Tuberculosis serosurveillance and management practices of captive African elephants (Loxodonta africana) in the Kavango-Zambezi Transfrontier Conservation Area

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Summary

Transfrontier conservation areas represent an international effort to encourage conservation and sustainable development. Their success faces a number of challenges, including disease management in wildlife, livestock, and humans. Tuberculosis (TB) affects humans and a multitude of non-human animal species and is of particular concern in sub-Saharan Africa. The Kavango-Zambezi Transfrontier Conservation Area encompasses five countries, including Zimbabwe, and is home to the largest contiguous population of free-ranging elephants in Africa. Elephants are known to be susceptible to TB, thus understanding TB status, exposure, and transmission risks to and from elephants in this area is of interest for both conservation and human health. To assess risk factors for TB seroprevalence, a questionnaire was used to collect data regarding elephant management at four ecotourism facilities offering elephant-back tourist rides in the Victoria Falls area of Zimbabwe. Thirty-five working African elephants were screened for *Mycobacterium tuberculosis* complex antibodies using the ElephantTB Stat-Pak and the DPP VetTB Assay for elephants. Six of 35 elephants (17.1%) were seropositive. The risk factor most important for seropositive status was time in captivity. This is the first study to assess TB seroprevalence and risk factors in working African elephants in their home range. Our findings will provide a foundation to develop guidelines to protect the health of captive and free-ranging elephants in the southern African context, as well as elephant handlers through simple interventions. Minimizing exposure through shared feed with other wildlife, routine TB testing of elephant handlers, and regular serological screening of elephants are recommended as preventive measures.

Keywords

African elephant, elephant handlers, human-wildlife disease interface, Mycobacterium tuberculosis, transfrontier conservation areas, tuberculosis diagnosis

Introduction

Transfrontier conservation areas (TFCAs) in Africa incorporate large tracts of adjacent private, communal, and public land between neighboring countries and represent a broad-scale approach to improving biodiversity conservation and socioeconomic development of local communities (Hanks, 2003). One goal of TFCAs is to increase connectivity of an otherwise fragmented landscape for wildlife, while boosting nature-based tourism (Osofsky, Cumming, & Kock, 2008). An inherent challenge in the transboundary landscape of TFCAs is disease management, given movement and interfaces among wildlife, livestock, and humans in countries with varying disease prevalence and management strategies (Osofsky et al., 2008). A variety of diseases, including zoonoses, have been identified as threats to human and animal health within TFCAs (Cumming, 2011). Movement of diseases within TFCAs is a major concern: for example, work by Caron et al. (2016) has documented the spread of bovine tuberculosis (TB) and other diseases in African buffalo (Syncerus caffer) within the Great Limpopo TFCA.

Sub-Saharan African countries are particularly affected by TB in livestock, wildlife, and humans (Corbett, Marston, Churchyard, & De Cock, 2006; de Garine-Wichatitsky et al., 2013). Human TB, caused by Mycobacterium tuberculosis (M. tb), has high prevalence in the region as a result of high incidence of human immunodeficiency virus (HIV) and limited healthcare infrastructure and disease control measures (Corbett et al., 2006; World Health Organization, 2015). Sub-Saharan African countries feature prominently in the high-burden country lists for TB, TB/HIV coinfection, and multidrug-resistant (MDR) TB (World Health Organization, 2016). Angola, Botswana, Namibia, Zambia, and Zimbabwe all appear on at least one of these three lists, and both Angola and Zimbabwe appear on the lists for high TB, TB/HIV, and MDR-TB burden (World Health Organization, 2016). These five countries contribute land to the Kavango-Zambezi (KAZA) TFCA (Figure 1), the largest proposed TFCA at approximately 520,000 km^2 (Anonymous, 2014).

