RRH: PATOUILLAT ET AL. - STREPTOCOCCAL OUTBREAK IN WILD LONG-TAILED MACAQUES

Recurring Streptococcal Outbreak Threats in Urban Long-Tailed Macaque (*Macaca fascicularis*)

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ABSTRACT:

Streptococcus equi ssp. zooepidemicus is a zoonotic bacterium known to cause severe respiratory tract infections in primates, while most documented mortality episodes occurred in captivity. Here, we report a mass mortality event in a wild population of long-tailed macaques (*Macaca fascicularis*) inhabiting an urban mosaic habitat in central Bali, Indonesia, and describe the demographic impact and clinical patterns of the outbreak. Mortality was observed in three of the nine social groups which shared overlapping ranges, and the infection spread progressively over time. Clinical signs included lethargy, respiratory distress, and locomotion disorders. Over a 2-mo period during March-May 2022, 170 carcasses were found. Demographic surveys revealed a mortality rate ranging from 16-53% among the three affected groups. Adult females and adult males were the most affected age-sex classes, representing 36% and 35% of the carcasses found, respectively. Necropsy findings from four individuals, combined with bacteriological culture, histopathology, qPCR, and 16S rRNA metabarcoding analysis, all suggested *Streptococcus equi ssp. zooepidemicus* as the most likely causative agent. Similar streptococcal outbreaks had occurred in this population in 1994 and 2012, raising concerns about the recurrent introduction of the bacterium or potential reservoirs either within or outside the macaque population. The recurrence of *Streptococcus equi ssp. zooepidemicus* outbreaks in this population highlights the impact of lethal bacterial epidemics in wild primates, which remain poorly documented. Strengthening long-term surveillance, including non-invasive serological monitoring, is essential for better understanding infection dynamics and improving conservation strategies.

Key words: Bacterial infection, human-primate interface, primate tourism, mortality, wildlife health, wildlife conservation

INTRODUCTION

Infectious diseases, including bacterial infections, pose a significant threat to primates (Didier and Kondova-Perseng 2024), but they remain largely under-documented in wild primate populations, especially outside Africa; most research has focused on great apes (*Pan troglodytes* and *Gorilla spp*.) and baboons (*Papio spp.*; Nunn and Altizer 2006; Hopkins and Nunn 2007; Patouillat et al. 2024). This knowledge gap is particularly concerning for primates inhabiting anthropogenic environments, where exposure to human-associated pathogens and habitat disturbances may increase susceptibility to infectious disease outbreaks (Balasubramaniam et al. 2019; Devaux et al. 2019). Severe bacterial epizootics have been reported in primates living at interfaces with humans, sometimes resulting in significant morbidity and mortality (Brotcorne et al. 2015; Meesawat et al. 2023; Napit et al. 2023), yet detailed documentation of these events remains scarce and fragmented.

Among the bacterial pathogens responsible for fatal outbreaks in primates, *Streptococcus equi subsp. zooepidemicus* (SEZ) has been identified as a recurrent and concerning agent. This gram-positive, β-hemolytic bacterium from the Lancefield group C (Fulde and Valentin-Weigand 2012) is a commensal bacterium that typically inhabits the upper respiratory and lower genital tracts of horses, where it can act as an opportunistic pathogen and cause pneumonia (Newton et al. 2008; Lowenstine and Osborn 2012). Transmission can occur directly through nasal secretions and respiratory droplets, or indirectly via contaminated equipment, food, water, or the environment (Sweeney et al. 2005). *Streptococcus equi subsp. zooepidemicus* has been identified in many species, including dogs (*Canis familiaris*; Chalker et al. 2003; Priestnall and Erles 2011), poultry (Garmyn et al. 2020), guinea pigs (*Cavia porcellus*; Gruszynski et al. 2015), cattle (*Bos taurus*; Jovanović et al. 2008), pigs (*Sus scrofa*; Soedarmanto et al. 1996; Costa and Lage 2020), and primates (Schiller et al. 1989; Soedarmanto et al. 1996; Brack et al. 1997; Mätz-

Rensing et al. 2009; Brotcorne et al. 2015). Human SEZ infections, although rare, can be serious and have often been linked to unpasteurized dairy products or transmission from infected animals through respiratory route or wound contamination (Kuusi et al. 2006; Jovanović et al. 2008; Abbott et al. 2010; Eyre et al. 2010; Priestnall and Erles 2011; Pelkonen et al. 2013).

