Mycobacterium bovis infection in the lion (*Panthera leo*): current knowledge, conundrums and research challenges

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Highlights

• We review published literature on *Mycobacterium bovis* infection in lions.

• Specific and non-specific *M. bovis* disease pathology in lions are summarised.

• Sources and route of infection, diagnostics, and co-morbidities are reviewed.

• Tuberculosis threat to lion population is reviewed.

• Knowledge gaps are identified and discussed.

Abstract

Mycobacterium bovis has global public-health and socio-economic significance and can infect a wide range of species including the lion (*Panthera leo*) resulting in tuberculosis. Lions are classified as vulnerable under the IUCN Red List of Threatened Species and have experienced a 30% population decline in the past two decades. However, no attempt has been made to collate and critically evaluate the available knowledge of *M.bovis* infections in lions and potential effects on population. In this review we set out to redress this. Arguments suggesting that ingestion of infected prey animals are the main route of infection for lions have not been scientifically proven and research is needed into

other possible sources and routes of infection. The paucity of knowledge on host susceptibility, transmission directions and therefore host status, manifestation of pathology, and epidemiology of the disease in lions also needs to be addressed. Advances have been made in diagnosing the presence of *M. bovis* in lions. However, these diagnostic tests are unable to differentiate between exposure, presence of infection, or stage of disease. Furthermore, there are contradictory reports on the effects of *M. bovis* on lion populations with more data needed on disease dynamics versus the lion population's reproductive dynamics. Knowledge on disease effects on the lion reproduction and how additional stressors such as drought or co-morbidities may interact with tuberculosis is also lacking. Filling these knowledge gaps will contribute to the understanding of mycobacterial infections and disease in captive and wild lions and assist in lion conservation endeavours.

Keywords: Bovine Tuberculosis; *Mycobacterium bovis; Panthera leo;* Lion; Mycobacterial disease; Wildlife conservation.

1. Introduction

Mycobacterium bovis forms part of the pathogenic *Mycobacterium tuberculosis* complex group of organisms (Brosch et al. 2002). Its ability to infect a wide range of livestock and wildlife species, as well as humans, highlights its global public-health and socio-economic significance (Ayele et al. 2004; Michel et al. 2006; Renwick et al. 2007; OIE, 2012). Additionally, *M. bovis* can be considered an invasive species in ecosystems where it historically did not occur (Michel et al. 2006; Ferreira & Funston 2010). In 1929 a report on the presence of *M. bovis* in greater kudu (*Tragelaphus stresiceros*) and other small ungulates in the Eastern Cape Province of South Africa suggested a potential transmission of *M. bovis* from domestic cattle to African wildlife species (Michel et al. 2006; OIE, 2012). De Vos et al. (2001) stated that *M. bovis* showed all indications of causing ecological imbalance in the Kruger National Park (KNP) ecosystem and had at that stage already taken on epidemic proportions.

Since 1929, other African wildlife species reported to have been infected with *M. bovis* include African buffalo (*Syncerus caffer*), wildebeest (*Connochaetes taurinus*), bushpig (*Potamochoerus porcus*), chacma baboon (*Papio cynocephalus*), cheetah (*Acinonyx jubatus*),

common duiker (Sylvicapra *grimmia*), eland (*Taurotragus oryx*), honey badger (*Mellivora capensis*), impala (*Aepyceros melampus*), large spotted genet (*Genetta tigrina*), leopard (*Panthera pardus*), lechwe (*Kobus leche*), lion (*Panthera leo*), spotted hyaena (*Crocuta crocuta*), and warthog (*Phacochoerus aethiopicus*) (Keet et al. 1996; de Vos et al. 2001; Cleaveland et al. 2005; Michel et al. 2006; Trinkel et al. 2011; OIE, 2012). All species do not appear to have the same susceptibility to infection with *M. bovis* and their role in the epidemiology can be roughly grouped into spillover hosts and maintenance hosts (Ayele et al. 2004). In maintenance hosts, infection can persist in a population without reinfection events from other species. In contrast, spillover host populations need to be reinfected from other sources in order for the infection to persist. Cattle and other bovids are arguably the most well known maintenance hosts (Ayele et al. 2004).

African buffalo are a maintenance host of *M. bovis* in much of their range with *M. bovis* endemic in the buffalo populations of the KNP and the eastern KwaZulu-Natal (KZN) province of South Africa. *M. bovis* in hosts such as buffalo can potentially be transmitted to other susceptible species – including domestic cattle - that can either also serve as additional maintenance hosts or spillover hosts (de Vos et al. 2001; Renwick et al. 2007). The presence of *M. bovis* in certain lion populations has been ascribed to transmission from infected buffalo (Renwick et al. 2007; Michel et al. 2009).

