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Characterizing tuberculosis progression in wild meerkats (Suricata suricatta) from faecal samples and symptom data

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Abstract:	Tuberculosis (TB) is an increasing threat to wildlife, yet tracking its spread is challenging because infections often appear asymptomatic, and gold-standard diagnostic tools are invasive and resource-intensive. Our understanding of TB biology and epidemiology in wildlife is therefore limited to a small number of well-studied species, hindering wildlife conservation efforts and the development of management policies. In this study, we combine observation data of external TB symptoms with PCR-based testing of 388 faecal samples to characterize longitudinal dynamics of TB infection in 66 wild meerkats (Suricata suricatta) naturally exposed to TB-causing agent Mycobacterium suricattae between 2000 and 2018. Our specific objectives are to 1) test whether faecal samples can be used to monitor TB infection; 2) characterize TB progression between three infection states (PCR-negative exposed, PCR-positive asymptomatic, and PCR-positive symptomatic); and 3) estimate heterogeneity in TB susceptibility (i.e. the propensity to contract TB upon exposure) and resistance (i.e. the ability to survive with an existing TB-infection) across individuals. We found the TB PCR sensitivity rate from faecal samples for symptomatic individuals was 12.5% (CI: 7.4 - 20.4%), and therefore reliable detection required up to 10 replications per test. Despite this low detection sensitivity, the adjusted protocol revealed hidden TB infections in 59% of meerkats prior to the onset of symptoms. On average, meerkats became PCR-positive approximately 14 months after initial exposure to TB, developed symptoms approximately one year after becoming PCR positive, and died within five months of developing symptoms. Individual variation in disease outcome was high, with meerkats developing symptoms from immediately after exposure to 3.4 years later, with the latter potentially acting as superspreaders. Overall, this study generates novel insights into wildlife TB progression, provides baseline information on susceptibility and resistance that can inform future epidemiological models, and can help guiding adapted management strategies for TB-susceptible wildlife populations.

JWD cover letter Tuesday 13th April 2021

Dear *Journal of Wildlife Disease* editors,

A major limitation in studying and managing wildlife diseases is the ubiquity of pathogens that are characterized by sub-clinical and often symptomless infections. Symptomless infections are challenging to identify and hinder our understanding of basic pathology, which in turn limits our ability to model pathogen dynamics and epidemiology. Wildlife tuberculosis (TB) is one such disease that is notoriously difficult to monitor due to its long latent period in hosts, and the fact that animals often need to be captured for gold-standard tests (e.g. saliva and blood sampling). We therefore have little understanding of how TB progresses in the vast majority of wildlife species that are susceptible to TB, with the notable exception of badgers, despite it being a major zoonotic pathogen that threatens vulnerable mammalian populations globally.

In our manuscript, titled "**Characterizing TB progression in wild meerkats (*Suricata suricatta*) from faecal samples and symptom data**", which we hereby submit for consideration at *Journal of Wildlife Diseases* as a full length manuscript, we characterise longitudinal TB progression over life in 66 wild meerkats (*Suricata suricatta*) with known exposure to TB and which were monitored daily for external symptoms, using PCR tests on faecal samples collected over a 20 year period. We aimed to test whether PCR on faecal samples can be used to detect sub-clinical infections prior to the onset of visible symptoms, and to estimate natural variation in how long individuals harbour sub-clinical TB infections. We show that many of the meerkats tested were positive for TB many years prior to the onset of symptoms, and we quantify the average length of non-symptomatic and symptomatic infection. Whilst estimated sensitivity of the PCR test was low, it nevertheless more than doubled the length of time of known infection for almost all meerkats. Moreover, we also identified a proportional of individuals that never became infected despite consistent TB exposure, suggesting some level of innate immunity. We therefore conclude that, in conjunction with symptom data, PCR testing of faecal samples can be a useful tool for monitoring TB in individually-identifiable animals without the need for capture.

Our study is uniquely powerful because it follows individually-marked animals across life, allowing a rare opportunity to characterise longitudinal infection dynamics within individuals over a number of years. Our results demonstrate that infected animals can appear seemingly healthy for many years prior to the onset of symptoms, and highlights the importance of being able to identify such individuals. Given the prevalence of wildlife TB and its importance in wildlife conservation and human-wildlife conflict, we believe our findings will be of interest to the broad readership of Journal of Wildlife Diseases.

The data analysed in the manuscript has not been published elsewhere, and all authors have read and approved the final version, with all having made meaningful contributions to the work.

Sincerely,

Dr Alice Risely, on behalf of all authors

Running head: Characterizing tuberculosis progression from faecal samples and symptom data (Donadio et al.)

Characterizing tuberculosis progression in wild meerkats (*Suricata suricatta*) from faecal samples and symptom data

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Abstract

Tuberculosis (TB) is an increasing threat to wildlife, yet tracking its spread is challenging because infections often appear asymptomatic, and gold-standard diagnostic tools are invasive and resource-intensive. Our understanding of TB biology and epidemiology in wildlife is therefore limited to a small number of well-studied species, hindering wildlife conservation efforts and the development of management policies. In this study, we combine observation data of external TB symptoms with PCR-based testing of 388 faecal samples to characterize longitudinal dynamics of TB infection in 66 wild meerkats (*Suricata suricatta*) naturally exposed to TB-causing agent *Mycobacterium suricattae* between 2000 and 2018. Our specific objectives are to 1) test whether faecal samples can be used to monitor TB infection; 2) characterize TB progression between three infection states (PCR-negative exposed, PCR-positive asymptomatic, and PCR-positive symptomatic); and 3) estimate heterogeneity in TB susceptibility (i.e. the propensity to contract TB upon exposure) and resistance (i.e. the ability to survive with an existing TB-infection) across individuals. We found the TB PCR sensitivity rate from faecal samples for symptomatic individuals was 12.5% (CI: 7.4 - 20.4%), and therefore reliable detection required up to 10 replications per test. Despite this low detection sensitivity, the adjusted protocol revealed hidden TB infections in 59% of meerkats prior to the onset of symptoms. On average, meerkats became PCR-positive approximately 14 months after initial exposure to TB, developed symptoms approximately one year after becoming PCR positive, and died within five months of developing symptoms. Individual variation in disease outcome was high, with meerkats developing symptoms from immediately after exposure to 3.4 years later, with the latter potentially acting as superspreaders. Overall, this study generates novel insights into wildlife TB progression, provides baseline information on

susceptibility and resistance that can inform future epidemiological models, and can help guiding adapted management strategies for TB-susceptible wildlife populations.