Most SEZ infections in primates have been documented in captive settings and have often resulted in high mortality. Notable cases have included outbreaks in red-bellied tamarins (*Saguinus labiatus*) and Goeldi's marmosets (*Callimico goeldii*) at the National Zoological Park, Washington, D.C., US, in 1984, probably due to infected horse meat (Schiller et al. 1989). Other SEZ-associated mortalities include three lion-tailed macaques (*Macaca silenus*) at NaturZoo Rheine, Rheine, Germany in 1997 (Brack et al. 1997) and six rhesus macaques (*Macaca mulatta*) at the German Primate Center, Göttingen, Germany, in 2007, with suspected reverse zoonotic transmission from humans (Mätz-Rensing et al. 2007). In wild populations, SEZ outbreaks have been reported solely in long-tailed macaques (*Macaca fascicularis*) in Bali, Indonesia. The first outbreak, in 1994, caused significant mortality among macaques and also affected pigs in nearby farms, which were suspected as the source of transmission. Necropsies revealed polyarthritis, bronchopneumonia, pleuritis, epicarditis, endocarditis, and meningitis (Soedarmanto et al. 1996). A second outbreak occurred in the same macaque population in 2012, with similar clinical signs, though confirmation of SEZ was inconclusive (Brotcorne et al. 2015). Here, we document a recent SEZ outbreak in the same population of long-tailed macaques in Bali, Indonesia, which occurred between March and May 2022. We describe the demographic impact of the outbreak and provide pathological and microbiological findings. This recurrent pattern of SEZ outbreaks in a highly anthropogenic habitat highlights the critical need for systematic documentation and

monitoring of epizootic events in wild primates to inform conservation and management strategies effectively.

MATERIALS AND METHODS

Study site and groups

The epizootic event occurred in a monkey forest of south-central Bali (8°31 S–155°15 E). This 20-ha secondary forest sanctuary serves as a tourist site and a Hindu temple complex daily used by villagers. The site is located in an urban landscape and is bordered by rivers, roads, infrastructure, and rice fields (Howells et al. 2022). By late 2021, the forest was home to a free-ranging population of approximately 1,260 long-tailed macaques distributed across nine social groups: Selatan, Utara, Temple, East, Central, Michelin, Cemetery, Atap, and New Forest (including the daughter-group called Ashram). These groups occupied overlapping ranges within and around the forest sanctuary (Fig. 1) and the macaques were provisioned daily by the management team (Howells et al. 2022).

Demographic impact

Since 2009, annual demographic censuses have been conducted on this macaque population using total counts during group travel events (Brotcorne et al. 2015, Giraud 2023). Individuals' ages classifications are based on morphological characteristics: adult male (>6 yr), adult female (≥ 3.5 yr), immature (1-3.5 yr for females, 1-6 yr for males), and infant (≤1 yr) (Brotcorne 2014; Pal et al. 2018).

To quantify the demographic effects of the 2022 epizootic, we combined two data sources: (a) The number of carcasses found during the outbreak period, and (b) comparison between demographic censuses conducted before (September 2021) and after (August 2022) the

113 outbreak. For the first method, carcasses were assigned to a group based on their location within
114 known ranges (Fig. 1), and on individual markings such as tattoos when available. Because
115 carcass inventory was probably incomplete, the method based on demographic censuses enabled
116 a more comprehensive assessment of population changes by comparing group sizes between
117 2021 and 2022. The annual growth rate (intrinsic rate of increase, *r*)(Cowlishaw and Dunbar
118 2000) for each group was calculated as follows, where Nt and N0 represent the population sizes
119 in 2022 (post-outbreak period: August 2022 census) and 2021 (pre-outbreak period: September
120 2021 census), respectively, and *t* is the one-year interval between surveys.

121 $$r = \frac{\ln(\mathrm{Nt}) - \ln(\mathrm{N0})}{t}$$

122

123 We compared the intrinsic rate of increase between the groups affected by the epizootic
124 and those not affected. Additionally, we calculated the annual intrinsic rate of increase for each
125 group from 2018-2021 (the years prior to the outbreak) to establish baseline growth rates in the
126 absence of epizootic events.

