threshold. Further validation is needed.**

Supporting Images:



Yee, JoAnn L., et al. "Tuberculosis detection in nonhuman primates is enhanced by use of testing algorithms that include an interferon-γ release assay." *American journal of veterinary research* 83.1 (2022): 15-22.

**Abstract:**

**OBJECTIVE**

To develop a testing algorithm that incorporates multiple assays to evaluate host cellular and humoral immunity and antigen detection concerning *Mycobacterium tuberculosis* complex (MTBC) infection in captive nonhuman primates.

**ANIMALS**

Cohorts of captive-bred and wild-caught macaques from 5 different geographic regions.

**PROCEDURES**

Macaques were tested for MTBC infection by use of a γ interferon tuberculosis (GIFT) assay, an interferon-γ release assay, and other assays. In the first 2 cohorts (n = 15 and 181), initial validation of the GIFT assay was performed by use of experimentally infected and unexposed control macaques. In the next 3 cohorts (n = 59, 42, and 11), results were obtained for opportunistically collected samples from macaques exposed during spontaneous outbreaks. **RESULTS**

Sensitivity and specificity of the GIFT assay in the control cohorts were 100% and 97%, respectively, and were variable but enhanced by incorporating results from multiple assays in spontaneous outbreaks.

**CLINICAL RELEVANCE**

The detection and management of MTBC infection in captive nonhuman primate populations is an ongoing challenge, especially with animal imports and transfers. Despite standardized practices of initial quarantine with regular intradermal tuberculin skin testing, spontaneous outbreaks continue to be reported. Since infection encompasses a range of disease manifestations over time, a testing algorithm that incorporates multiple assays, such as the GIFT assay, to evaluate host cellular and humoral immunity in addition to agent detection is needed. Testing a combination of samples from controlled studies and spontaneous outbreaks of MTBC infection in nonhuman primates would advance the development and validation of a functional algorithm that incorporates promising tools such as the GIFT assay.

**Background:**

* The detection and elimination of *Mycobacterium tuberculosis* complex (MTBC) is an ongoing management problem for nonhuman primates (NHP) in captivity. It is also a concern of wild NHP populations where TB represents a risk to endangered species. The tuberculin skin test (TST) is the primary tool for diagnosis and surveillance.
* A series of negative TSTs is required by the CDC to clear import quarantine of NHPs. However, there are multiple documented cases of animals successfully clearing quarantine and then later developing clinical disease after shipment to other facilities.
* TST continues to be used, despite documented evidence of its limitations, including unreliable identification of animals with latent MTBC. The use of the TST has also been impacted by problems in production and quality control of mammalian old tuberculin. Utility of the TST and other diagnostic tests may also be affected by the species of NHP being tested, potentially further complicating test interpretation.
* From observations of case studies and outbreaks, we have postulated that reliable surveillance/diagnosis of MTBC in NHPs will require a testing algorithm with multiple assays to evaluate host cellular immunity, host humoral immunity, and agent detection.
* The Primate Assay Laboratory at the California National Primate Research Center (CNPRC) developed the γ-interferon tuberculosis (GIFT) test for NHPs. It is an interferon-gamma release assay (IGRA).