Keywords: disease ecology, latent infection, *Mycobacterium*, non-invasive detection method, TB detection, Tuberculosis, wildlife

Introduction

Emerging infectious diseases (EIDs) in wildlife are increasing due to human encroachment into wildlife habitats, thereby facilitating transmission of pathogens between wildlife, livestock, and humans (Jones et al. 2008; Neiderud 2015). However, monitoring wildlife disease and investigating pathogen epidemiology in wildlife is challenging, because it often requires long-term, high frequency, and invasive sampling of animals. Cryptic pathogens that do not manifest in phenotypic symptoms immediately, or are undetectable during latent stages, present particularly complex and difficult cases (Gilch et al. 2011), and often play a disproportionately large role in pathogen transmission (Bosch et al. 2018). For such pathogens, the absence of non-invasive diagnostic tests (e.g. from faecal samples) to detect infections in asymptomatic hosts without capture limits our understanding of variation in disease susceptibility and resistance across wildlife populations. This in turn hinders the development of epidemiological models, the identification of superspreaders (Woolhouse et al. 1997; Lloyd-Smith et al. 2005), and restricts management policies that help prevent pathogen transmission within wildlife populations and potential spill-over into livestock and humans (Wood et al. 2012).

Wildlife tuberculosis (TB) is an example of cryptic multi-host pathogens that can remain undetected for prolonged periods in infected hosts, and is becoming one of the major emerging challenges for conservation globally (Alexander et al. 2002). The *Mycobacterium*

tuberculosis complex consists of a genetically related group of multi-host *Mycobacterium* species that cause TB in large number of mammalian species. For example, TB frequently occurs in African buffalos, different seal species, Asian elephants, banded mongoose, meerkats and European badgers, among others (Wirth et al. 2008). However, our knowledge of basic TB progression, such as infection duration and pathogen latency in hosts, are limited to a small number of long-term and longitudinally-monitored model systems. In wild badgers, four relevant infection states have been recognized: negative (all cultures and antibody tests negative), exposed (antibody positive), excretor (positive culture) and super-excretor (multiple positive cultures), with the assumption that the progression to each stage is an irreversible process and that excretors and super-excretors are infectious (Graham et al. 2013). Negative effects of TB infection on survival and energetics appear to be minimal (Cross et al. 2009; Graham et al. 2013; Barbour et al. 2019), and individuals can live for up to eight years after being tested positive for TB (Tomlinson et al. 2013), potentially acting as superspreaders (McDonald et al. 2018). Nevertheless, the generality of these findings across other host systems is unclear, since longitudinal studies on wildlife TB are rare. Longitudinal studies on a wider range of host systems that quantify infections prior to symptom onset are crucial for understanding TB transmission patterns across wildlife populations.

A major constraint to increasing our understanding of wildlife TB is the limited availability and application of non-invasive diagnostic tools, such as faecal samples. TB infections are often contained within the lungs and kidneys and therefore are not externally visible (Gallagher et al. 1998). Whilst saliva and blood tests remain the gold standard for TB detection (McDonald and Hodgson 2018), they are highly invasive, relatively insensitive (e.g. Drewe et al. 2010), and difficult to obtain for many wildlife species. As such, detection of TB infections in wildlife is often restricted to external symptoms (e.g. Patterson et al. 2017), which may underestimate true infection rates and duration. A potentially valuable method to

non-invasively detect and monitor TB in wildlife could be to test faecal samples for TB, since faecal material can contain *Mycobacteria* when infected saliva is swallowed (Bassessar et al. 2014). TB detection from stool is used in humans when patients are unable to provide saliva (El Khéchine et al. 2009; Mazidur Rahman et al. 2018), although test specificity is highly variable and averages between 40-72% (Mesman et al. 2019). Faecal samples have been successfully used in conjunction with oral and nasal swabs in wild boar, where they performed equally well as nasal and slightly worse than oral swabs (Barasona et al. 2017). However, there are currently no other studies consistently using faecal samples as indicators of TB status in wildlife, so the utility of this method for wildlife disease surveillance is unclear.

In this study, we combined faecal TB diagnosis with symptom data to examine longitudinal TB progression in 66 wild, TB-exposed meerkats over an 18-year period. Meerkats are highly social mammals inhabiting arid regions of southern Africa, and periodically experience TB outbreaks caused by *Mycobacterium suricattae* (Parsons et al. 2013). Meerkats are cooperative breeders that live in groups of 2–50 individuals, with a dominant pair largely monopolizing reproduction, and a variable number of subordinate helpers of both sexes that support pup rearing (Clutton-Brock and Manser 2016). Individuals roving between groups provide TB transmission opportunities (via grooming or biting) between social groups (Drewe 2010), and TB outbreaks within social groups may last many years, with pups born during outbreaks being exposed from birth. Typical symptoms of TB infection in meerkats are submandibular swellings, emaciation and lethargy, and eventual death (Drewe et al. 2009a; Patterson et al. 2017), which match the symptoms recorded in other wildlife species (de Lisle et al. 2002). A previous study estimated a latent period (defined there as the time between a meerkat developing antibodies and becoming infectious to others) of 385 days, based on 39 TB-infected meerkats over a two-year study period (Drewe et al. 2011). However, how long

meerkats are TB-positive before developing the characteristic external symptoms, or how TB progresses across an individual lifetime, is still unclear.