Lions are apex predators in the African habitat with their presence or absence determining the survival of various other animals (carnivores and herbivores) which in turn can even affect the flora and overall biodiversity in a specific area. Lions are a major tourist attraction and thus economically important. Together with other species in the large predator guild they have the capacity to act as flagship or sentinel species for conservation efforts (Dalerum et al. 2008).

It is therefore important to establish the effect of *M. bovis* infections on lions in order to make informed decisions concerning their management and conservation. This review is aimed at summarising current publications of tuberculosis in lions, critically analysing them and identifying additional research required to allow informed policy and intervention strategies.

2. Overview of tuberculosis case reports in lion

The lion is classified as vulnerable under the IUCN Red List of Threatened Species and has experienced a 30% population decline over the past two decades (Nowell et al. 2012). This is primarily due to the killing of lions to protect human life and livestock as well as a reduction in wild prey availability and habitat loss. Additionally, disease is also considered a threat to lion populations (Nowell et al. 2012).

The first reported cases of lions contracting tuberculosis (TB) came from two different zoos (Eulenberger et al. 1992; Morris et al. 1996). In 1992, Eulenberger et al. reported on the cases of tuberculosis and the management thereof in primates and felids in the Leipzig Zoological Gardens in Germany from 1951-1990. Although this report focussed on all felid species housed at the zoo - including leopard (*Panthera pardus*), tiger (*Panthera tigris*), puma (*Puma concolor*), lynx (*Lynx lynx*) - the species most often diagnosed with TB was the lion (Eulenberger et al. 1992). The high level of infection recorded (12 cases in 39 years) was not regarded as an indication of lion susceptibility to TB, but rather ascribed to the manner in which the zoo lion population was housed. They did not specify the *Mycobacterium sp.* that infected the lions. However, of all the felids, seven of the TB cases were confirmed to be due to *M. bovis* infections, while 19 cases were undetermined. No cases of *M. tuberculosis* were reported (Eulenberger et al. 1992). The onset of disease was relatively sudden after the felids experienced high stress situations such as after repeated periods of pregnancy and lactation. Other signs of disease observed in the felids were a lack of movement associated with an overall loss of condition and body weight as well as severe dyspnoea (Eulenberger et al. 1992). The lungs were the main organ affected in felids, suggesting that the route of infection in these cases was through airborne droplets. Additionally, alterations in the intestine and intestinal lymph nodes suggested the possibility that infection could also have occurred through the ingestion of infected meat (Eulenberger et al. 1992).

The report from the second zoo was published in 1996 and reported on TB due to *M. bovis* in only lions. An eight year old male lion in the Knoxville Zoo, USA, was euthanised in 1985 due to its continued deteriorating health (Morris et al. 1996). The first signs that the lion was diseased were a three week history of weight loss and anorexia. Other clinical signs observed are listed under section 3 of this review. Diagnosis of *M. bovis* infection was

demonstrated post mortem by isolation from a trachebronchial lymph node (Morris et al. 1996). This lion was in direct contact with a lioness and in indirect contact with two other younger lions. Three years after the diseased lion was euthanised, follow-up examinations were done on the remaining three lions with normal results obtained for physical examinations, whole blood counts and serum chemistry. Morris et al. (1996) were not able to establish the route of infection for the male lion. No mention was made concerning the age or origin of the infected lion at the time of procurement by the Knoxville Zoo. Considering that *M. bovis* can be latent for a long period of time in some animal species, infection could have occurred before arrival at the zoo.

The first report of *M. bovis* in free ranging African lions was in 1996 in two lionesses in the KNP, South Africa (Keet et al. 1996). These two lionesses were both approximately 10 years of age with one emaciated to the degree that she could hardly stand. Both of the females had lung lesions that were morphologically similar. Acid-fast bacilli were detected in smears made from the exudate of these lesions and culture confirmed the presence of *M. bovis* (Keet et al. 1996). Since this report many more lions with tuberculosis have been identified in the KNP (Keet et al. 2000; Keet et al. 2010). Most of the confirmed tuberculosis cases in lion came from the central and southern regions of KNP, corresponding with the regions of high *M. bovis* prevalence in buffalo herds (Renwick et al. 2007). Keet et al (1996), while referring to the report of Eulenberger et al. (1992), proposed that the most likely route of exposure was via the alimentary route from eating contaminated buffalo carcasses. Many diseased lions have since been euthanised and generated considerable data (Kirberger et al. 2006; Keet et al. 2010; Trinkel et al. 2011)(see details in later sections).

The presence of *M. bovis* in wildlife in general has also been reported in KZN with confirmation of lions being infected in the Munyawana Game Reserve (Michel et al. 2009) and the Hluhluwe-iMfolozi Park (HiP) (Michel et al. 2006; Michel et al. 2009; Trinkel et al. 2011). In HiP, infection with *M. bovis* was confirmed from post mortem inspection and culture of samples from lions that had died naturally (presumably from tuberculosis) or that were euthanised due to advanced emaciation (Trinkel et al. 2011). The main route of exposure for HiP lions was believed to be ingestion of contaminated buffalo carcasses (Trinkel et al. 2011). Unfortunately, disease pathology was not described in this study.