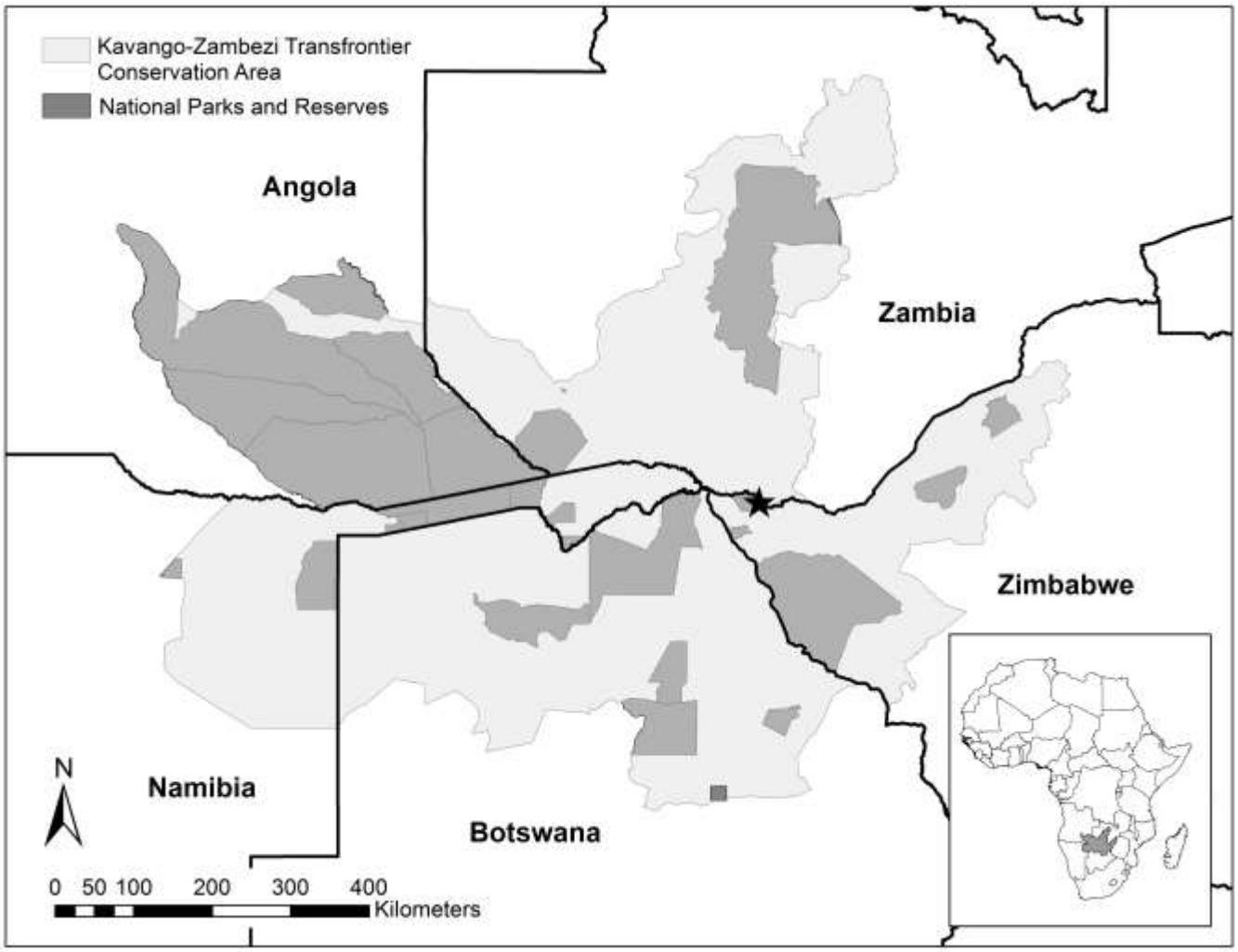

Figure 1. Map of the Kavango-Zambezi Transfrontier Conservation Area. The black star indicates Victoria Falls, Zimbabwe.

The KAZA TFCA supports the largest contiguous population of African elephants in the world (Loxodonta africana) (Van Aarde, Jackson, & Ferreira, 2006), concentrated in Botswana and Zimbabwe (Anonymous, 2014). Threats to conservation of elephants include disease epidemics, human-elephant conflict, and illegal killing for ivory (Sukumar, 2003). Illegal killing of elephants for ivory has driven a steep decline in African elephant populations (Wittemyer et al., 2014), with overall population declines of 8% per year (Chase et al., 2016). Elephants are among the top-ranked species that visitors are interested in viewing (Lindsey, Alexander, Mills, Romañach, & Woodroffe, 2007), and the loss of elephants due to poaching results in an estimated $25 million of lost tourism revenue to African

countries annually (Naidoo, Fisher, Manica, & Balmford, 2016). Declining elephant numbers appear to drive tourism losses rather than vice versa (Naidoo et al., 2016), thus further losses of elephants will only exacerbate tourism losses and concomitant economic impacts.

Investing in the sustainable conservation of elephants is therefore of economic benefit to range countries in addition to promoting biodiversity. As a result, diseases affecting elephants, including TB, are of particular interest within the TFCA. Elephants in zoological collections around the world have been diagnosed with TB (Lewerin et al., 2005; Mikota et al., 2001; Vogelnest, Hulst, Thompson, Lyashchenko, & Herrin, 2015; Zlot et al., 2015), primarily caused by M. tb or, more rarely, M. bovis (Mikota & Maslow, 2011). Infections with zoonotic pathogens transmitted from humans to animals (reverse zoonosis or zooanthroponosis) are more frequently reported in captive animals than their wild counterparts (Epstein & Price, 2009). A case of TB caused by M. tb has been documented in a wild African elephant in Kenya, and, although the source of that animal's infection could not be definitively traced, it had known extended prior contact with humans (Obanda et al., 2013). In 2016, an elephant was found dead in Kruger National Park and, at necropsy, evident TB lesions were found in the lungs with subsequent culture and identification of M. tb (M. Miller, pers. comm. 2017). Spillover of M. tb from humans has been documented in free-ranging and captive wildlife in South Africa (Michel et al., 2013).