**Key Points:**

* This study included samples from macaques in controlled, captive environments with (case study 1 controls) and without (case study 2 controls) TB, as well as from both wild-caught (case study 3) and captive (case studies 4 and 5) macaques in which spontaneous infection was found or suspected
* Case 1: experimentally infected rhesus macaques
	+ 15 rhesus macaques that received intrabronchial MTB
	+ The GIFT assays demonstrated reactivity beginning at day 14, when 8 (53%) of 15 animals had positive results. By day 28, all animals with available samples had positive results
* Case 2: captive-bred colony rhesus macaques
	+ GIFT samples collected for routine surveillance from 181 rhesus macaques of both sexes ranging in age from 0.5 to 25 years and housed outdoors in social groups at the CNPRC or ONPRC.
	+ Samples for GIFT assay were collected in parallel with TST at both the California and Oregon locations on 181 animals. No skin test reactors were found. Based on nonexposure history and skin test results, 5 GIFT false positives were identified for an overall specificity of 97.2% or 97% (95/98) at the ONPRC and 98% (81/83) at the CNPRC
* Case study 3: wild-caught cynomolgus macaques
	+ Samples for GIFT assay collected from 59 wild-caught cynomolgus macaques.
	+ Overall, 48 samples were nonreactive and interpreted as negative; 11 samples were reactive and included 8 positive and 3 indeterminate interpretations. Four of the GIFT-reactive samples were also TST reactive, and the other 7 were not. Seven of the 11 were culture positive at later dates.
	+ IGRA and TST results corresponded in some but not all animals. Diagnostic sensitivity could be increased by interpreting reactivity to either the IGRA or TST as indicative for infection.
* Case study 4: captive pigtailed macaques
	+ A spontaneous outbreak in a captive research colony provided an opportunity to test samples from 42 southern pigtailed macaques imported from Indonesia.
	+ The GIFT assay yielded 13 reactive samples. Seven were interpreted as positive, and 6 were interpreted as indeterminate. The remaining 29 were nonreactive and interpreted as negative results.
	+ Ten samples were reactive in the ELISPOT assay.
	+ Overall, 26 samples were nonreactive on both assays and 7 were reactive on both; 6 were reactive on GIFT assay alone, and 3 were reactive on ELISPOT assay alone
	+ Combining the IGRA and ELISPOT reactivity in case study 4 similarly improved sensitivity
* Case study 5: captive cynomolgus macaques
	+ This group comprised samples from 11 captive-bred cynomolgus macaques for which the GIFT assay was performed following incidental findings of gross and histologic lesions indicative of TB at necropsy and MTBC-positive results of PCR assay from another member of their cohort.
	+ No samples were positive on GIFT assay.
* IGRAs, similar to the Quantiferon blood test used in humans, are an alternative to the TST for detecting cell-mediated immunity
* When applied to spontaneous outbreak situations described in case studies 3, 4, and 5, the GIFT assay did detect samples from infected animals that were not positive by use of the CDC-mandated skin test in those populations.
* These samples were from populations in which an animal was characterized as infected based on pathogen detection or necropsy. Lack of parallel tests from the same time points precluded a direct comparison assays and determination of analytical sensitivity.
* Our findings suggested that cell-mediated immunity is a useful marker, and assays such as the GIFT assay would be an important component of a TB testing algorithm.
* Decreasing cell-mediated and increasing humoral immunity over time suggest that assays for both will be needed in a TB testing algorithm.
* For direct agent detection, the PCR assay for MTBC detection is both sensitive and specific; however, it is often difficult to obtain an appropriate antemortem sample.
* Although thoracic radiography and acid-fast bacteria smears may not distinguish MTBC organisms from environmental and other mycobacteria, they may also be useful in a testing algorithm. Microbial culture has historically been considered the gold standard; however, it is logistically difficult and may lack sensitivity.
* To determine the optimal testing algorithm for detection of TB in macaques would require testing large numbers of uninfected and infected animals. The potentially confounding influence of various NHP species and MTBC strains would need to be addressed.
* The development of reliable algorithms for the diagnosis of TB is urgently needed and should be based on the following considerations: time course of cellular and humoral immune responses during infection and disease progression, NHP species–specific differences, the source of the animals (captive bred vs wild caught or imported), and validation with data sets from both laboratory and field studies.

**Takeaway:**

The detection and management of MTBC infection in captive nonhuman primate populations is an ongoing challenge, especially with animal imports and transfers. Despite standardized practices of initial quarantine with regular intradermal tuberculin skin testing, spontaneous outbreaks continue to be reported. Since infection encompasses a range of disease manifestations over time, a testing algorithm that incorporates multiple assays, such as the GIFT assay, to evaluate host cellular and humoral immunity in addition to agent detection is needed. Testing a combination of samples from controlled studies and spontaneous outbreaks of MTBC infection in nonhuman primates would advance the development and validation of a functional algorithm that incorporates promising tools such as the GIFT assay.