Elsewhere in Africa *M.bovis* was reported in free ranging lions in the Serengeti National Park, Tanzania (Cleaveland et al. 2005). Lion blood serum samples collected over the period 1984 to 2000 were subjected to *M.bovis* antibody enzyme immunoassay (EIA). The serological results suggested that mycobacterial infection was present in the Serengeti lions from as early as 1984. Although the serology results could not identify the species of the *Mycobacterium tuberculosis* complex involved, isolation of *M. bovis* from lion prey species suggested it as a likely candidate (Cleaveland et al. 2005).

3. Clinical signs and physiological changes accompanying *M. bovis* infection in lions

The degree to which lion health is impacted by tuberculosis is largely unknown. Progress of tuberculosis in lions is apparently slow, with the majority of infected lions appearing healthy while being sub-clinically infected (Keet et al. 2010). Unfortunately, clinical signs of active disease appear only when the disease has progressed to an advanced stage. Antemortem clinical signs associated with progressive tuberculosis are: marked alopecia and old, poorly healed bite wounds (Keet et al. 1996; Keet et al. 2000), emaciation (Morris et al. 1996; Keet et al. 1996; Keet et al. 2010; Trinkel et al. 2011), corneal opacity (Keet et al. 1996; Keet et al. 2000); dyspnoea and tachypnoea (Eulenberger et al. 1992; Morris et al. 1996; Cleaveland et al. 2005), bilateral sub-mandibular swelling (Cleaveland et al. 2005), ataxia and hypermetria (Cleaveland et al. 2005) and bilateral pulmonary disease (observed by means of thoracic radiography) (Morris et al. 1996). Cytology of bronchoscopic aspirate revealed pyogranulomatous exudates with many macrophages, moderate numbers of mature non-degenerate neutrophils, and a few plasma cells and lymphocytes (Morris et al. 1996). Abnormal whole blood counts included leukocytosis with a mature neutrophilia and slight toxic granulation, and monocytosis, while serum chemistry abnormalities included hypoalbuminaemia, hyperglobulinaemia, and hypercalcaemia (Morris et al. 1996). Keet et al. (2000) reported similar haematology and blood chemistry findings and suggested that *M. bovis* in lions causes haematological changes similar to that seen in alimentary tract infections associated with malabsorption (Keet et al. 2000). In humans there is a strong association between active tuberculosis and diabetes mellitus (DM) with indications that DM is a significant risk factor for developing active TB and/or vice versa (Broxmeyer, 2005;

Harries et al. 2009; Mao et al. 2011; Gupta et al. 2011). Human TB is associated with altered energy metabolism and homeostasis in patients with active TB (Broxmeyer, 2005; Bell et al. 2007; Bottasso et al. 2010; Santucci et al. 2011) that could actually aid in the development of type-2 diabetes (Broxmeyer, 2005). Whether or not such associations exist in lions is unknown.

Tuberculous lesions in lions differed macroscopically from that described in ungulates and non-human primates (Keet et al. 2000; Renwick et al. 2007; Keet et al. 2009). Only pulmonary lesions were macroscopically diagnostic while all other lesions were difficult if not impossible to identify macroscopically (Keet et al. 2000).

The lesions observed in various organs were granulomatous, typical of tuberculosis lesions in other species. Histologically they consist of macrophages, epithelioid cells, lymphoplasma cells and neutrophils (Keet et al. 2010). In addition lung and lymph node lesions showed extensive fibrosis and scant focal necrosis. Bronchiectasis and exudative tuberculous bronchitis was also observed in the lungs (Keet et al. 2000). Table 1 summarises the lesion characteristics and the macroscopic and microscopic pathology associated with the various organ systems.

Since there are over 130 known species of Mycobacteria, caution is required to not over diagnose *Mycobacterium tuberculosis* complex by simple lesion observation or smear testing (Botha et al. 2013). Acid-fast bacilli were often sparse or absent from histological sections, even from some culture-positive cases (Keet et al. 2010). Non-tuberculous mycobacteria (NTM) were frequently cultured from lions and might have been responsible for the observation of acid-fast bacteria in suspicious and microscopic lesions (Keet et al. 2010). As a result, Keet et al. (2010) did not use histology to enhance the specificity and/or sensitivity of their culture gold standard while validating the lion intradermal tuberculin skin test (Keet et al. 2010).

Table 1: Tuberculosis lesions: characteristics and micro- and macro-pathology associated with the different lion organ systems.