The aim of this study was to characterize the longitudinal dynamics of TB infection across life by combining PCR-based TB detection from faecal samples with observed symptom data to categorize TB infection into three distinct states in wild meerkats: PCR-negative exposed, PCR-positive asymptomatic, and PCR-positive symptomatic; Figure 1; see Table 1 for glossary). Whilst we cannot distinguish between infectious and non-infectious states here, we assume that PCR-negative exposed individuals are not infectious, PCR-positive yet asymptomatic individuals may potentially be infectious, and PCR-positive symptomatic individuals are very likely to be infectious to others. Our specific aims were to 1) test whether faecal samples can facilitate the monitoring of TB infection, 2) characterize TB progression between three infection states, estimating average time periods individuals spend in each infection stage, and 3) estimate heterogeneity in TB outcome across individuals. The study provides valuable baseline data for epidemiological modelling and conservation management.

Materials and methods

Study system and sample collection

Our study population consisting of habituated social groups is located in the Southern Kalahari/South Africa (Clutton-Brock et al. 1998). Available social groups have been monitored 4 to 5 days a week since 1993, providing extensive life history data, birth and death dates, as well detailed information on signs of disease, including typical TB symptoms, like submandibular or inguinal lumps (Clutton-Brock and Manser 2016). Faecal samples from individually identified animals were collected upon defaecation and either freeze-dried and stored at room temperature, or frozen immediately and stored at -80 °C.

We focused on meerkats belonging to three well-monitored social groups that experienced an extended TB outbreak. Most meerkats in these groups are assumed to have died of TB, as many exhibited acute clinical TB symptoms verified by a vet at the end of their life. We selected a total of 66 adult individuals (Vivian: N = 21 (13 males; 8 females); Van Helsing: N = 20 (12, 8); Baobab: N = 18 (11, 7); other groups: N = 7 (1, 6)) where faecal samples had been consistently collected up until the point of death. The seven individuals not part of the three focus groups were selected for a pilot study to optimize the PCR protocol (outlined in detail below), because they were confirmed to have died from TB and showed extended clinical symptoms, and are included in the results of this study. Of the others, 37 individuals showed clinical symptoms of TB at the end of their life, whilst 22 never exhibited symptoms, but were good candidates for subclinical infections as they were known to be exposed to TB. Overall, we analyzed 388 samples (a mean of 7 ± 4 samples per individual; min = 1, max = 17) collected over the course of the study individuals' lives (2.4 ± 1.5 years, max = 6.7 years).

DNA extraction and PCR amplification

Faecal DNA was extracted using the 'NucleoSpin® 96 Soil' kit (Macherey and Nagel, Germany), following the manufacturer's protocol. Approximately 80 mg of faecal sample were used per extraction. To amplify TB-causing *Mycobacterium* agents, we designed a primer pair primer TB 421F (5' – CCCCGATGGTTTGCGGTGG-3') and TB 574R (5' – GCGGCTGATGTGCTCCTTGA-3'), targeting the highly conserved tuberculosis insertion element IS6110 region (Thierry et al. 1990). The primers were chosen, because they performed the most reliable in the pilot study, when compared with three other sets of candidate primers (Supplementary Table S1). Target DNA fragments were amplified in a 10 µl volume using 2 µl of template DNA, 2.4 µl H_2O, 5 µl AmpliTaq Gold 360 Master Mix

(Thermo Fisher Scientific, USA) and 0.3 mM of each primer. The PCR run consisted of a denaturation phase of 10 min at 95 °C and 40 cycles of 30 sec at 95 °C, 15 sec at 60 °C and 15 sec at 72 °C, and finished with 3 min at 72 °C. Successful amplification was confirmed by gel electrophoresis on a 1.5% agarose gel.

Seven individuals with confirmed death by TB were used to establish the PCR protocol. Because the pilot study indicated relatively low sensitivity, and because our aim was to detect TB in samples with potentially low *Mycobacterium* concentration, each sample was tested repeatedly up to 10 times or until it was recorded positive, and samples that remained negative after 10 replications were categorised as TB negative. We used a one-way classification system of no return commonly applied to TB in wildlife (Tomlinson et al. 2013), assuming that individuals that test TB-positive once remain positive until death, since there is no evidence for natural TB infection clearance without treatment (Houben and Dodd 2016). The first TB positive PCR product of an individual was sequenced using Sanger sequencing to ensure the amplified product aligned (>99,1% of base pairs) to the reference sequence *Mycobacterium tuberculosis* strain H37Rv (Cole et al. 1998).

PCR sensitivity analysis

We assessed PCR sensitivity retrospectively by estimating the probability of a positive result given the meerkat was symptomatic at the time of sampling and therefore known to be infected. Because most of the 388 samples included in this study were collected prior to the onset of symptoms, our sensitivity analysis included 66 samples from 33 symptomatic individuals, that were PCR tested in total 374 times. We predicted PCR outcome with a binomial Generalized Linear Model (GLM) using the *lme4* package and including sample number as a random effect, and no fixed effects.

Characterising TB progression, susceptibility, and resistance

For each individual, we created a timeline categorising the individual status into three conceptually and epidemiologically meaningful infection states based on PCR results and TB symptom status at the time of sampling: PCR-negative exposed, PCR-positive asymptomatic, and PCR-positive symptomatic (Figure 1). All TB related terms and their definitions are summarized in Table 1. We defined the exposure date as the first date an individual was seen co-habiting with an individual known to be infected, either due to be being symptomatic or shown to be PCR-positive to TB in this study. We defined meerkats as symptomatic when an animal showed submandibular lumps, genital lumps or had TB mentioned in any record, defining the first date of whichever case as the symptom date. If an animal showed a subcutaneous lump or swelling that was not explained by an injury, snake bite or similar, and developed clear TB symptoms within one year, the date of the subcutaneous lump was used as the symptom date. We defined death date as the date an individual with advanced TB symptoms disappeared from the study population (N = 36), was found dead (N = 12), or was euthanised due to showing very advanced stages of TB (N = 18). We calculated the mean, median and range of periods (in days) between the infection stages (exposure, becoming PCR-positive, becoming symptomatic, and death). For each time span calculation, we only included individuals where exact start date of the infection stages was known to avoid any bias. Lastly, we aimed to understand variation in TB progression patterns across the tested individuals. We thus estimated individual TB susceptibility, defined as the time span between exposure to TB and becoming PCR-positive, and individual TB resistance, defined as the time span between becoming PCR-positive and death. All analyses were performed in R version 3.6.2 (R Core Team 2019).