****

Fowler Zoo and Wild Animal Medicine 10th ed, Chapter 28. Ecosystem and Multiple Species Effects of Tuberculosis in Kruger National Park.

**Abstract:**

Kruger National Park, South Africa, is endemic for bovine tuberculosis (bTB) caused by *Mycobacterium bovis*, with more than 15 wildlife species affected. As a multihost disease, bTB may impact individual animal health and lead to mortality, may have indirect effects by influencing fitness for hunting and reproduction, or result in greater susceptibility to predation. At a population and ecosystem level, effects are more difficult to discern from other influences such as drought, environmental changes, and other diseases. Despite being a chronic disease in a complex ecosystem, bTB has been increasingly detected in new species and at apparently high prevalence in buffalo (*Syncerus caffer*), lion (*Panthera leo*), wild dog (*Lycaon pictus*), and warthogs (*Phacochoerus africanus*). Therefore long-term studies are needed to determine the overall impact on the Kruger ecosystem.

**Key Points:**

* Overview of Kruger National Park Ecosystem
	+ Kruger National Park (KNP) is situated in the low-lying savanna of northeastern South Africa bordering Mozambique in the east and Zimbabwe in the north. The Park is approximately 20,000 km2 in area.
	+ In 2002, the Greater Limpopo Transfrontier National Park (GLTNP) was created bringing together KNP, South Africa, Limpopo National Park, Mozambique, and Gonarezhou, Zimbabwe.
	+ The creation of this enlarged conservation area with freedom of movement of wildlife is expected to significantly increase interactions between wildlife, livestock, and humans.
* History of Bovine Tuberculosis in Kruger National Park
	+ Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* (*M. bovis*), is an enzootic disease in Kruger National Park
	+ In KNP, a single case of mycobacteriosis was identified in an impala (*Aepyceros melampus*) in 1967; however, *M. bovis* was first isolated from a buffalo (*Syncerus caffer*) carcass in 1990
	+ In less than two decades, the disease had spread throughout the Park and reached the northern boundary by 2005.
* Species Affected by Bovine Tuberculosis
	+ *M. bovis* has a broad host range and is the principal cause of tuberculosis (TB) in wildlife. The disease has been diagnosed in more than 15 KNP species.
	+ Buffaloes are the main maintenance host in KNP, with species like greater kudu and warthogs having a potential role in the continued presence of the disease.
	+ Other species, such as lion, leopard, cheetah, and other carnivores, are considered spillover hosts, although more recent evidence suggests that lions and wild dogs may shed *M. bovis* in respiratory secretions.
	+ In a multiple-host system such as KNP, multiple routes exist for inter- and intraspecific transmission of, including respiratory, alimentary, and percutaneous.
* Species Specific Effects of Tuberculosis in Kruger National Park
	+ Buffalo
		- Infected buffalo develop lesions in lymph nodes of the head, tonsils, and lungs within 3 to 6 months of infection. Adult buffalo may remain infected for 3 to 5 years before developing fatal disease. Likelihood of being infected increases with age.
		- Environmental conditions, genetic resistance, and comorbidities influence susceptibility and progression of disease.
		- bTB is a chronic disease in buffaloes, making it difficult to determine the effects at a population level.
		- African buffaloes typically occur in breeding herds of approximately 30 to 1000 individuals in KNP with adult males, females, and subadults moving between herds. These movements promote the spatial spread of *M. bovis*.
		- The KNP buffalo population experiences steep declines in times of drought, and animals in herds with high bTB prevalence lose condition faster during dry conditions.
		- As lions and other large predators target compromised prey animals, it is predicted that diseased buffalo are preferentially predated, resulting in spillover, but also removing infected individuals in the herd.
	+ Rhinoceros
		- Between 2016 and 2017, a single black and six white rhinoceros in KNP were diagnosed with *M. bovis* infection
