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Intradermal tuberculin testing of wild African lions (*Panthera leo*) naturally exposed to infection with *Mycobacterium bovis*

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ABSTRACT

African lions in the southern half of Kruger National Park (KNP) are infected with *Mycobacterium bovis*. Historically, reliable detection of mycobacteriosis in lions was limited to necropsy and microbiological analysis of lesion material collected from emaciated and ailing or repeat-offender lions. We report on a method of cervical intradermal tuberculin testing of lions and its interpretation capable of identifying natural exposure to *M. bovis*. Infected lions ($n = 52/95$) were identified by detailed necropsy and mycobacterial culture. A large proportion of these confirmed infected lions (45/52) showed distinct responses to bovine tuberculin purified protein derivative (PPD) while responses to avian tuberculin PPD were variable and smaller. Confirmed uninfected lions from non-infected areas ($n = 11$) responded variably to avian tuberculin PPD only. Various non-tuberculous mycobacteria (NTM) were cultured from 45/95 lions examined, of which 21/45 were co-infected with *M. bovis*. Co-infection with *M. bovis* and NTM did not influence skin reactions to bovine tuberculin PPD. Avian tuberculin PPD skin reactions were larger in *M. bovis*-infected lions compared to uninfected ones. Since NTM co-infections are likely to influence the outcome of skin testing, stricter test interpretation criteria were applied. When test data of bovine tuberculin PPD tests were considered on their own, as for a single skin test, sensitivity increased (80.8–86.5%) but false positive rate for true negatives (18.75%) remained unchanged. Finally, the adapted skin test procedure was shown not to be impeded by persistent Feline Immunodeficiency Virus$_{Ple}$ co-infection.

1. Introduction

Mycobacterium bovis has infected part of the African buffalo (*Syncerus caffer*) population in the Kruger National Park (KNP) causing bovine tuberculosis (bovine TB) (Bengis et al., 1996; De Vos et al., 2001). Since the African buffalo is considered a preferential prey species, African lions (*Panthera leo*) residing in areas of KNP with a high prevalence of bovine TB in buffaloes acquired infection through consumption of tuberculous carcasses (Keet et al., 1996, 2000). We confirmed, by genetic typing, that spillover of *M. bovis* from buffaloes to lions occurred in the KNP (Michel et al., 2009). The lion index case in KNP was diagnosed through necropsy and culture in 1995 (Keet

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et al., 1996). Subsequently a number of emaciated and ailing lions were euthanized and it was established that multiple organ systems were affected, suggesting several possible routes of infection followed by haematogenous and/or lymphatic spread of mycobacteria (Keet et al., 2000). It is estimated that between 16,500 and 30,000 wild lions occur in Africa of which approximately 2200 occur in the Kruger Ecosystem (Bauer and Van Der Merwe, 2004). Since African lions are listed as Vulnerable on the IUCN Red Data List (2006), this precarious population status demanded a sensitive research approach regarding various infectious diseases especially bovine TB in lions (Cousins and Florisson, 2005).

There are currently still no reliable immunodiagnostic tests for feline tuberculosis (Rhodes et al., 2008). Morris et al. (1996) referred to an ELISA test that was used on three zoo lions exposed to a lion with advanced pulmonary bovine TB. A similar MPB70 ELISA test suggested a seroprevalence of 4% in Serengeti lions (Cleaveland et al., 2005) but this result was not substantiated by mycobacterial culture of lion lesion material. The same ELISA test was used on 26 confirmed and highly developed *M. bovis*-positive cases from KNP. Only 12/26 of these infected cases could be identified by this test (Unpublished data). Serologic assays for rapid detection of *M. bovis* infection have been described for a number of other free-ranging wildlife species (excluding lions) with promising results (Lyashchenko et al., 2008).