Elephants are used as working animals in logging camps or tourist resorts in some countries where they are native (Sukumar, 2003). Working elephants have close contact with humans, providing a potential interface for bidirectional pathogen transmission. Tuberculosis infection or seroconversion has been documented in working Asian elephants (Elephas maximus) in Thailand (Angkawanish et al., 2010, 2013), India (Verma-Kumar et al., 2012), Malaysia (Ong et al., 2013; Yakubu et al., 2016), Laos (Lassausaie et al., 2014), and Nepal (Mikota et al., 2015). In contrast, the health of working African elephants has only been studied in the contexts of stress response (Millspaugh et al., 2007) and animal

welfare governance (Duffy & Moore, 2011), not their risk of TB or other diseases. Transmission of M. tb has been documented both from humans to elephants (Michalak et al., 1998) and vice versa (Murphree, 2011), thus the interface between humans and working elephants presents a risk for pathogen transmission to both species.

Approximately 50–60 captive African elephants are used in ecotourism facilities in the KAZA TFCA for the purpose of elephant-back safaris, and may therefore be at higher risk of exposure to M. tb than their wild counterparts. Standard practices at these facilities allow elephants to freely forage during the day, with the potential for contact with wild elephants. Thus, these captive elephants represent an important human-wildlife interface scenario, having contact with both humans in a high-burden M. tb area as well as an important population of free-ranging African elephants.

The goals of this study were twofold: 1) to screen this population of elephants to determine the seroprevalence of TB antibodies and 2) to explore associations between elephant demographics and management characteristics (risk factors) and seropositivity among captive African elephants in the Victoria Falls area of Zimbabwe in the KAZA TFCA. Based on our findings, management recommendations are provided to mitigate risk of M. tb exposure to captive elephants, as well as free-ranging wildlife and humans.

Materials and Methods

Study Population and Potential Risk Factors Questionnaire

Four ecotourism facilities offering elephant-back safaris in the Victoria Falls area of Zimbabwe consented to participate in the study. To maintain confidentiality of participating facilities, each facility was designated as A, B, C, or D. A questionnaire was developed to gather information about elephant demographics and management through in-person interviews, and survey responses were used to define potential risk factors for seropositive status. The survey included 45 closed- and open-ended

questions regarding the working elephants, including questions about: (1) demographics (sex, age, birthplace); (2) breeding status (sexual maturity, pregnancies, and births); (3) management style (free vs. protected contact with handlers); (4) social structure (housing, social grouping, contact with wild elephants); (5) public contact; and (6) staff numbers, type (full-time, part-time, or volunteer), and TB testing frequency. Staff at each facility were interviewed in English, Tonga, Ndebele, or Shona during September–October 2014. All survey responses were recorded in English. Follow-up interviews to clarify responses were conducted in English in February 2016.

Serologic Testing

Seroprevalence was determined by testing elephant blood samples for antibodies to Mycobacterium tuberculosis complex (MTBC) bacteria using two serologic assays used for elephants. A total of 35 African elephants were sampled at the four facilities in September 2014. Five animals were not sampled because they could not be sampled safely. Blood samples were collected from the auricular or saphenous vein, and allowed to clot at room temperature before testing, then centrifuged to separate serum. Each serum sample was tested using two serologic tests for MTBC antibodies that have been validated for elephants, the ElephantTB STAT-PAK® (no longer commercially available; Chembio Diagnostic Systems, Inc., Medford, NY, USA) and the DPP® VetTB Assay for Elephants (Chembio Diagnostic Systems, Inc., Medford, NY, USA), according to manufacturer's instructions. STAT-PAK detects antibodies to ESAT-6, CFP10, and MPB83 (Lyashchenko et al., 2006) and DPP detects antibodies to MPB83 and a CFP10/ESAT-6 fusion (Lyashchenko et al., 2012). Each STAT-PAK and DPP test result was interpreted as reactive (clearly visible test line), suspect (a faint color change response), or negative (no visible test line). During January 2015, blood samples were collected as part of another study from a subset of eight of the original 35 elephants, and STAT-PAK and DPP tests were repeated. In February 2016, 10 of the original elephants were tested again using STAT-PAK. If the STAT-PAK was reactive on

this occasion, the sample was also tested using DPP. For each sampling period, STAT-PAK and DPP results were interpreted in parallel, such that if an elephant had any reactive result to either the STAT-PAK or DPP, it was considered seropositive.

All serological testing was performed with the written consent of elephant owners. Approval for this study was obtained from the animal ethics committee at the University of Pretoria, the Department of Livestock and Veterinary Services, and the Division of Research, Diagnostics and Technical Services in the Zimbabwe Ministry of Agriculture, Mechanisation and Irrigation Development.