		- Extensive TB pulmonary lesions and associated pathology contributed to the death of the black rhinoceros. In contrast, lesions were incidental findings in white rhinoceros. All cases appeared during a period of severe drought, which resulted in nutritional stress and is suspected to increase susceptibility to infection from environmental sources.
		- Current evidence suggests that bTB is not a significant health threat to the KNP rhinoceros populations, and there is no evidence that they present a risk of spread to other animals.
		- Movement of rhinos out of KNP is restricted, which may impact conservation efforts
	+ African Elephant
		- Since 2016, two young bull elephants have been diagnosed postmortem with bTB. In both cases, small nonspecific lesions were detected at necropsy, and diagnosis was based on mycobacterial culture
		- Estimated a seroprevalence of 6% to 9% of *M. tuberculosis* complex in KNP elephants. A single fatal case of *M. tuberculosis* disease in an adult bull elephant in KNP suggests that transmission occurred indirectly via contaminated human material in the environment.
		- Elephants may no longer be translocated from KNP in support of conservation efforts.
	+ Warthog
		- Recently been recognized as a potential maintenance host of bTB in KNP. Seroprevalence varied from 16% to 38%.
		- As warthogs may move across fences, they may present an underrecognized threat for spillover to other hosts.
	+ Greater Kudu
		- Kudus are considered to be a maintenance host in KNP because they develop discharging parotid lymph node fistulae which have the potential of disseminating bacteria over a wide area
		- Infected kudu develop generalized disease and wasting, which makes them more susceptible to predation, and kudus are adept at jumping game-management fences
	+ Lion
		- Lions are important predators of buffalo, and ingestion is the most common route of bTB spillover to this predator
		- Estimated prevalence in lions in the southern KNP was 54%, with 33% prevalence in the central area where buffalo bTB prevalence is also lower.
		- Transmission may also occur via aerosol or percutaneous infection through aggression or grooming between pride members
		- Although studies have shown that lions may shed *M. bovis* in respiratory secretions, it is still uncertain whether lions are maintenance or primarily spillover hosts in KNP
		- Infected lions develop clinical signs, including emaciation, poorly healing skin lesions, elbow hygromas, osteomyelitis of limbs, lameness, and blindness. The estimated time from infection to death is between 2-5 years
		- Mathematical models suggest that over 50 years, bTB poses a serious threat to survival of this species in KNP, with a possible 35% to 75% decrease in the current population numbers.
* Effects of Bovine Tuberculosis in Other Mammals
	+ The impact of bTB on other smaller mammals is relatively unstudied and therefore little is known.
	+ Cases of bTB in wild dogs have been sporadically recorded in KNP since 2012
	+ In wild dogs, even animals younger than 1 year old, have been found with generalized disease. Preliminary data suggest that wild dogs may shed *M. bovis* in respiratory secretions, and therefore may be able to spread disease.
	+ Infection with *M. bovis* has been confirmed in other mammals, including cheetah, leopard, honey badger, banded mongoose, chacma baboon, and giraffe. These species are considered spillover hosts and anecdotal evidence suggests that population effects are limited
* Effects of Bovine Tuberculosis at Interfaces
	+ The GKNPC is separated on the western boundary from the surrounding agricultural areas by a disease-control fence designed to prevent buffalo moving into the surrounding rural communities. This fence limits but does not eliminate the possibility of disease transfer between buffalo and cattle.
	+ In 2012 to 2013, the KNP M. bovis strain (SB0121) was detected in cattle directly bordering the GKNPC ecosystem supporting spillback. With such spillback events, there is the added risk of zoonotic transfer to humans. The significance of zoonotic TB in humans in South Africa is currently unknown.
	+ Communal farming communities with their livestock live within the GLTFCA, creating an extensive wildlife-livestock-human interface.
	+ Satellite telemetry shows buffalo leave KNP and traverse large distances within and beyond the borders of the GLTFC.