Organ or System	Lesions	References
Respiratory system	More often associated with advanced cases of tuberculosis; Consolidation with ill-defined lesions, sometimes confluent, firm, pliable foci approximately 4 cm in diameter; Varying levels of acid-fast bacilli. Pneumonia consisted of an amorphous, multifocal to coalescing, expansile (non-encapsulated) granulomatous inflammatory reaction without necrosis, giant cells or calcification;	Keet et al. (1996), Keet et al. (2000), Keet et al. (2010)
Intestine	Miliary distributed, microscopic, lesions with small granulomas in submucosa; Mononuclear macrophage predominance suggestive of mycobacterial mural enteritis.	Keet et al. (2000), Keet et al. (2010)
Skeletal	Granulomatous osteitis, periostitis and osteosis, frequently associated with myositis; *M. bovis* induced osseous lesions were more likely to involve the joints; Proliferative septic arthritis, joint capsule mineralization, and bone slivers may be good indicators of *M. bovis* infection; Unilateral lesions in the proximal tibia (more often seen in adult males) and tibio-tarsal joints, the proximal radius and ulna, and the thoracic vertebrae with various degrees of hind limb atrophy and lameness; Numerous intracellular acid-fast organisms present in elbow joint hygromas in adult lions; Elbow hygromas more frequently seen in females.	Keet et al. (2000), Keet et al. (2009), Keet et al. (2010), Kirberger et al. (2006),
Lymphatic system	Visible, palpable and marked enlargement of superficial lymph nodes not present in lions; Miliary distribution of lesions in nodes in early stage infection; Marked lymphoid atrophy with no evidence of tuberculosis; Generalised lymphadenopathy accompanied by cystic dilation in the paracortical areas observed after necropsy; Lesions present in many of the lymph node groups, regardless of degree of infection; Granulomatous lesions containing eosinophils and parasitic remnants observed.	Keet et al. (1996), Keet et al. (2000), Keet et al. (2009), Keet et al. (2010)
Eyes	Granulomatous panophthalmitis, choroiditis, uveitis, and conjunctivitis, with or without retinal detachment.	Keet et al. (2000)
Kidneys	Amyloidosis in the medulla being more severe in advanced tuberculosis cases.	Keet et al. (1996), Keet et al. (2000)
Other	Microscopic lesions in liver and bone marrow; Non-specific muscle atrophy accompanied by cachexia, decubitus ulcers; Testis atrophy; None of 86 necropsied females were pregnant.	Keet et al. (2000), Keet et al. (2009)

4. Routes of exposure

Domestic cats may be infected with *M. bovis* from infected food sources (Little et al. 1982; Morris et al. 1996). Eulenberger et al. (1992) were the first to suggest that lions could be infected with *Mycobacterium sp.* by ingesting infected meat. They also suggested horizontal transmission between lions by means of aerosol droplets (Eulenberger et al. 1992). Hence, Keet et al. (1996) suggested that wild lions become infected with *M. bovis* firstly through consumption of infected prey after which spread of the disease to other pride members could occur via droplet transmission. Additionally, the possibility also exists that lions can be exposed to *M. bovis* while suffocating prey (e.g. African buffalo) by biting over the muzzle of the prey (Renwick et al. 2007). The finding that lions are able to shed viable *M. bovis* through the respiratory system argues for droplet transmission (Miller et al. 2015). However, definitive evidence for these routes of infection is lacking. Other routes of

transmission may also be involved, for example percutaneous infection occurring during social aggression, to explain hygromas.

The epidemiology of tuberculosis in lions may differ between areas. In the KNP and HiP genotyping of *M. bovis* isolated from buffaloes and lions showed matching sequences although the sequences in the two areas were not the same. This suggested that *M. bovis* infection in these lions was due to transmission from infected buffaloes (Renwick et al. 2007; Michel et al. 2009).It was also considered that lion behaviour patterns (preferential prey species, individual prey selection, scavenging, and intra-species specific aggression) led to the frequent exposure of lions to *M. bovis* from different and varied species(Keet et al. 2000; Renwick et al. 2007). It is now commonly accepted that the principle route of infection for lions is through consumption of infected prey (Keet et al. 2000; Cleaveland et al. 2005; Kirberger et al. 2006; Keet et al. 2010). In this regard, Renwick et al. (2007) raised the concern that if *M. bovis* was to become established in additional prey species with maintenance host capabilities, the opportunity for contact with infectious prey would increase. Indeed the main suspected source of *M. bovis* infection for Serengeti lions was considered to be wildebeest and not the buffalo (Cleaveland et al. 2005).

That lions are a maintenance host has not been conclusively established. Lesions in many different sites have been described in infected lions, suggesting the possibility of several different routes of infection (Keet et al. 2000). Interestingly Renwick et al. (2007) stated that lions do not seem able to maintain *M. bovis* infection in the absence of infected maintenance hosts in an ecosystem. This suggests that lions may be end hosts(Renwick et al. 2007) in which there is little or no chance of onward transmission occurring. Anecdotal evidence for this comes from Morris et al. (1996), who described an infected male lion housed with a lioness who did not contract the disease. Serum samples from this lioness subjected to ELISA tests showed a decline in reactivity to both *M. bovis* and *M. avium* antigens at progressively longer time intervals after the male was euthanised (Morris et al. 1996). This could indicate that although the lioness was exposed to *M. bovis* her immune system managed to prevent progression to a diseased state. To our knowledge no other studies have provided further evidence for changes in reactivity over time.