127 We defined the mortality rate as the number of deaths relative to the population size at a
128 given period of time (CDC 2012). More precisely, we calculated mortality rates for each group
129 and each age-sex class by dividing the number of carcasses found during the epidemic by the
130 total number of individuals present in each group or age-sex class before the epidemic (i.e., 5 mo
131 earlier, based on the 2021 census), and we computed 95% confidence intervals (CI) to account
132 for statistical uncertainty. Given the atypical profile of high acute mortality, deaths during the
133 epizootic period were attributed to the disease unless a clear alternative cause of death, such as
134 injury from a fight or car collision, was identified.

135

Pathological and microbiological analyses

During the outbreak, necropsies were conducted on four carcasses from different affected groups within 3-24 h postmortem. Tissues from the brain, trachea, lungs, spleen, heart, liver, kidney, and small intestine were sampled for histopathologic and molecular analysis, with cardiac blood collected from three carcasses. Samples were either stored frozen at -20 C or preserved in 10% formalin. For histopathologic examination, samples from the lung, brain, heart, trachea, kidney, liver, and intestine were formalin-fixed, paraffin-embedded, and stained with H&E. Bacteriological analyses on lung and brain samples included bacterial cultures on general blood agar plate medium (sheep blood) incubated for 24 h at 37 C, plus Gram staining and catalase tests to help identify bacteria and distinguish aerobic from anaerobic species.

DNA and RNA extraction

In the week after sample collection and freezing, DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany) at the Veterinary Virology Laboratory of the Veterinary Medicine Faculty of Udayana University (Denpasar, Bali), following the manufacturer's protocol. Additionally, RNA was extracted from 250 µL of blood or supernatant using TRIzol LS reagent (Invitrogen, Carlsbad, California, USA), following standard protocols. Detailed procedures are provided in Supplementary Materials (Supp. Mat. 1). Specific molecular diagnostic tests included those for SARS-Cov-2 and SEZ, as defined hereafter.

SARS-CoV-2 reverse-transcription -PCR

Since this epizootic occurred during the Covid-19 pandemic, reverse transcription (RT)-PCR analyses for SARS-CoV-2 were performed to rule out the involvement of this virus. This used a

10 µL reaction mixture containing 5 µL of mastermix (SuperScript™ III One-Step RT-PCR System with Platinum Taq DNA Polymerase, ThermoFisher Scientific, Waltham, Massachusetts, USA), 0.6 µL of each 10 µM primers, 0.25 µL of SuperScript Reverse Transcriptase (SuperScript III Reverse Transcriptase, ThermoFisher Scientific, USA), 1.55 µL of nuclease-free water (Invitrogen, Carlsbad, California, USA), and 2 µL of RNA. We applied a primer set targeting the spike gene of SARS-CoV-2, specifically designed to amplify the most conserved region of the spike gene sequences available in the Global Initiative on Sharing All Influenza Data (GISAID) database at that time. The SARS-CoV-2 primers were NCOV2F: AGATTCAACTGGCAGTAACCAGAAT and NCOV2R: ACAGTTTGCTGTTTCTTCTGTCTCT (Gusti N.K. Mahardika, unpublished data) and they had previously been validated using 10 human samples confirmed positive for SARS-CoV-2 by real-time RT-PCR at Udayana University Education Hospital (Badung Regency, Bali) a recognized Covid-19 testing center in Bali. The hospital used standard primer sets recommended by the Ministry of Health of Indonesia (Agustiningsih et al. 2020). Validation of our in-house primer set was further confirmed by sequencing the RT-PCR products using the Sanger method. The reaction mixture was amplified using a Bio-Rad MJ Mini thermocycler (Bio-Rad, Hercules, California, USA), with the following conditions: initial step at 50 C for 1 h, denaturation at 94 C for 5 min, followed by 35 cycles of 94 C for 45 s, annealing at 50 C for 1 min, and 72 C for 1 min, and a final elongation step at 72 C for 5 min.