**Takeaway:**

Kruger National Park, South Africa, is endemic for bovine tuberculosis (bTB) caused by *Mycobacterium bovis*, with more than 15 wildlife species affected. As a multihost disease, bTB may impact individual animal health and lead to mortality, may have indirect effects by influencing fitness for hunting and reproduction, or result in greater susceptibility to predation. At a population and ecosystem level, effects are more difficult to discern from other influences such as drought, environmental changes, and other diseases. bTB has been increasingly detected in new species and at apparently high prevalence in buffalo (*Syncerus caffer*), lion (*Panthera leo*), wild dog (*Lycaon pictus*), and warthogs (*Phacochoerus africanus*). Long-term studies are needed to determine the overall impact on the Kruger ecosystem. Effective bTB control is of importance with an understanding of the complex systems influencing human livelihoods and wildlife health within the Greater Limpopo Transfrontier conservation area (GLTFCA), which extends across international borders.

SEROPREVALENCE OF ANTIBODIES AGAINST MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS AND ITS RELATIONSHIP TO AGE AND SEX OF TEXAS WHITE-TAILED DEER (ODOCOILEUS VIRGINIANUS) IN COAHUILA, MEXICO

E. Alejandro Lozano-Cavazos,1 J. Vicente Vela ́zquez-Morales,2 Marco A. Santilla ́n-Flores,3

Luis A. Tarango-Ara ́mbula,4 Genaro Olmos-Oropeza,4 J. Felipe Mart ́ınez-Montoya,4 and César Cortez-Romero2,4,5

ABSTRACT: Paratuberculosis (PTB) is a disease that affects cattle (Bos taurus), goats (Capra aegagrus hircus), sheep (Ovis aries), and wild animals, such as white-tailed deer (Odocoileus virginianus), since all ruminants are susceptible. The causal agent is Mycobacterium avium subsp. Paratuberculosis (MAP). The disease is chronic, consumptive, and incurable; it causes chronic granulomatous gastroenteritis with lymphangiectasis and lymphangitis leading to a syndrome of malnutrition and eventually to death. Mycobacterium avium subsp. paratuberculosis is transmitted in feces mainly orally; however, it can also be transmitted vertically. Thus, the objective of this study was to determine the seroprevalence of MAP antibodies and its relationship to age and sex of Texas white-tailed deer in the subclinical stage of PTB in Coahuila, Mexico. The entire population (n1⁄499) belonging to the Wildlife Management and Conservation Unit (WMCU) San Juan, Monclova, Coahuila, Mexico was captured. Mycobacterium avium subsp. paratuberculosis was diagnosed using an enzyme-linked immunosorbent assay by serologic test. Seroprevalence variables of adult vs. young females and males vs. females were compared. The treatments were assigned at random. For the analysis of data, the chi-square test was used. Total seroprevalence in an intensive WMCU was 16% (16/99). Total seroprevalence by sex was 5.0% (5/99) for males and 11% (11/99) for females, and total seroprevalence by age was 7% (7/99) for young and 9% (9/99) for adult. Within sex, the seroprevalence in males was 16% (5/31) and 16% (11/68) in females. There were no statistical differences for any of the comparisons. Total seroprevalence of the white-tailed deer population in the WMCU was 16%, and PTB seroprevalence was independent of sex or age of the sampled individuals of this population.

* Paratuberculosis: Johne’s disease
	+ Common clinical signs: chronic diarrhea, weight loss, decline in body condition
* Horizontal and vertical (suspected- intrauterine transmission more common in farmed deer)
* PTB has been reported in red deer and rabbits
* No statistical differences between young/adult or males/females
* The value of seroprevalence tended to be higher in adult animals than in young animals since MAP has a long incubation period
* Serological, molecular, and culture-based tests can be used to estimate the number of infected individuals
* Little relationship between age and deer; for red tailed deer most susceptible animals are 8-15 mo old
* Risk of infection increases due to high animal concentration, exposure of offspring to contaminated feces, milk and water, low nutritional value feed, acidic soils, stress factors (transport, birthing, and lactation)
* Bacteriologic culture of feces: allows for clinical and subclinical detection of infection
	+ Specificity 100% but 50% sensitivity
* This study recommended nested PCR test: sensitivity 67.8% and specificity 84%