5. *M. bovis* diagnostics for lions

The diagnosis of *M. bovis* infection in lions may involve gross post mortem examination with associated histopathology, bacteriological examination of clinical and post mortem samples, and immunological assays. The development and application of diagnostics for M. bovis infection is dependent on a number of factors. This includes whether the target is a living or dead animal, the degree of false positive and false negative findings for the test, test sensitivity, the practicality for field application and expense. The accepted gold standard for diagnosis of mycobacterial infection is still culture based (generally from tissues obtained at necropsy). This remains the benchmark for the validation of other diagnostic protocols or algorithms combining several tests. It should be mentioned that based on cattle studies the sensitivity of culture diagnosis of *M. bovis* is reliant on the technique and effort employed during post mortem examination and sampling of carcases (Corner, 1994). The first reported infections of *M. bovis* in wild lions employed culture of samples obtained from lungs and identification by standard bacteriological methods (Keet et al. 1996). *M. bovis* has also been isolated from the tracheobronchial lymph node (Morris et al. 1996).

Another option to obtain samples for culture is through bronchoalveolar lavage (BAL). Miller et al. (2015) managed to identify *M.bovis* infection in 6% of 134 lions tested in the KNP. However, this method can produce significant false negative findings. Aspiration liquid does not reach all areas of the lung and therefore can fail to recover viable organisms for culture (Somu et al. 1995; Miller et al. 2015) in infected lions. Additionally, processing (freeze-thaw) and transportation might also decrease viability of the samples. Therefore prevalence of shedding of *M. bovis* is likely to be higher in the KNP population (Miller et al. 2015). Infection in other organs or lymph nodes cannot be reached through BAL sampling, and sampling of these tissues requires biopsy (e.g. fine needle aspirate) which can be effective for some nodes. To our knowledge other clinical samples such as urine, faeces, saliva and nasal mucus has not been investigated as sources of organisms for culture. Keet et al. (2010) highlighted the importance of attempting culture from all organ systems irrespective of observable macroscopic lesions. For lions this is particularly important since multiple organ systems can be affected while lesions, other than pulmonary ones, are not readily identifiable macroscopically (Keet et al. 2010). Kirberger et al. (2006) managed to culture *M. bovis* from

affected joints and a hygroma. Interestingly, histological slides of microscopic lesions stained with Ziehl-Neelsen had a notable absence of acid-fast bacilli despite the lions being shown to be culture positive (Keet et al. 2010).

An option for antemortem diagnosis of *M. bovis* infection is the use of ELISA/EIA antibody tests using *M. bovis* and *M. avium* antigens. Morris et al. (1996) suggested that they could serve as a sensitive supplement to traditional diagnostic tests. Morris et al. (1996) utilised ELISA tests on blood samples from an infected male lion, an in-contact lioness, and two juvenile lions indirectly in-contact with the male lion. Comparing the ELISA results to those obtained from nine healthy control lions showed that the diseased male had much higher antibody levels while the two juveniles had basal values. The in-contact female had intermediate levels of antibodies. The in-contact female was sampled one and three years after the male was euthanised and showed a decrease in antibody levels. The lioness and the juveniles were still asymptomatic respectively five years and nine years after the diseased lion was euthanised (Morris et al. 1996). In future useful information on infection and progression to disease (or lack thereof) could be gained from serological tests repeated over a few years in infected and exposed lions. While this might be suitable for a captive lion study, repeat sampling of free roaming lions would be more difficult. A more, *M. bovis* specific once-off antemortem diagnosis would be more optimal for wildlife management. However, it needs to be recognised that the presence of *M. bovis* specific antibodies simply indicates that the animal has been exposed to *M. bovis* at some time and does not indicate current infection or active disease.

Two separate studies from the Serengeti National Park and the KNP utilised a similar serological test using MPB70 antigen (Cleaveland et al. 2005; Keet et al. 2010). MPB70 is thought to be highly specific to *M. bovis* and was initially purified from *M. bovis* bacillus-Calmette-Guerin (BCG) (Nagai et al. 1981; Harboe et al. 1990). Four percent of 184 Serengeti lion tested seropositive for tuberculosis. No cultures were done to confirm infection with *M. bovis* (Cleaveland et al. 2005). Since MPB70 is highly expressed by *M. bovis* (Wiker et al. 1998) it is reasonable to assume that the Serengeti lions were indeed exposed to *M. bovis*. However, there may be some level of non-specificity as Rhodes et al. (2011) showed antibody production to MPB70 in cats infected with *Mycobacterium* complex species other

than *M. bovis* (Rhodes et al. 2011). Due to the limited sensitivity of the ELISA Cleaveland et al. (2005) stated that the 4% seroprevalence might suggest the minimum prevalence of *M. bovis* in the Serengeti lion population. In the KNP study only 12 of 26 confirmed *M. bovis* positive lions with advanced clinical disease gave seropositive results (Keet et al. 2010) also indicating low sensitivity.