Statistical Analysis

Descriptive statistics including counts, frequencies, 95% confidence intervals, mean, standard deviation, and ranges were calculated to assess seropositivity and the data distribution for potential risk factors. For each facility, seroprevalence was calculated as the proportion of seropositive elephants among the total number of elephants tested during each sampling period. For the risk factor analysis, a group of factors/characteristics from the survey data were selected based on potential to serve as biological risk factors. Risk factors were assessed as categorical variables, and included the following: sex, time in captivity (< 15 years or ≥ 15 years), overnight contact with other elephants (yes or no), contact with wild elephants (yes or no), birthplace (captive or wild-born), shared feed with wildlife (yes or no), and facility (A, B, C, or D). To evaluate potential associations between data collected under different survey questions, correlation between variables was assessed using Spearman's correlation, with correlation values of $\rho \geq 0.6$ being considered correlated. When a strong correlation between variables was found, the decision about which of the variables to use for the risk factor analysis was made based on biological plausibility. For the risk analysis, a candidate model set of 6 models was built using a simple univariable logistic regression model for each factor and an intercept-only model to represent a null model; serological status (positive or negative) was the outcome variable in this

analysis. Akaike's Information Criterion adjusted for small sample sizes (AICc) was calculated for each model and was used to select the best model where the highest-ranked model carries the lowest AICc value and highest Akaike weight (Burnham & Anderson, 2002). All analyses were performed using the AICcmodavg package (Mazerolle, 2016) in R 3.3.2 (R Core Team, 2016).

Results

Survey Results

The number of elephants at each facility ranged from 2–17 throughout the study. The sex ratio of sampled elephants was approximately equal (17 females, 18 males). The mean reported age of the elephants was 22.0 years (SD 11.4, range 4–41 years), and 51% of the population sampled had reached breeding age. However, some ages were approximate, because ages for wild-born elephants were estimated and the majority (83%) of elephants were wild-born. The average estimated time under human management was 13 years, but actual time under human management for older animals was greater given that history at any previous facility may be unknown.

Wild-born elephants at the facilities originated from within Zimbabwe. Elephants were routinely used for morning and evening rides for tourists, lasting approximately 45–60 minutes each. In addition, tourists had the opportunity to feed, touch, and take photos with the elephants for periods of 5–25 minutes. During the day, elephants were allowed to forage on the facilities' property under the supervision of staff. Wildlife within the Victoria Falls National Park had access to the facilities (Figure 2), and free foraging during the day could bring working elephants into contact with species including wild elephants, buffalo, greater kudu (Tragelaphus strepsiceros), impala (Aepyceros melampus), bushbuck (Tragelaphus scriptus), waterbuck (Kobus ellipsiprymnus), common duiker (Sylvicapra grimmia), common warthogs (Phacochoerus africanus), Burchell's zebra (Equus quagga burchellii), giraffe (Giraffa

camelopardalis), banded mongoose (Mungos mungo), chacma baboons (Papio ursinus), and vervet monkeys (Chlorocebus pygerythrus).

Figure 2. Warthogs and an elephant feeding from the same trough at an ecotourism facility in Zimbabwe.

Elephants at all four facilities were kept in conditions that allowed for natural breeding. All facilities had at least one breeding bull elephant, and three facilities had at least one previous known elephant pregnancy. Three facilities used gonadotropin-releasing hormone (GnRH) vaccine to prevent musth and aggressive behavior in bulls. Elephants at two facilities were permitted overnight contact with other elephants in the herd, while overnight contact was restricted at the other two facilities. There were no indoor elephant enclosures at any of the facilities. During the day when foraging, elephants had direct and close contact with the other elephants and caretakers. In addition to foraging, the elephants' diet was supplemented with one or more of the following items: additional browse, local

hay (with molasses or salt additives), commercial game cubes, green bana grass (Pennisetum hybrid), commercial horse feed, or vegetables. At three facilities, warthogs were reported to have contact with elephant feed or were observed in close contact with elephants, especially during feeding in stable areas, including sharing game cubes and vegetables from feeding containers (Figure 2). Baboons and mongoose were also reported or observed to have contact with elephant feed. Potential contact with other wildlife was indirect through shared foraging areas. At three facilities, contact with wild elephants was possible but discouraged.

Elephants at all facilities were managed using unprotected contact. Handlers were not assigned to work with the same individual elephants but rotated through the herd. Full-time elephant handlers ranged from 8–14 per facility; part-time or volunteer staff were not used. Staff testing for TB was not required at any facility.

Serological Test Results

Thirty-five elephants were tested with both STAT-PAK and DPP (Table 1) during the first round of sampling in September 2014. Six elephants were reactive to STAT-PAK (17.1% seroprevalence, 95% CI: 4.7–29.6%), but none were reactive to DPP. In the second round of sampling during January 2015, eight of the 35 elephants were re-tested using both STAT-PAK and DPP. All eight elephants had been seronegative during the first round of sampling, and all STAT-PAK and DPP tests were non-reactive during the second round of sampling. In the third round of sampling during February 2016, 10 of the 35 elephants were re-tested using STAT-PAK. Two of these elephants had been seropositive in the first round of sampling, and an additional four elephants had also been tested during the second round of sampling. Nine of the tested elephants had a non-reactive STAT-PAK, and one was reactive. The reactive elephant, which had previously had a reactive STAT-PAK in the first round of sampling, was tested using DPP and that result was negative. In total, six out of 35 (17.1%; 95% CI 4.7–29.6%)

elephants were classified as TB seropositive based on parallel interpretation of STAT-PAK and DPP results. One of these elephants was classified as seropositive during two sampling sessions. All other elephants were classified as TB seronegative.