ANTIBODY PREVALENCE TO AFRICAN SWINE FEVER VIRUS, MYCOBACTERIUM BOVIS, FOOT-AND-MOUTH DISEASE VIRUS, RIFT VALLEY FEVER VIRUS, INFLUENZA A VIRUS, AND BRUCELLA AND LEPTOSPIRA SPP. IN FREE-RANGING WARTHOG (PHACOCHOERUS AFRICANUS) POPULATIONS IN SOUTH AFRICA

Donald Neiffer,1,11 Jennie Hewlett,2,8 Peter Buss,3 Leana Rossouw,3 Guy Hausler,4 Lin-Mari deKlerk-Lorist,5 Eduard Roos,4,9 Francisco Olea-Popelka,6,10 Baratang Lubisi,7 Livio Heath,7 and Michele Miller4

ABSTRACT: The warthog (Phacochoerus africanus) can be used as a model for investigating disease transmission at the human, wildlife, and livestock interface. An omnivore and scavenger, a warthog moves freely between natural ecotypes, farmland, and human communities and is susceptible to diseases of zoonotic, agricultural, and conservation concern. A retrospective study using 100 individual serum samples collected from May 1999 to August 2016 was performed to determine antibody prevalence to seven pathogens in warthogs from five locations in northeastern South Africa. Higher prevalence of antibodies to African swine fever virus and Mycobacterium bovis were detected in warthogs from the Greater Kruger National Park ecosystem in comparison to lower prevalence of antibodies to M. bovis and no antibodies to African swine fever virus in warthogs from uMhkuze Game

Reserve. Low prevalence of antibodies to foot-and-mouth disease virus, Rift Valley fever virus, and influenza A virus was detected in all locations, and no antibodies against Brucella and Leptospira spp. were detected. No statistically significant difference in antibody prevalence was found between sexes for any disease. At the univariate analysis, M. bovis seropositivity was significantly different among age categories, with 49% (35/71) of adults found positive versus 29% (4/14) of juveniles and 9% (1/11) of sub-adults (Fisher’s exact test, P1⁄40.020), and between the sampling locations (Fisher’s exact test, P1⁄40.001). The multivariate model results indicated that juvenile warthogs had lower odds of testing positive to M. bovis antibodies than adults (juveniles’ odds ratio [OR]1⁄40.17, 95% confidence interval

[CI]: 0.02–1.0), although this result was not statistically significant at the 5% level (P1⁄40.052). For warthogs sampled at Satara Buffalo Camp, the odds (OR1⁄40.22, 95% CI: 0.035–0.96) of being M. bovis antibody positive were significantly lower (P1⁄40.043) than for warthogs sampled at Skukuza. Of particular interest in this study was the detection of warthogs seropositive for influenza A virus.

* In Sub Saharan Africa: warthog can be used for investigation between human, wildlife and livestock interface
* Evaluated 100 banked serum samples for 7 different diseases
* Reactive antibodies were found to ASFV (84%) with higher prevalence in GKNP 98% than MZ (0%), and Mycobacterium bovis (42%)
* Avian influenza was 9%, low reactive antibodies for FMDV and RVFV (Rift valley fever)
* Antibodies to brucella and lepto were not detected
* No significant differences between males/females for any pathogen
* ASFV: lower in subadults compared to adults and juveniles
* No significant difference between age categories formyccobacterium bovisadults higher positive samples compared to juveniles or sub adults
	+ Samples from MP had 3.6 times more likely to be positive compared to samples from SZ (not statistically significant)
* Bovine tuberculosis= most likely occurs at shared food and water resources
	+ Antibody positive warthogs is similar to what is found in African buffaloes and lions
* Note: lepto has been noted in kidneys (carrier state) in suids
* 9% positive for Avian influenza: concern b/c warthogs are an unknown vessel for mixing