The low sensitivity of the serological assays is not limited to lions. Serological assays, while in some instances specific, do not have the necessary sensitivity to serve as reliable antemortem diagnostic tests for individual animals (Harboe et al. 1990; de Lisle et al. 2002; Chambers et al. 2008). However, increased sensitivity of serological assays may be seen in animals with advanced tuberculosis (Harboe et al. 1990; de Lisle et al. 2002; Chambers et al. 2008). Use of serological tests in concert with cellular immune based diagnostic tests could increase the sensitivity of identifying *Mycobacterium* infected animals (Harboe et al. 1990; Gutiérrez et al. 1998; de Lisle et al. 2002). In most animal species a specific cell-mediated response is detectable following infection but in advanced cases cell-mediated immune responses may decline and high levels of antibody may become apparent (Harboe et al. 1990; de Lisle et al. 2002).

The lack of reliable serological or cell-mediated immune assay led Keet et al. (2010) to explore the use of the intradermal tuberculin skin test as an antemortem diagnostic test for *M. bovis* in lions. Although skin testing of free roaming wildlife species presents logistical difficulties, Keet et al. (2010) managed to show the inherent value of doing such diagnostic tests on lions. By modifying the method used to test domestic cattle, it was possible to identify over 86.5% of lions (n=52) in which *M. bovis* infection was confirmed through mycobacterial culture. The two main alterations to the established cattle protocol were: the use of 0.2ml tuberculin per injection site (double the volume prescribed for cattle); and while both avian and bovine tuberculin were injected at separate sites, they only considered the result of the bovine tuberculin reaction (Keet et al. 2010). However, 13.5% of culture positive lions tested negative (false negative) and 18.8% of true negative animals tested positive (false positive) (Keet et al. 2010). Additionally, skin testing of free ranging lions is a logistical challenge as it requires holding the animals for or recapturing them after 72 hours to record a response.

As discussed above, assays of the cell-mediated and humoral immune responses could be evaluated in parallel to increase the accuracy of diagnosis. Keet et al (2010) suggested that serological diagnostics could serve to compliment the intradermal tuberculin test, specifically in cases of non-reactor lions with advanced tuberculosis. However, the progression of *M. bovis* infection could be accelerated by feline immunodeficiency virus (FIV) co-infection. However, co-infection could ultimately also lead to reduced antibody levels (Keet et al. 2010) thereby negatively affecting serological assays. If this is the case it could also help to explain the low *M. bovis* seroprevalence (4%) described for Serengeti lions by Cleaveland et al. (2005) (See Section 7 for FIV prevalence).

Another test that is at least as sensitive as the skin test in other species, but with the ability to detect infection marginally earlier, is the IFN-γ test that also relies on the CMI (de la Rua-Domenech et al. 2006). Unfortunately this test does not distinguish between infection status (e.g. recent, latent or advanced/diseased) (Pal et al. 2008) and can be affected by diverse pathophysiological and physiological events . Maas et al. (2010) described the genetic sequence of lion INF-γ. They compared it with the cheetah and domestic cat sequences and found that the sequences are highly conserved between these species. They suggested the possibility that a lion or cat specific INF-γ ELISA if developed could be used for other feline species (Maas et al. 2010). Further investigations to establish cross reactivity with existing INF-γ tests or one developed with cat INF-γ antibodies is also needed (Maas et al. 2010). In humans, co-morbidities with parasites and some viral infections as well as iron deficiencies and even younger age can increase the likelihood of an indeterminate INF-γ assay results (Banfield et al. 2012). This might have implications for lions with co-morbidities of other pathogens such as FIV or canine distemper virus, thus raising doubts on the sensitivity of the test.

6. Effect of *M. bovis* on lion populations

Keet et al. (1996) raised the concern that due to the interactions of lions, the disease could become established in the KNP lion population. If not already the case, lions might then serve as an additional maintenance host for *M. bovis* together with the buffalo. From the available literature it is clear that lions are spillover hosts but it is unclear if lions are end or

maintenance hosts (Keet et al. 1996; Michel et al. 2006; Renwick et al. 2007; Michel et al. 2009) (see earlier). Interestingly disease characteristics determined for buffalo also appear to be manifested in lions (Michel et al. 2006). These include mortality due to disease, correlations between age and infection, as well as correlations between infection and body condition (Michel et al. 2006).