Table 1. Seroprevalence of Mycobacterium tuberculosis complex (MTBC) antibodies in captive elephant facilities in Zimbabwe across sampling periods.

	First sampling (Sept. 2014)			Second sampling (Jan. 2015)			Third sampling (Feb. 2016)		
Facility	# elephants	# screened	# seropositive (%)	# elephants	# screened	# seropositive (%)	# elephants	# screened	# seropositive (%)
A	16	12	1 (8.3)	17	4	0 (0.0)	17	5	0 (0.0)
B	4	4	0 (0.0)	2	1	0 (0.0)	2	0	0 (0.0)
C	9	8	3 (37.5)	4	0	0 (0.0)	3	2	0 (0.0)
D	11	11	2 (18.2)	11	3	0 (0.0)	11	3	1 (33.3)
Total	**40**	**35**	**6 (17.1)**	**34**	**8**	**0 (0.0)**	**33**	**10**	**1 (10.0)**

Risk Factors

Proportions of seropositive elephants and associated 95% confidence intervals, calculated for each risk factor, are shown in Figure 3. Strong correlations were noted between time in captivity and birthplace ($\rho = 0.67$), and between contact with wild elephants and facility ($\rho = 0.86$). Based on their perceived biological relevance, time in captivity and contact with wild elephants were included in the logistic regression analyses and models were ranked using Akaike weights (Table 2). The top-ranked model ($w = 0.41$) was for time in captivity, with elephants that had spent a shorter time in captivity (<15 years) having higher seroprevalence (36.4%) compared to elephants that spent >15 years in captivity (8.3%). The intercept-only model ranked second ($w = 0.18$) suggesting some model selection uncertainty, and the model for shared feed with warthogs or other wildlife ($w = 0.13$) ranked third, with elephants sharing feed with wildlife having higher seroprevalence (19.4% compared to 0%). Models for contact with wild elephants, overnight contact with elephants, and sex ranked approximately equally ($w = 0.08–0.10$). For these variables, seroprevalence was higher in elephants that had contact with wild

elephants (21.7% compared to 8.3%), no overnight contact with other captive elephants (25% compared to 13%), or were female (22% compared to 11%).