Ante-mortal diagnosis of bovine TB in free-living wildlife is notoriously difficult, as animals need to be located and immobilized to collect blood for *in vitro* diagnostic assays (Grobler et al., 2002; Palmer and Waters, 2006). In the event of skin testing, free-ranging animals need to stay in captivity or be released, tracked and immobilized again after 72 h to assess skin response. Intradermal tuberculin testing is the cornerstone of successful tuberculosis-control schemes in cattle (de la Rua-Domenech et al., 2006) and it has been used on a number of wildlife species, with varying degrees of sensitivity and specificity (Cousins and Florisson, 2005). There are other limitations to the use of intradermal tuberculin tests in wildlife, since information is lacking concerning proper test sites for different species, concentrations, dosages and preparations of tuberculins to use and guidelines for interpretation (Miller, 2008). Regarding the use of tuberculin in carnivores, reports on the use of intradermal testing in domestic cats and dogs were not favourable because they do not react adequately to intradermally administered tuberculin and results were found to be unreliable (Backues, 2008; Miller, 2008).

A large proportion of lions in KNP are serologically positive for FIV_{Ple} (Van Vuuren et al., 2003). Over and above the effect that infection with NTM may have on *M. bovis* specific immune responsiveness, hence on the results of skin testing in lions in the KNP, these test results may also be affected by co-infection with FIV_{Ple}. In domestic cats FIV_{Fca} critically impairs cell-mediated host responses and moderate to severe CD4+ depletion has been demonstrated in infected African lions (Roelke et al., 2006). A large proportion (53%) of bovine TB infected lions in areas of KNP where bovine TB is rife, are co-infected with FIV_{Ple} (Keet, unpublished data). In cattle, co-infection with *M. bovis* and viruses that depress the function of lymphocytes and macrophages (such as bovine viral diarrhoea virus) has been shown to affect diagnostic assays for bovine TB (Charleston et al., 2001). Similarly diminished *Mycobacterium tuberculosis*-specific cell-mediated immune (CMI) responses have been documented in humans where HIV immune suppression was involved (Vermund and Yamamoto, 2007). In our study FIV_{Ple} status of animals was carefully considered as a potential confounding factor in bovine TB diagnosis by skin testing.

The primary objective of this study was to determine whether the intradermal bovine tuberculin PPD test can identify lions naturally infected with *M. bovis* and which configuration of the intradermal skin test would achieve the highest sensitivity and specificity. Secondly the ability of NTM to evoke non-specific skin responses was evaluated. Concurrently, evaluation of possible attenuating effects of FIV_{Ple} on skin test response was required.

2. Materials and methods

2.1. Study population

A cohort of *M. bovis*-infected lions ($n = 52$) was identified from a group of emaciated and ailing ($n = 61/84$), or repeat-offender lions ($n = 23/84$) reported on an *ad hoc* basis by field staff and tourists (a repeat-offender lion being a recognized habitual cattle killer and/or an emigrating vagrant). Cases were collected from anywhere within and immediately around the KNP, with the preponderance of compromised lions originating from bovine TB infected areas. Lions from the uninfected far north of the KNP ($n = 11$) were assessed as non-infected control animals. These two cohorts of lions (84 and 11) were euthanized and their status confirmed through a comprehensive necropsy and mycobacterial culture process. An additional group of control lions ($n = 33$) from the uninfected area of the park were randomly selected and skin tested. They were not destroyed to confirm their status as they were in prime condition and not repeat-offenders. These lions were considered as negative specimens because they originated from an area where bovine TB had not been diagnosed in buffaloes or lions despite concerted efforts. This lion sub-population had been separated from the infected sub-population by a buffer zone of approximately 120 km wide. The buffer zone had a very low prevalence of bovine TB in buffalo (1.5%: 0.4–4.0, 95% CI) (Rodwell et al., 2001) and no lion cases were diagnosed.

2.2. Test procedure

Lions were immobilized with a combination of tiletamine and zolazepam (Zoletil® 100, Virbac Animal Health, Halfway House, South Africa) and transported to predator isolation units in Skukuza, the KNP headquarters. Venous blood samples were obtained from the medial or lateral saphenous veins immediately after anaesthesia. Age of the animal was estimated by examining dental attrition, its condition was scored and it was weighed. The animals were then tuberculin-tested according to the protocol described below and confined for 72 h. For skin testing