There may be multiple behavioural and social effects of infection within an infected population. The lameness caused by a *M. bovis* infection might impact on hunting success of free ranging lions (Kirberger et al. 2006) or compromise the ability of an individual to compete in a natural environment. Michel et al. (2006) speculated that *M. bovis* was driving social changes within prides, contributing to lower lion survival and breeding success. This included faster territorial male coalition turnover with consequent infanticide, and the eviction of entire male and female prides from territories (Michel et al. 2006; Keet et al. 2009). A comparison between infected and non-infected sub-populations showed that the non-infected sub-population was significantly longer lived and had a higher cub survival rate even though the infected sub-population had a higher birth rate (Michel et al. 2006; Keet et al. 2009).

Due to the social nature of lions it is possible for TB compromised individuals to retain support from the rest of the pride. This gives the affected individual a better chance of surviving than would be the case for solitary animals. Estimated time from infection to death is between two and five years (Renwick et al. 2007). While most of the lions that died as a consequence of tuberculosis were older than five years, some reports also included younger lions, the youngest euthanised being 16 months old (Morris et al. 1996; Keet et al. 1996; Cleaveland et al. 2005; Kirberger et al. 2006; Trinkel et al. 2011). However, currently there is insufficient data to make definitive statements concerning the average age lions may reach when experiencing active disease. There is also no data on indirect mortalities due to *M. bovis*, such as cub mortalities owing to inadequate parental care from diseased adults.

Environmental factors may also influence how tuberculosis affects lion populations. Cleaveland et al. (2005) did not report on actual effects of *M. bovis* infection on the Serengeti lion population. They did, however, speculate that conservation practices might have an effect on how tuberculosis establishes in wildlife species and how the wildlife adapts to a new disease. In South Africa *M. bovis* is considered a relatively new pathogen introduced to naïve

wildlife populations. Stringent control measures in livestock farming and the fact that South African wildlife and livestock are usually separated by fences may have limited the transmission of *M. bovis* to wildlife (Cleaveland et al. 2005). In contrast, Cleaveland et al. (2005) suggested that the continued practice of fenceless husbandry in East Africa might have permitted the spread and establishment of *M. bovis* infection in wildlife over a longer time period, perhaps even permitting a more stable endemic pattern of infection.

Ferreira and Funston (2010) disputed the conventional wisdom of how disease can influence carnivore populations. They suggested that *M. bovis* in prey appears to have little detectable influence on lion demography in KNP while prey biomass is of more importance. Additionally, differences in lion body condition scores were apparently not associated with *M. bovis* prevalence in prey (Ferreira & Funston 2010). They were concerned, however, that the situation might change under different circumstances. Additionally, they pointed out some of the biases that could have affected their results . This included:

- The assumption that lion population vital rates were stationary within the study zones for some time before the survey started.
- That they assessed the relative importance of only two variables, namely prey biomass and *M. bovis* prevalence in prey. Therefore they could not separate the interactions in the northern part of KNP where they only had low disease prevalence, compared to the south where they had both medium and high prevalence.
- The survey was done in a relatively wet period with high quality prey biomass. This is important since during wet periods KNP lions predate mostly on wildebeest and zebra (*Equus quagga)* and to a lesser extent on buffalo. Prey switching occurs during dry periods when the importance of buffalo as prey increases. Therefore, under drought conditions lions are more likely to come into contact with *M. bovis* infected prey (Ferreira & Funston 2010).

In single host systems each pathogen and species of animal infected has a specific threshold population density that would allow a pathogen to infect and then be maintained in a population (Renwick et al. 2007). Determining persistence of pathogens in multiple host systems is more complicated with community thresholds changing in accordance to inter- and

intra-species interactions (Renwick et al. 2007). Additionally persistence of a disease in a population is also influenced by the reproductive rate (R_0) of a pathogen. For a pathogen to survive indefinitely R_0 must be ≥ 1. Diseases can, however, persist and be a continued threat for significant periods when R_0 <1, that is during the extinction phase, especially for chronic infections (Renwick et al. 2007). *M. bovis* is considered to be such a pathogen (Renwick et al. 2007).

Infection thresholds and disease dynamics have not been described for lions, therefore, we currently have very little idea concerning the ultimate effect of *M. bovis* on lion populations. There is no data available to show if disease dynamics is faster or slower than the lion's reproductive population dynamics. The definition or scale of population one adheres will also influence the results when interrogating population effects of tuberculosis and might be different from conservation area to conservation area. Even within large conservation areas like the KNP, quantifying disease versus population dynamics and community thresholds could be complicated by the variation in lion density in different regional zones. Keet et al. (2009) attempted some quantification for the KNP lion population. However, much of the data used in the model were derived from species other than lion and expert opinion.