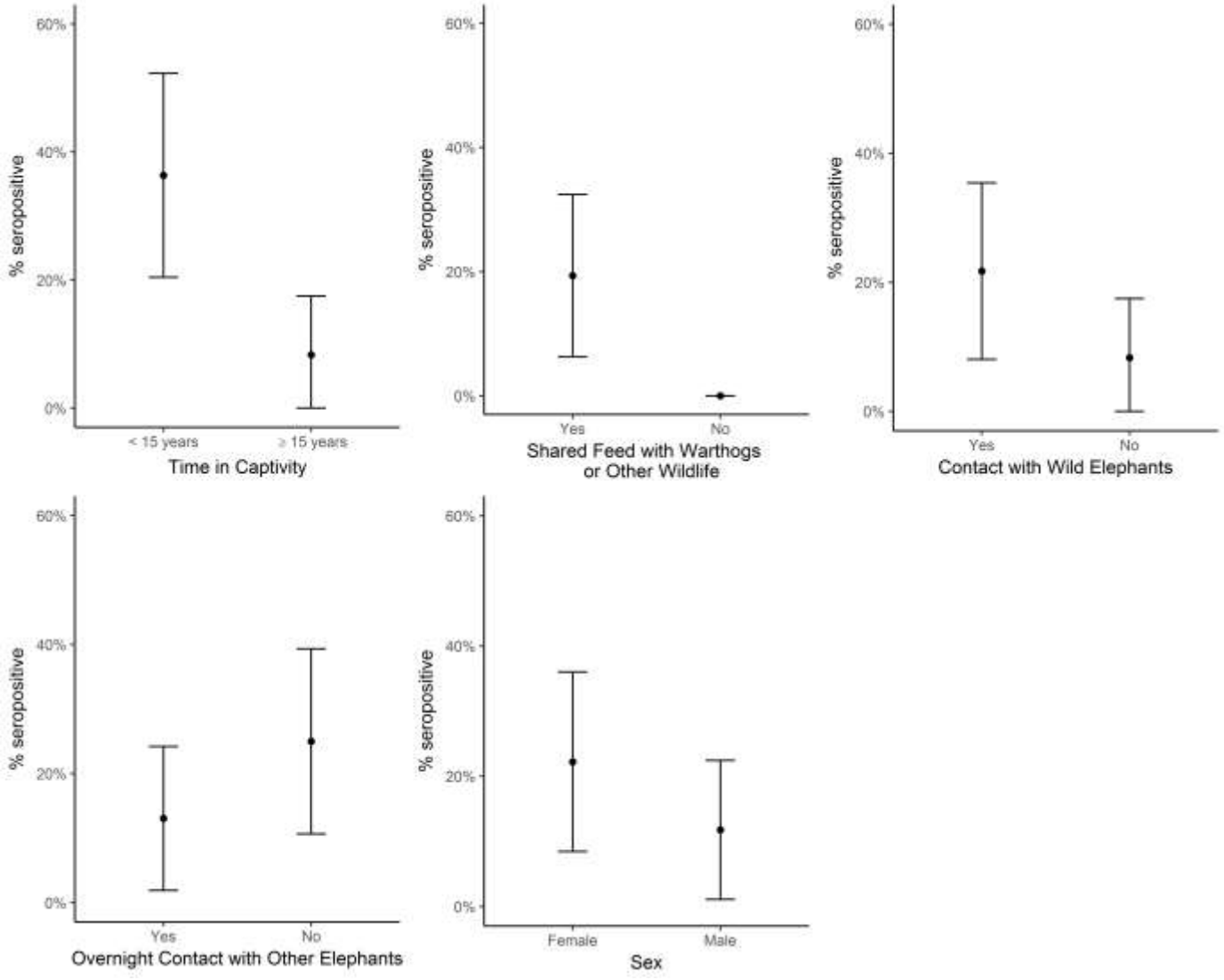

Figure 3. Proportions of captive African elephants that were Mycobacterium tuberculosis complex (MTBC) seropositive, reported for five potential risk factors: A) time in captivity, B) shared feed with warthogs or other wildlife, C) contact with wild elephants, D) overnight contact with other elephants, and E) sex s. Error bars indicate 95% confidence intervals.

Table 2. Candidate logistic regression models for Mycobacterium tuberculosis complex (MTBC) seropositivity in captive elephants in Zimbabwe.

Model	LL	K	AICc	ΔAICc	*w*
Time in captivity	-14.09	2	32.56	0	0.41
Intercept only	-16.04	1	34.19	1.63	0.18
Shared feed	-15.23	2	34.84	2.27	0.13
Contact with wild elephants	-15.48	2	35.34	2.78	0.10
Overnight contact with elephants	-15.65	2	35.68	3.12	0.09
Sex	-15.69	2	35.76	3.20	0.08

K: number of parameters; LL: log likelihood; AICc: Akaike's information criterion; ΔAICc: difference in AICc from minimum AICc model; w_i: Akaike weight

Discussion

This study provides the first assessment of TB seroprevalence in captive African elephants in a range country and investigates factors associated with positive serological responses. At the facilities under study, 17.1% of 35 elephants tested were seropositive during the first round of sampling. Seroprevalence in later sampling sessions, where a subset of elephants were tested, ranged from 0–10%. In addition, this study describes management of captive African elephants in Zimbabwe and explores potential factors associated with TB seropositive status. The risk factor most strongly associated with seropositive status was time in captivity. Models for contact with wild elephants, sex, and overnight contact with other captive elephants carried less weight.

It is important to note that this study only screened elephants for antibody responses to MTBC antigens, thus, a seropositive result cannot be considered equivalent to a diagnosis of clinical TB. Accurate and affordable tests for diagnosing mycobacterial infection are needed for elephants and other non-domestic species. However, serological testing provides a valuable starting point for screening elephants potentially at risk for MTBC exposure. Other immunoassays, such as the interferon gamma release assay, for detecting TB in elephants have been developed and show promise for antemortem diagnosis (Angkawanish et al., 2013; Paudel et al., 2016). However, these assays require special processing of blood samples and a laboratory with the expertise to run ELISAs, which can be a significant constraint to using these tests in areas with limited resources. While mycobacterial culture is considered

the gold standard for TB diagnosis in elephants (Mikota & Maslow, 2011), there are also major challenges to obtaining samples for culture among elephant populations under human care in range countries. First, elephants must be trained to perform a trunk wash procedure to collect samples for culture (Mikota & Maslow, 2011). Trunk wash training is not a routine procedure at ecotourism facilities in Africa, and requires specialized training of both elephants and their handlers. This training is under way at the four study facilities in order to be able to perform trunk washes in the future. Mycobacterial culture is also time-consuming, requires sample submission to a laboratory capable of growing and differentiating mycobacterial species, and may have a reduced sensitivity because samples frequently can be contaminated. Because elephants constantly interact with their environment using their trunks, trunk wash samples in culture are prone to overgrowth of environmental microorganisms (Mikota & Maslow, 2011). At ecotourism facilities, where elephants are housed outdoors in natural habitat, environmental contamination is more likely to interfere with culture.

In lieu of culture, serology allows for simplified and expedited routine testing of elephants that may be at risk of TB. Blood samples can be obtained more easily than trunk washes, tests can be performed on site in the field, and results are obtained quickly (within approximately 20 minutes). STAT-PAK and DPP have high reported sensitivity (100%) and specificity (95% and 100%, respectively) in isolated studies in captive elephants in the US and Europe (Greenwald et al., 2009). However, interpretation of test results must take into consideration differences in host species, local factors (e.g., presence of cross-reacting non-tuberculous mycobacteria), and true disease prevalence (Dohoo, Martin, & Stryhn, 2009). Serologic tests may not conclusively identify infected elephants; for example, captive Asian elephants in Thailand that were confirmed to have M. tb infection had one or more negative STAT-PAK tests, including an individual that died of severe clinical disease hours after testing (Angkawanish et al., 2010). Species differences between Asian and African elephants may influence serologic test performance, given that only the immune response of Asian elephants to TB has been characterized in