Streptococcus equi ssp. zooepidemicus **quantitative PCR (qPCR)**

Each qPCR reaction was performed on a Mic thermocycler (Bio molecular systems, Upper Coomera, Australia), using the ICESz1 primer set described (Cordoni et al. 2015), but with a

fluorescein amidite fluorophore. Each reaction mixture of 15 µL contained 4 µL pure water (Sigma-Aldrich, St. Louis, Missouri, USA), 7.5 µL of qPCR MasterMix (Luna Universal Probe qPCR Master Mix Biolabs, Ipswich, Massachusetts, USA), 1.5 µL of ICESz1GC primers & probe mix (20 µL of 10 µM F Primer + 20 µL of 10 µM R Primer + 10 µL of 10 µM probe) and 2 µL of DNA. The cycle applied was: 95 C for 5 min (initial denaturation), and 45 cycles at 95 C for 10s, 50 C for 20s, and 72 C for 20s. The cycle threshold (Ct) values were determined using the micPCR version 2.12.7 software (Bio molecular systems). We detailed in supplementary materials (Supp. Mat. 2) the procedure related to the genomic B-actin qPCR used as an internal control.

Metabarcoding 16S rRNA analysis

Metabarcoding 16S sequencing was performed to detect microbial composition in one cardiac blood sample and three lung tissue samples (*n*=4). High-throughput sequencing of 16S rRNA genes targeting the V3-V4 regions was carried out using specific primers (341F/805R, Illumina, San Diego, California, USA). The PCR products were pooled, end-repaired, and sequenced on an Illumina NextSeq 2000 platform (Illumina). Clean data were processed using QIIME2 (Bolyen et al. 2019) to generate amplicon sequence variants and annotate species. A distribution histogram of relative abundance for the top 10 taxa at each taxonomic level was plotted. Full details are provided in the Supplementary Materials (Supp. Mat. 3).

RESULTS

Outbreak timeline and demographic impacts

From 5 March to 10 May 2022, 170 carcasses were discovered across three of the nine macaque social groups. Selatan group was first affected, with 94 deaths occurring from 5 March to 9 May. Mortality then spread to the New Forest group, where 18 deaths were recorded between 3-14 April, and subsequently to the Temple group, which experienced 52 deaths from 10 April to 10 May (Fig. 2). These three affected groups occupied overlapping ranges in the southwestern part of the site (Fig. 1). No carcasses were found within the known ranges of the six other groups located in the northern and eastern parts of the sanctuary (Fig. 1). However, of the 170 carcasses, six could not be assigned to a specific group.

Mortality rates varied by group, with the Selatan group having the highest mortality rate, 53% (95% CI: 45-60), followed by the Temple group (27%, CI: 21-34) and the New Forest group (10%, CI: 6-15%) (Table 1). Deaths occurred across all age-sex classes, with adult females being the most affected class (N = 75). Mortality rates were recorded as 36% (CI: 29-42) for adult females, 35% (CI: 24-48) for adult males, 26% (CI: 20-32) for immature individuals, and 16% (CI: 10-27) for infants (Table 2).

In addition to the carcasses discovered, the population census conducted in 2021 and 2022 (pre- and post-outbreak period, respectively) confirmed significant declines in the sizes of the affected groups (i.e., Temple, Selatan, and New Forest), with the highest demographic decline being seen in the Selatan group ($r = -0.98$), followed by the Temple group ($r = -0.61$) and the New Forest group ($r = -0.27$), with a mean intrinsic growth rate of $r = -0.61$ across all affected groups (Fig. 3). In contrast, unaffected groups showed either no change or slight variations in intrinsic growth rates between 2021 and 2022 (e.g., Central group: $r = 0.11$, East group: $r = -0.01$, Michelin group: $r = -0.05$), averaging $r = 0.02$ (Fig. 3). Compared to previous years, the mean annual growth rate across all groups was positive and averaged $r = 0.11$ during

the four-year period before the epizootic (2018–2021), emphasizing the substantial demographic impact of the 2022 outbreak.

Pathological and microbiological findings

During the outbreak, affected macaques exhibited lethargy, locomotion disorders, and respiratory distress. Some carcasses showed epistaxis, frothy exudate in the oral cavity, and/or pallor indicating anemia. Necropsies on four animals revealed consistent hemorrhagic presentations, with splenomegaly and suspected pneumonia and meningitis, although hemorrhagic condition complicated definitive diagnoses. Histopathology showed multifocal necrosis, particularly in the liver and small intestine, with three cases also showing signs of neutrophilic and fibrinous meningitis. Lung histology was challenging due to extensive hemorrhage, precluding detailed lesion assessment.