Although Ferreira & Funston (2010) suggest that the persistence of lions in KNP is not threatened, they concede that the situation might change under different climatic conditions and in the presence of additional stressors. While it has been reported that *M. bovis* prevalence in prey does not have a negative influence on lion demography (Ferreira & Funston 2010), *M. bovis* infection in lions definitely affects individual lions and can therefore also affect pride dynamics. Should the lion population decline, inbreeding may become a problem with potentially serious consequences. In the HiP it was believed that the initial inbred status of the lion population in concert with tuberculosis had a bigger effect on the population with the effect subsequently being reduced when genetic diversity was introduced into the lion population (Trinkel et al. 2011).

7. FIV and *M. bovis* co-morbidities

Other diseases can have an effect on or be affected by the presence of an infectious disease such as tuberculosis. Feline immunodeficiency virus (FIV) and more specifically the

lion specific FIV strain (FIVple) is thought to be a relatively old disease in lions(Roelke et al. 2009). FIV has been shown to cause CD4+ immune cell depletion in lions (Roelke et al. 2006), cells that, at least in cattle, are significant producers if IFN-γ that plays an important role in the hosts immune response (Roelke et al. 2006). Neurological effects and wasting have been associated with FIV infection in captive lions (Roelke et al. 2009). In many instances increased cases of wasting were, associated with the outbreak of diseases such as tuberculosis or canine distemper virus with immunosuppressive effects (Roelke et al. 2009). Interestingly, some of the non-specific FIVple blood based abnormalities(Roelke et al. 2009) were similar to some of the non-specific *M. bovis* blood based abnormalities of a FIVple negative lion (Morris et al. 1996).

Some attempts have been made to determine if an FIV infection predisposes or exacerbates *M. bovis* infection. Keet et al. (1996) observed a high seroprevalence (83%) of FIV in the KNP lions who readily were infected with tuberculosis and that may have increased the susceptibility of lions to *M. bovis* infection. On the other hand, studies by Cleaveland et al. (2005) and Kirberger et al. (2006) described a low number of lions wit *M. bovis*-FIV co-infection and suggested that FIV seemingly has no influence on *M. bovis* infection in lions. These suggestions are, however, called in to question if one considers the low sensitivity of the tuberculosis EIA used to identify *M.bovis* infected lions in the Serengeti (Cleaveland et al. 2005) and the small samples size (n=6) reported on by Kirberger et al. (2006).

Subsequently Trinkel et al. (2011) considered that the proposed increase in susceptibility to *M. bovis* infection of lions with FIV (Keet et al. 1996) was indeed true for KNP lions. They compared this with the case of the Serengeti lions (Cleaveland et al. 2005) and concluded that *M. bovis* -FIV co-infection need not necessarily become an important problem in the HiP (Trinkel et al. 2011).

Additionally, *M. bovis* diagnostic tests utilising the CMI response could possibly be affected by the fact that FIVple is supposed to diminish the levels of CD4 subsets (Roelke et al. 2006; Keet et al. 2010). While looking at the viability of intradermal tuberculin testing as an ante-mortal diagnostic tool, Keet et al. (2010) concluded that FIVple co-infection did not significantly affect the intradermal skin test results.

It is clear that much more follow-up studies on lions infected with either FIV or *M. bovis* or both is needed to arrive at definitive conclusions about the possible synergies between FIV and *M. bovis*.

8. Conclusion and knowledge gaps

Relatively little has been published on *M. bovis* infection and ensuing pathology in lions. The interplay between a host and pathogenic microbe in a free-ranging ecosystem is highly complex and calls for a multidimensional and integrated approach in order to draw definitive conclusions. This review set out to critically review all publications on lion infection with *M. bovis* and tuberculosis and to identify crucial gaps in our knowledge which need to be addressed. Such knowledge gaps currently exist for the source and route of infection, and disease pathology. This includes our lack of understanding of host susceptibility, transmission directions (horizontal and vertical) and host status (maintenance, spill over or end host). We have no evidence concerning the mycobacterial dose and frequency of exposure required to establish infection and subsequent disease pathology in lions, factors which cause a lion to progress from infected to clinically diseased. We do not know whether some lions are resistant to *M. bovis* or what effect environmental stress could play in the onset and advance of active disease. While wasting and debilitation is strongly associated with end stage tuberculosis cases there are some suggestions that these are not *M. bovis* specific (Ide, 2002) end conditions. Little knowledge is available on the mechanism underlying emaciation and what effect this has on reproductive competence. At this time, there are contradictory findings and opinions on the effects of *M. bovis* on lion populations and population stability or size. We do not know with any confidence whether co-morbidities such as feline immunodeficiency virus (FIV) impact severely on individual animals or populations. Even our diagnostic ability in lions is poor and we are not confidently able to distinguish between exposure, infection and clinical disease in the lion. Such knowledge is vital to our approach to disease management of lions, and successful conservation strategies. Until such time as knowledge gaps such as those identified in this review can be addressed, management and disease control decisions will therefore largely be based on assumptions rather than robust scientific information.

9. References

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