other studies (Landolfi et al., 2010, 2014). Conversely, STAT-PAK false positives are known to occur in cases of non-tuberculous mycobacterial infection (Greenwald et al., 2009; Lacasse et al., 2007) and with chronic inflammatory conditions like arthritis (Greenwald et al., 2009). Infection with non-tuberculous mycobacteria may alter the antibody profile that elephants produce (Lyashchenko et al., 2012). The elephants in this study may be more likely than zoo elephants to be exposed to both tuberculous mycobacteria, due to their location in a high TB burden country, and non-tuberculous mycobacteria, given their outdoor housing and foraging behavior. These factors should be taken into consideration when interpreting serological test results, which can still provide important information that, when used in combination with other diagnostics, can inform prevention and control strategies for reducing the risk of MTBC transmission.

Interestingly, all seropositive elephants in this study were reactive to STAT-PAK but not DPP. The STAT-PAK positive results could indicate early infections, as STAT-PAK uses a larger volume of serum than DPP (30 µl compared to 5 µl; Greenwald et al., 2009), and therefore has a lower threshold of detection. Based on the lower specificity of STAT-PAK compared to DPP, these results could also indicate false positive STAT-PAK results in this population, possibly as the result of factors such as non-tuberculous mycobacteria or inflammatory conditions. One elephant that was seropositive during the first round of testing became seronegative on the third round of testing. In this case, the elephant may have cleared a TB infection, as was hypothesized for elephants with intermittent borderline reactive DPP results after exposure to a TB-infected elephant (Vogelnest et al., 2015). In addition, there was one seroreactive elephant that remained seroreactive on the third round of testing; although DPP was nonreactive, this finding may increase suspicion of a true MTBC infection.

In humans, a positive serological test result without accompanying clinical disease or detection of M. tb is interpreted as a latent infection, but it is unclear if elephant serological responses should be interpreted similarly (Ong et al., 2013). Given their apparent predilection toward strong humoral

responses to mycobacterial infection, elephants may become persistently seropositive years before M. tb or M. bovis are isolated from trunk wash culture samples (Greenwald et al., 2009; Lyashchenko et al., 2012; Vogelnest et al., 2015). Increased monitoring with DPP (as STAT-PAK is no longer available) can show persistent reactions or those of increasing intensity that are suggestive of active TB disease (Vogelnest et al., 2015). Elephants with positive serological tests results should be monitored for clinical signs associated with TB, and subjected to increased TB diagnostic testing such as more frequent serological assays and trunk wash cultures.

Potential risk factors in this population may differ from other elephant populations where epidemiological studies have identified risk factors because of contrasts in environment and elephant management practices. For example, working Asian elephants with an assigned mahout (elephant handler) have an increased risk of TB seropositivity (Yakubu et al., 2016), but elephants at the African facilities do not have assigned handlers. Close and prolonged contact between humans and elephants, such as spending hours indoors with infected elephants (Zlot et al., 2015), or cleaning barns and aerosolization during pressure washing (M. Miller & Olea-Popelka, 2013) have been implicated in human cases of TB at animal facilities. However, indoor elephant housing does not exist at the facilities in this study, nor are pressure washers in use. Determining risk factors for transmission of TB among elephants and between elephants and humans at ecotourism facilities is crucial to enacting preventive measures to protect both elephants and humans.

In this analysis, time in captivity was the most important variable associated with higher risk for seropositive status in elephants based on AICc weight. The association of seropositive status with a shorter time in captivity is somewhat counterintuitive, as increased time in captivity would be expected to be associated with increased human exposure and therefore risk of TB. This may be in part due to the correlation observed between age and time in captivity, as elephants that have spent less time in captivity are generally young. Young elephants could be more curious in exploring the environment

with their trunks, leading to an increased exposure to environmental organisms. Young elephants or those that have spent less time in captivity may be handled more for training purposes, and increased handling time could increase risk of exposure to human TB if handlers are infected. One major potential factor not explored in this study is the TB status of elephant handlers, and TB transmission is most likely to occur from humans to elephants in high TB burden countries (Lassausaie et al., 2014). Alternatively, seropositive results may have been more likely in elephants that have been exposed or infected but have not had time to observe a waning humoral response. In cattle, antibody responses to mycobacteria can wane over time (Waters et al., 2010), thus if older elephants or those in captivity for longer were exposed to mycobacteria in the distant past, they may no longer have a robust antibody response.

The null or intercept only model, ranked second, serves as a comparison point for the models evaluated in this study. That the intercept-only model was highly ranked and all of the models were within 3-4 AICc units of each other suggests that there is some model selection uncertainty and that other factors that were not evaluated may be involved. With the relatively small sample size in our study, it was not possible to adequately investigate multivariable logistic regression models that could better reflect the multifactorial etiology of TB and allow for potential compounding or interactive effects among different factors. Nonetheless, the relative Akaike weights of the evaluated models for risk factors may allow for prioritization of variables to explore in future studies.