Results

PCR sensitivity to *Mycobacterium suricattae*

We retrospectively assessed PCR sensitivity on samples taken from symptomatic individuals, which showed a sensitivity rate of 12.5% (95% CI: 7.4 - 20.4%; N = 374 PCRs), indicating that our replication rate of ten PCR replications per sample should be adequate to detect low concentration of *Mycobacterium suricattae*. The sensitivity analysis indicated that variation in PCR outcome between samples was much higher than variation within samples (i.e. some samples were always likely to be positive and other samples were always likely to be negative), indicating that PCR outcome is likely to be highly dependent on *Mycobacterium* concentration, and that this can be highly variable between samples even when the individual is showing external symptoms of active TB infection.

Alignment of PCR and symptom data

We identified the earliest time point of infection using PCR for 66 TB-exposed meerkats, and aligned this information with field observations of symptoms to examine TB progression within individuals over their life (Figure 2). Overall, for 78.8% of meerkats tested, PCR results and observed symptoms matched by the end of life, i.e. PCR-negative individuals stayed asymptomatic and PCR-positive individuals developed symptoms. Of these, 53% of meerkats were PCR-positive and developed symptoms over time, whilst 25.8% of meerkats were never PCR-positive or symptomatic despite being exposed to TB. Of the 21.2% of meerkats whose PCR and symptom data did not align, 7.6% were tested PCR-positive yet remained in an asymptomatic state until death, whilst 13.6% displayed TB symptoms but never became PCR-positive (Supplementary **Error! Reference source not found.**).

TB progression

Most meerkats included in our study were born into a group infected with TB and thus exposed to TB at birth (

Figure **3**). After first TB exposure, animals became PCR-positive on average 427 ± 328 (s.d.) days (~1.2 years) later, although inter-individual variation was high (Table 2**Table**). The asymptomatic period between an individual becoming PCR-positive and developing symptoms was on average 349 ± 211 (s.d.) days (~1 year), although the maximum asymptomatic period was 834 days (~2.3 years; Table 2). Once an animal became symptomatic, and thus likely infectious, they survived on average 160 ± 173 (s.d.) more days (~0.4 years), although one meerkat exhibited symptoms for 595 days (~1.6 years). We did not detect sex-specific differences in our dataset (Table S3).

Individual variation in susceptibility and resistance

We found high variation in TB susceptibility across individuals, measured here as the time span between TB exposure and becoming PCR-positive (427 ± 328 (s.d.) days; Figure 4A). As outlined above, 25.8% of exposed meerkats (N = 17) never became PCR-positive nor symptomatic, suggesting a significant proportion of the population may not be susceptible to TB. Out of meerkats which did contract TB, the lowest susceptibility detected was 1518 days (~4.2 years) between exposure and PCR-positive, whilst other meerkats had very high susceptibility and appeared to contract TB immediately upon exposure. Variation in resistance, which we define as the period between first positive PCR sample and death, was also very high (377 ± 283 (s.d.) days (~ 1 year; Table 2), with some meerkats surviving for up to 1040 days (~2.8 years) after becoming PCR-positive (Figure 4B).

Discussion

Monitoring tuberculosis (TB) in wildlife is challenging because infections often appear asymptomatic, and gold-standard diagnostic tools (such as saliva and blood sampling) are invasive and require capturing the animals. Our study, which pairs observed external symptom data with PCR testing of faecal samples collected non-invasively across most of the lifespan of 66 naturally TB-exposed wild meerkats, suggest that faecal samples can be used to detect TB in wildlife and to estimate the parameters associated with TB progression, providing a markedly better estimate of infection duration and patterns than symptoms alone. Notably, the use of PCR testing on faecal samples revealed hidden TB infections on average a year prior to the onset of external symptoms, more than doubling the estimated infection period from symptom data alone.

Using information from faecal TB detection, we substantially contributed to the current understanding of TB epidemiology in wild meerkats. Firstly, a full quarter of consistently TB-exposed meerkats never became PCR-positive nor developed symptoms, suggesting a significant pool of non-susceptible individuals within the population. Second, we found that the average time between exposure and a meerkat becoming PCR-positive was 427 days, which is consistent with a latent period of 385 days proposed by a study that used antibody tests and culturing methods on 39 longitudinally monitored meerkats (Drewe et al. 2011). This provides further evidence that results from faecal samples are comparable to those from more invasive yet sophisticated detection methods. Thirdly, we show that TB progression is highly variable across individuals at every stage of the infection. For example, the time between exposure and first detection of TB via PCR was on average 427 ± 328 days, and individuals were symptomatic for approximately five months, but up to 1.6 years in extreme

cases. This long infection period, and particularly the long asymptomatic period, is likely to have strong consequences for TB maintenance, social structures, survival and reproduction, as with other pathogens (Jolles et al. 2005; Perez-Heydrich et al. 2012; Edmunds et al. 2016; Lopes et al. 2016). During this time, asymptomatic but TB-infected meerkats could function as undetected 'superspreaders' (Martin et al. 2019), being seemingly unaffected themselves, but having a major negative impact on the population in terms of TB. Identifying these infected yet asymptomatic individuals and assessing the role they play for TB transmission will be crucial for limiting the spread of TB within managed wildlife populations (Chisholm et al. 2018). Lastly, we found high heterogeneity in the susceptibility and resistance in TB in meerkats. The underlying factors generating such variation require further research, yet may be linked to variation in genetics and maternal effects (Marjamaki 2019).

Despite these advantages, TB detection from faeces has also limitations. The use of faecal samples to monitor TB is most useful in study systems where study animals can be individually identified and observed, and is less useful for cryptic species. In addition, PCR sensitivity was low at 12.5%, and in 13.6% of our study individuals, symptoms were observed yet samples never became PCR-positive, highlighting the possibility of false negative results due to methodological constraints. One reason for the low detected rate could be systematic differences in *Mycobacteria* shedding between individuals due to the form of TB infection: Most individuals displayed lesions in the head lymph nodes and lungs in post mortem examinations (Drewe et al. 2009a), which likely leads to swallowing of *Mycobacteria*, later detectable in the faeces. However, when inspected by a vet after death, substantial proportions of individuals showed lesions in liver and mesenteric or mediastinal lymph nodes (Drewe et al. 2009a), and these internal symptoms might not lead to *Mycobacterium* in the faeces. Due to the low PCR sensitivity, this method also required an adapted PCR protocol with multiple replications, increasing lab time and costs, which may not always be feasible. To date, the

gold standard of TB detection in wildlife is serological testing, which has been developed with a sensitivity of 83% in meerkats (Drewe et al. 2009b), paired with mycobacterial cultures of pooled tracheal washes and lymph node aspirate (sensitivity of 36% in meerkats, and 8% in badgers; Drewe et al. 2009b, 2010). While these methods can distinguish between latent and active infection stages, particularly if used in combination, their application can be severely limited in wildlife if animals cannot be regularly handled or trapped. We argue that, despite the limitations of TB detection from faecal samples, their application still provide a powerful diagnostic tool to assess TB status in systems where study animals can be easily observed, allowing for the monitoring of disease presence and progression prior to the onset of symptoms.

Our study also assumes that once a meerkat becomes TB-positive, it remains infected for life, since there is no evidence for natural TB infection clearance without treatment (Houben and Dodd 2016). Accordingly, we classified meerkats as either PCR-negative exposed, PCR-positive asymptomatic, or PCR-positive symptomatic, with an assumption that changes in infection state are non-reversible. However, there is anecdotal evidence that clearance upon exposure is possible (Verrall et al. 2014). A study of wild field voles (*Microtus agrestis*) trapped animals that were lesion-free after showing lesions in previous captures (Burthe et al. 2008), although the study concluded that lesions are a transient stage of an advanced infection and that animals never completely cleared the infection. In badgers, five animals that tested positive for *Mycobacterium bovis* infections later appeared to have resolved the disease completely, with post-mortems finding no sign on internal lesions (Gallagher et al. 1998). We acknowledge that our study does not have the power to distinguish between potentially more complex infection states, such identifying latent infections, which are by definition non-infectious. It is unclear whether meerkats that were exposed to TB, but never tested positive or developed symptoms, are potentially carrying latent infections or whether they are truly not

susceptible. Epidemiologically, however, this may be irrelevant since neither state can transmit the infection to others, but could play a large role on individual consequences of infections, such as immune-reproductive trade-offs, longevity, and ultimately fitness (Lochmiller and Deerenberg 2000).

Our study is one of the first to investigate TB progression over the entire life of wild animals using only non-invasive methods. We not only demonstrate the potential of PCR-based TB detection from faecal samples as a diagnostic tool in wildlife, but also provide valuable insights into the course of TB following exposure. Our results indicate that TB progression is characterised by extended asymptomatic period followed by a shorter symptomatic period, with high heterogeneity, potentially promoting TB persistence within even asymptomatic populations. This implies that meerkats with an average life expectancy of two to three years, can stay positive but asymptomatic for half their lifetime. Establishing routine non-invasive TB screening, particularly in longitudinal studies of known individuals, can thus be a suitable approach to investigate which mechanisms and factors determine individuals' susceptibility, resistance and potential differences in fitness related to TB in much greater detail than using purely observational data on symptoms. On the epidemiological level, being able to detect asymptomatic infections opens a range of possibilities to more accurately follow transmission routes and elucidate the relative contributions of symptomatic vs. asymptomatic individuals to disease transmission, potentially adding to our knowledge of transmission routes, allowing for more effective TB management of endangered species or populations.

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513 **TABLES**

514

515 **Table 1.** Summary table of the different terms in relation to TB used in this study, and how
516 they were measured.

517

Classification	Definition
PCR-negative exposed	Meerkats that were known to be exposed to TB after co-habitation with a TB-infected meerkat (i.e. either showing TB symptoms or being PCR-positive for TB) or became PCR-positive or symptomatic themself.
PCR-positive asymptomatic	Meerkats tested PCR-positive for TB but has no visual symptoms.
PCR-positive symptomatic	Meerkats tested PCR-positive for TB and showed visual symptoms of TB. Meerkats considered symptomatic when they showed visual symptoms, such as submandibular lumps, genital lumps or had clear TB signs (e.g. lesions) mentioned in database records.
Susceptibility	Likelihood that an animal becomes infected with TB after being exposed to TB, estimated here as time between exposure and becoming PCR-positive
Resistance	A measure of how well a meerkat is able to survive with TB infection, defined here as the time period between TB detection via PCR and death.

518

519 **Table 2.** Summary of the periods (in days) between the infection stages (exposure, becoming
520 PCR-positive, becoming symptomatic, and death) with the median, mean, standard deviation
521 and maximum for each time period. [a] from two individual exposure date was missing; [b] meerkats
522 that became symptomatic before becoming PCR-positive were excluded (N = 9) from the
523 calculations to avoid a bias due to deficient sampling or low PCR sensitivity.

524

Infection stages	**Min [days]**	**Median [days]**	**Mean [days]**	**Max [days]**	**SD [days]**
Age at exposure (N = 64)[a]	0	0	205	2360	498
Age at PCR-positive (N = 40)	32	363	581	3237	683
Age at showing symptoms (N = 44)	29	650	851	3136	657
Age at death (N = 66)	387	808	980	3352	569
Exposure – PCR-positive (N = 40)	0	363	427	1518	328
Exposure – Symptoms (N = 44)	0	650	644	1237	282
Exposure – Death (N = 64)	117	736	759	1549	263
PCR-positive – Symptoms (N = 26)[b]	2	330	349	834	211
PCR-positive – Death (N = 40)	4	358	377	1040	283
Symptoms – Death (N = 44)	12	98	160	595	173

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FIGURE LEGENDS

Figure 1. Illustration of TB infection in meerkats. After being exposed, TB progression depends on susceptibility (progression from exposed to PCR-positive and resistance (progression from PCR-positive to death). Non-susceptible meerkats may never become infected with TB, whereas meerkats with high resistance have slower progression and may even supress the development of symptoms.