Interactions with other wildlife through shared feed may also be important in elephant seropositive status. All seropositive elephants were housed at facilities that reported having warthogs or other wildlife around their stables. At one facility in this study, a banded mongoose colony appeared to use a hay barn as a semi-permanent refuge. Warthogs are known spillover hosts of TB (Renwick, White, & Bengis, 2007), and wildlife having access to stored food may be a factor worth further investigation, given the importance of shared feed in other ecosystems in which TB is present and

transmitted across species. For instance, European badgers (Meles meles) are known to consume cattle feed, and indirect contact through shared feed or direct contact during feeding may be important for M. bovis transmission between badgers and cattle (Garnett, Delahay, & Roper, 2002). In North America, supplemental feeding of wildlife is considered a risk factor for M. bovis transmission (R. S. Miller & Sweeney, 2013), and indirect transmission through feed sharing has been documented from experimentally infected white-tailed deer (Odocoileus hemionus) to cattle (Palmer, Waters, & Whipple, 2004). Securing food from free-ranging wildlife and preventing wildlife contact while elephants are feeding would reduce the potential risk of pathogen transmission via shared feed.

Seroprevalence was higher among elephants that had contact with wild elephants. It is unknown whether wild elephants in the area are infected with mycobacteria and could serve as a source of infection to captive elephants. However, this finding has important conservation implications, as seropositive captive elephants have the potential to transmit pathogens when in contact with wild elephants. Although contact between captive and wild elephants is discouraged, interactions between them have occurred. Such interactions have included captive elephants mating with wild elephants, captive elephants taken into a wild herd, and an orphaned wild-born elephant being adopted into a captive herd. These interactions demonstrate the potential for close contact between captive and free-ranging populations that could put elephants at risk for pathogen transmission.

The remaining factors carried less weight in terms of importance to seropositive status indicating that they are less important in shaping seroprevalence. Seroprevalence was higher among elephants without overnight contact with other captive elephants. Again, this finding is somewhat counterintuitive, as higher seroprevalence might be expected in elephants with overnight contact, given the opportunity for transmission via direct contact. This finding may be associated with other management differences between facilities that contribute to variation in risk but were not measured in this study. Seroprevalence was higher among female elephants (22.2%) than males (11.8%), which is in

contrast to trends reported in working Asian elephants (Lassausaie et al., 2014; Yakubu et al., 2016). Most of the male elephants in this study were housed at facilities that reported using GnRH vaccine to suppress musth. High testosterone levels during musth may result in immunosuppression (Sukumar, 2003), thus suppressing musth might indirectly benefit bull elephant immunity.

The captive elephants at the facilities under study are present in a unique community of mycobacteria and other wildlife. Humans in these communities are hosts to M. tb, and M. bovis is present in cattle in neighboring Zambia (Muma et al., 2013; Munyeme et al., 2009, 2010; Phiri, 2006). An emergent MTBC species, M. mungi (Alexander et al., 2010), has been identified in banded mongoose in northwest Zimbabwe (Alexander et al., 2016). Banded mongoose in South Africa were recently found to be hosts of M. bovis (Brüns et al., 2016), and nearly all of the other wildlife species reportedly seen on the facilities are known natural hosts of M. bovis or other tuberculous mycobacteria (Mukundan, Chambers, Waters, & Larsen, 2015). A variety of non-tuberculous mycobacteria have been cultured from tissues of South African wildlife species, including elephants (Botha, Gey van Pittius, & van Helden, 2013), and non-tuberculous mycobacteria can induce cross-reaction to MTBC antigens (Gcebe & Hlokwe, 2017). Relatively little is known about movement of mycobacteria among humans, wildlife, and domestic species, especially in southern Africa. Deceased working elephants and, when possible, other susceptible wildlife hosts should have a post-mortem examination conducted and any tuberculous lesions cultured for mycobacteria. Post-mortem examinations are a critical surveillance tool; however, implementation may be limited by finding fresh carcasses and the logistics associated with sampling large animals in field settings. Better understanding the dynamics of infection among and between species will be crucial to effectively preventing and controlling diseases within animal populations within TFCAs. In addition, ecotourism facilities in the area can take steps to limit risks to their elephants and staff. An important first step would be implementation of routine TB screening for elephant handlers at the time of hiring and annually, allowing timely treatment of TB infected handlers. Elephants should be

screened for TB using serologic testing with the DPP; elephants reactive on serology should receive increased monitoring for TB and increased testing frequency using serologic tests or culture. Additionally, other screening and diagnostic tests (e.g., interferon gamma assay and mycobacterial culture) should be evaluated and considered to continuing monitoring this population of African elephants in the KAZA TFCA. Securing feed sources from other wildlife would eliminate a known route for TB transmission. Continuing to explore potential risk factors, including others we did not evaluate, is warranted in future studies. Elephant facility owners should be proactive in working with local public health authorities, veterinarians, employees, and other stakeholders in creating an informed management plan to mitigate risk of pathogen transmission to or from humans, elephants, and other wildlife.

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