Figure 2. Timeline of PCR-based TB-identification (PCR neg/PCR pos) and TB-related phenotypic symptoms (asymptomatic/symptomatic) in individual meerkats (N = 66). After the detection of the first PCR-positive -sample, all subsequent samples of the same individual were considered positive. The triangle indicates the date of the first observed TB-related phenotypic symptoms. The stars and crosses indicate the birth and death dates, respectively, with bold crosses indicating TB-related euthanasia of the individual. The numbers on the y-axis refer to individual IDs.

Figure 3. TB progression in wild meerkats (N= 66). The density lines show the age distribution of individuals during the four major TB progression events (exposure, becomes PCR-positive, becomes symptomatic, death). The red lines indicate the median, the blue lines the first and third quartile, respectively.

Figure 4. Histograms showing the heterogeneity of TB progression in meerkats. A) TB susceptibility (time between exposure and becoming PCR-positive), B) TB resistance (time between PCR-positive and death).

Figure 1

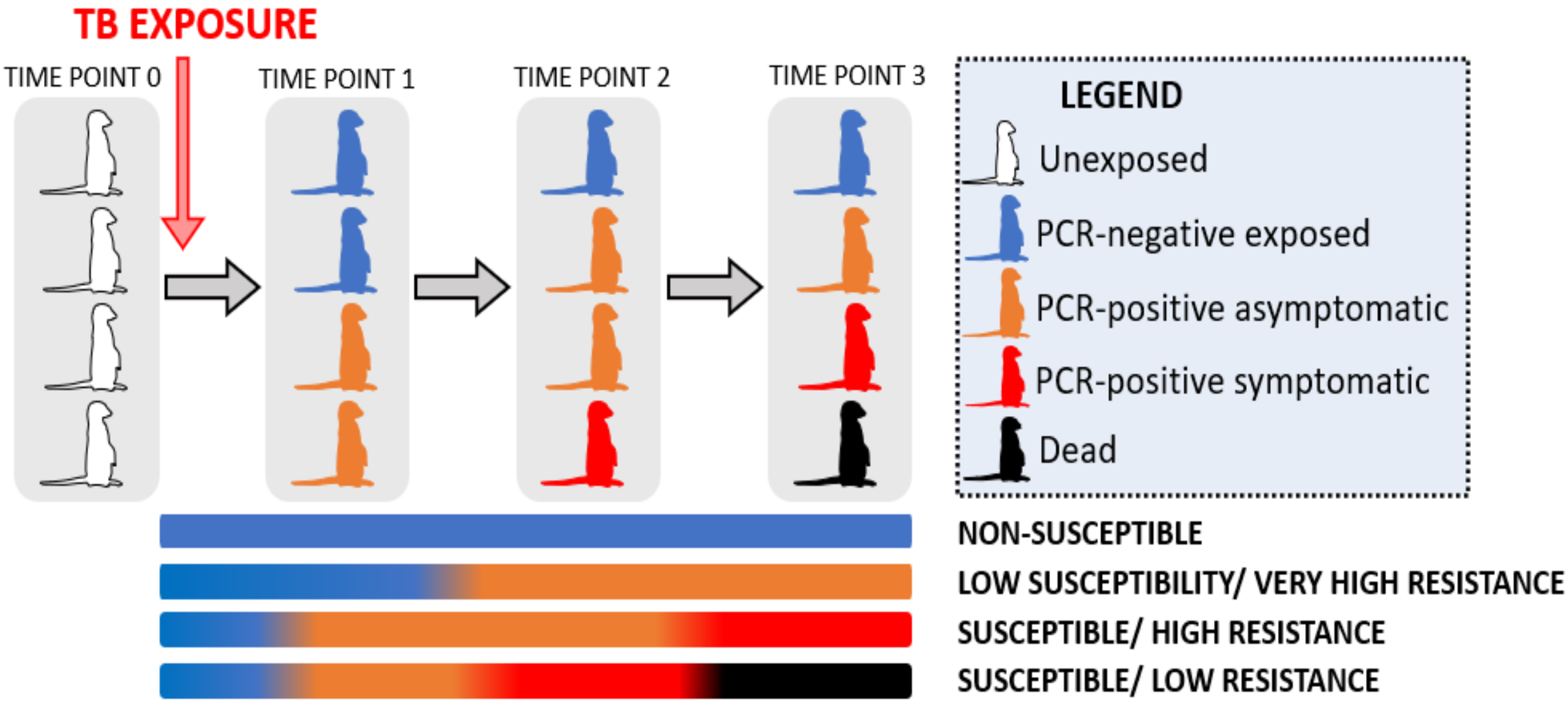

Figure 2

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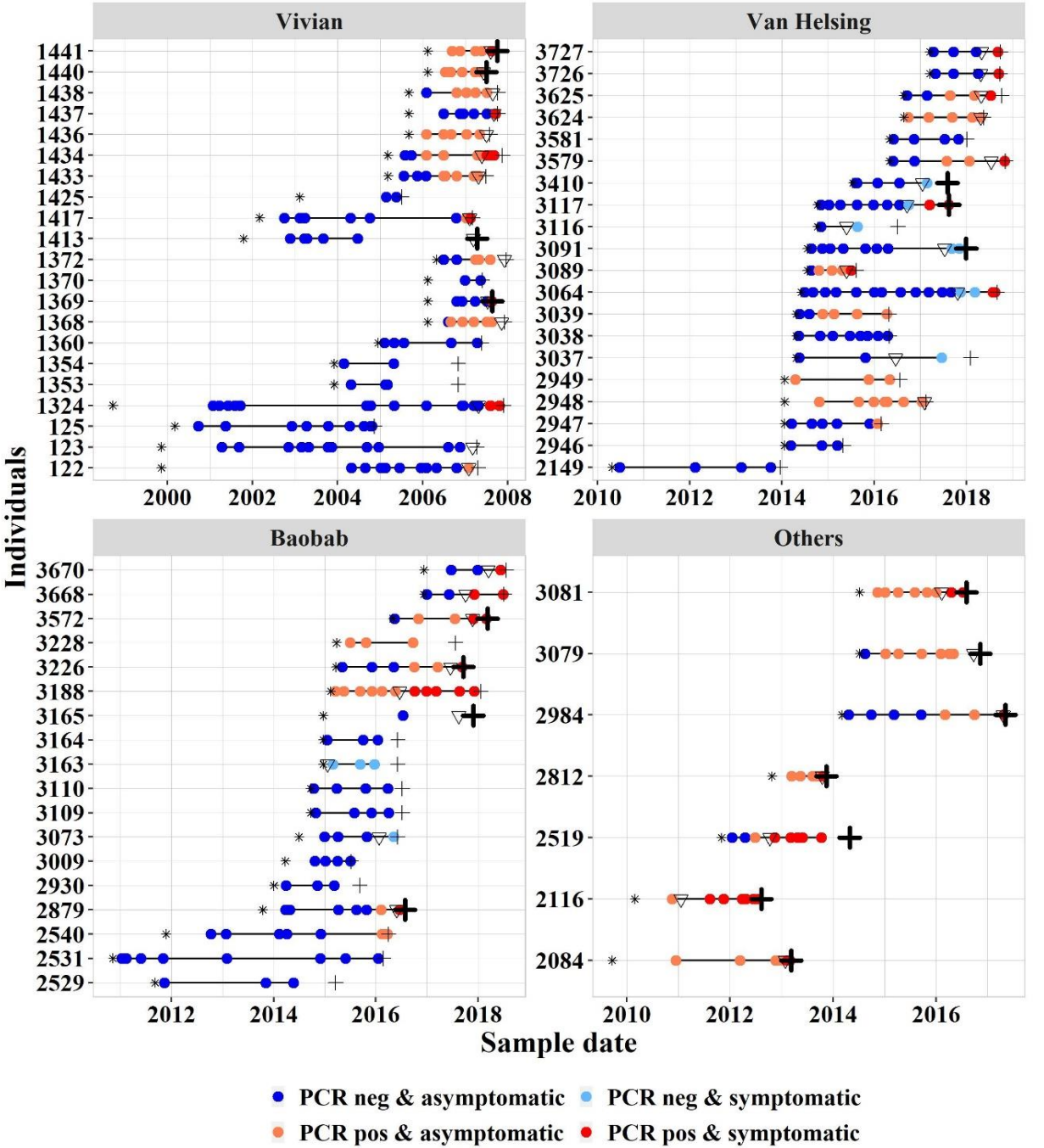

Figure 3

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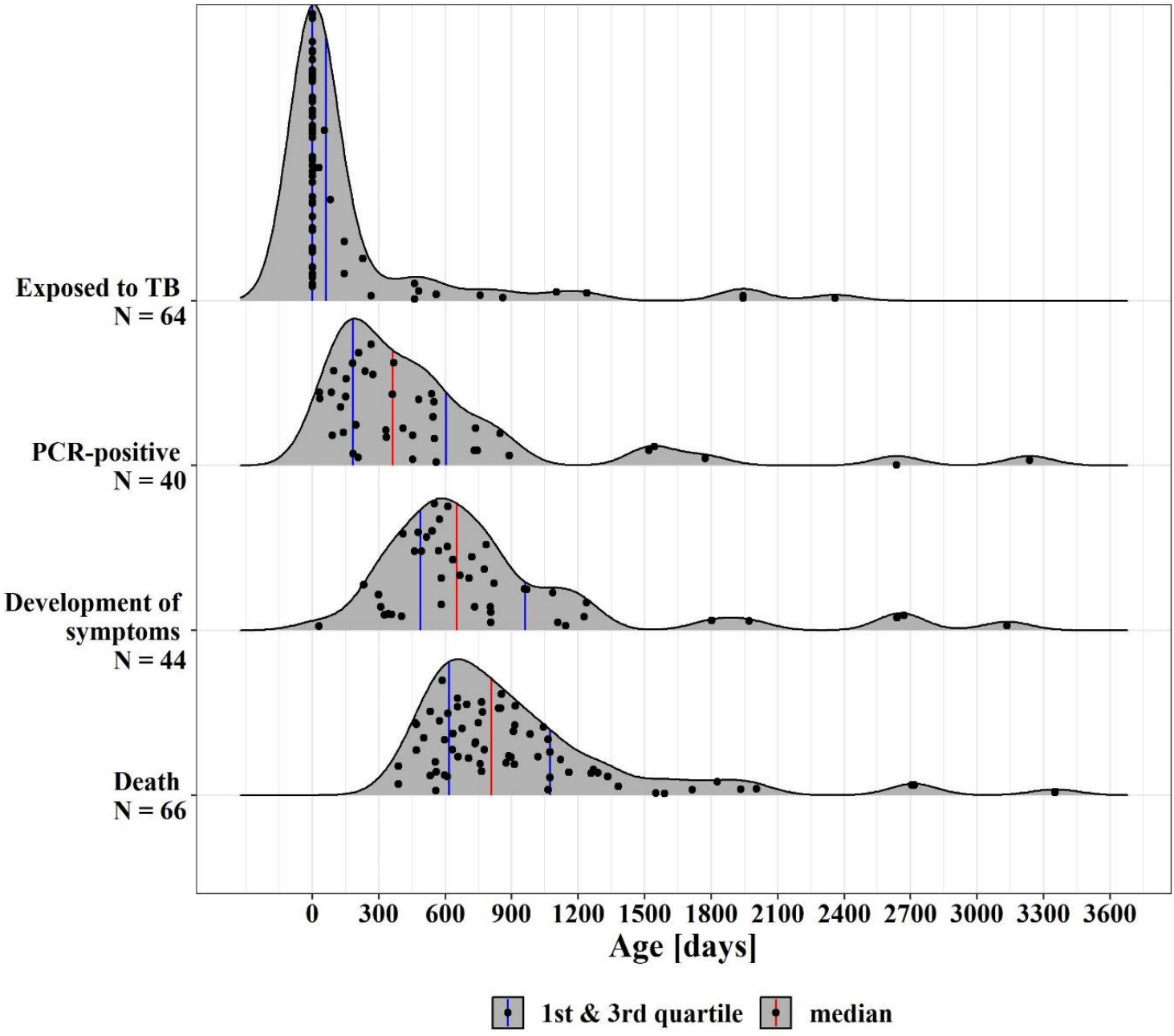

Figure 4

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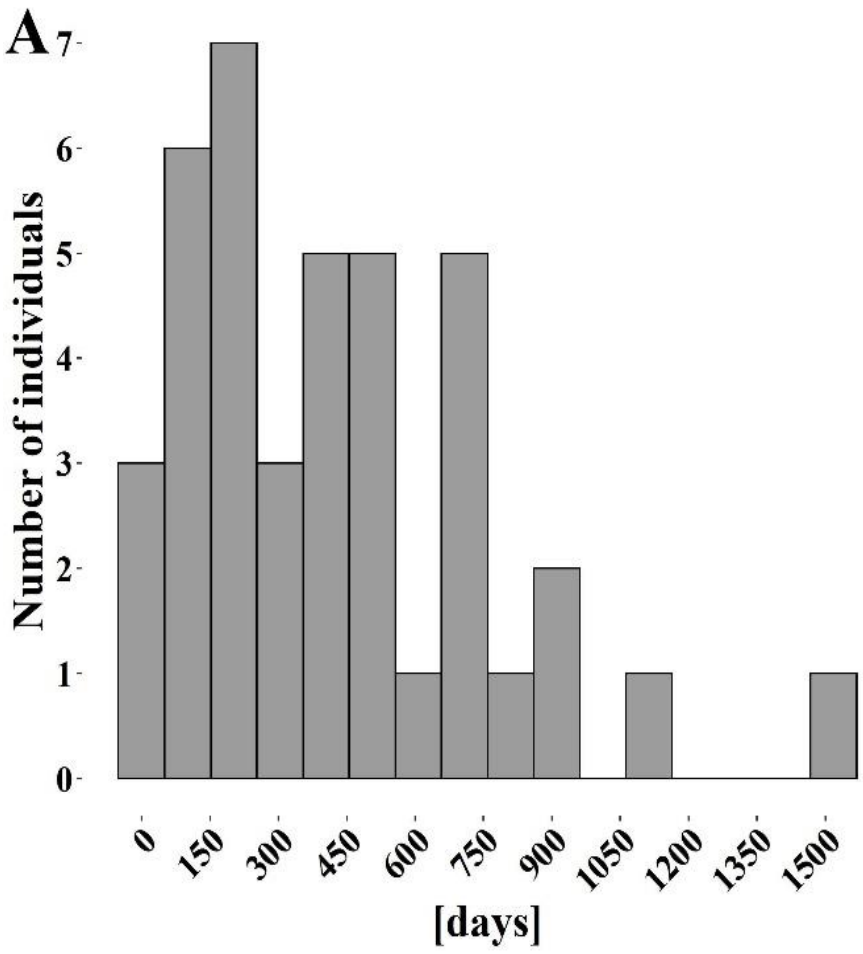

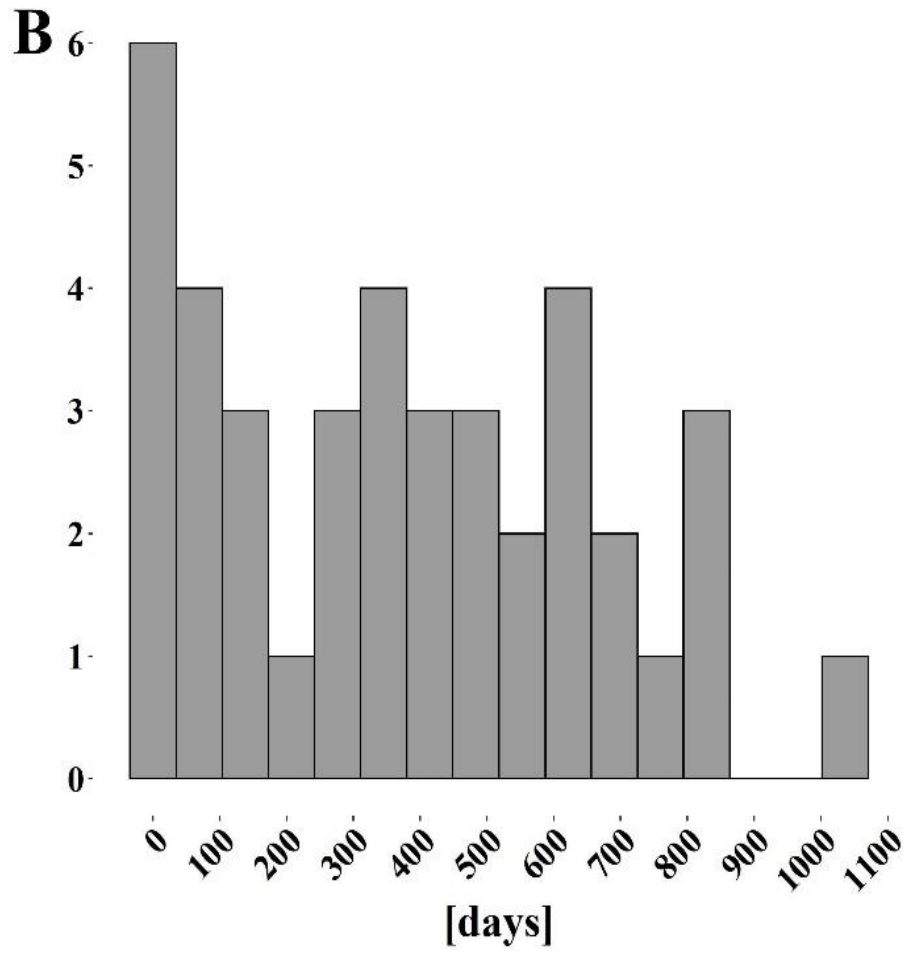

Supplementary tables S1-S3