Bacteriologic culture analysis Gram staining and catalase tests identified bacterial colonies of the genus *Streptococcus* in 2/3 lung samples and 2/4 brain samples. These findings, along with historical instances of streptococcal epidemics in this population in 1994 and 2012 (Soedarmento et al. 1996; Brotcorne et al. 2015), suggested a respiratory infection with *Streptococcus sp.*, specifically SEZ. Due to the similarity of findings with viral respiratory infections such as SARS-CoV-2 (Johansen et al. 2020), RT-PCR analysis was conducted on the extracted RNAs; this conclusively ruled out SARS-CoV-2 involvement, as all cases consistently yielded negative results. In contrast, qPCR tests confirmed the presence of SEZ DNA in lung and cardiac blood samples, with Ct values of 30.3-34.4 across cases (Table 3). At least one tissue sample from each of the four cases tested positive for SEZ, while all negative controls remained consistently negative, confirming the absence of contamination in the qPCR assays.

Metabarcoding 16S rRNA gene sequencing further characterized bacterial relative abundance in lung and blood samples from the four carcasses. *Streptococcus equi* was the most abundant species in all samples, with the highest relative abundances (approximately 0.85 and 0.80) in Cases 2 and 3 respectively (Fig. 4).

DISCUSSION

Understanding the causes, dynamics, and consequences of epizootic outbreaks in wild primate populations is essential for effective population management and species conservation (Nunn and Gillespie 2016; Lappan et al. 2020). The rapid increase in mortality over the period, March-May 2022 affecting three of nine long-tailed macaque groups in a sanctuary of central Bali strongly suggested an infectious pathogen as the causative agent.

Necropsy and histopathologic findings, bacteriological cultures, and molecular diagnostics collectively indicated SEZ as the most probable causative agent for the acute mortality observed in macaques during this period. This organism was detected in all tested samples via qPCR, although relatively high Ct values (mostly >30) were observed. These values, consistent with those for the beta-actin gene, probably reflected DNA degradation due to delays of up to 24 h between death, and DNA extraction. Such delays may also have facilitated postmortem bacterial proliferation, contributing to the detection of diverse bacterial populations through 16S rRNA gene metabarcoding, including *Lactobacilli* species (Palmiere et al. 2016). Nevertheless, SEZ remained the predominant bacterium detected, strongly supporting its role in the outbreak.

We screened for SARS-CoV-2 due to its emergence as a significant respiratory pathogen at the time of the study and its known ability to infect non-human primates (Johansen et al. 2020). All samples tested negative. It remains possible that other respiratory viruses may have

273 contributed to the initial infection or co-infection with SEZ, potentially exacerbating disease
274 outcomes. . In primates, particularly great apes, single or co-infections involving respiratory
275 viruses such as HRSV, HMPV parainfluenza 3 and opportunistic bacteria (e.g., *Streptococcus*
276 *pneumoniae* or *Pasteurella spp.*) frequently exacerbate respiratory disease severity and related
277 mortality (Jones et al. 1984; Chi et al. 2007; Köndgen et al. 2008; Szentiks et al. 2009; Unwin et
278 al. 2013). The lack of systematic testing for potential co-infections, particularly respiratory
279 viruses such as influenza, respiratory syncytial virus (HRSV), metapneumovirus (HMPV),
280 parainfluenza, and measles, remains an important limitation of this study.
281 The cumulative death curve in spring 2022 exhibited a clear epidemic pattern,
282 characterized by acute mortality and the progressive spillover of infection across the three
283 affected groups (Fig.2). However, this curve does not provide a precise measure of disease
284 transmission dynamics, as key epidemiological factors such as infection latency, variability in
285 clinical signs, and survival times remained unknown. Mortality rates during the outbreak ranged
286 from 10-53% across affected groups, exceeding the 13-38% mortality rates recorded during the
287 2012 SEZ outbreak in the same population (Brotcorne et al. 2015). However, mortality estimates
288 derived from carcass counts should be interpreted cautiously, as this method is prone to
289 underestimation, due to probable undetected carcasses, or overestimation, when all carcasses are
290 attributed to the outbreak in the absence of an obvious alternative cause of death. Conducting
291 regular and systematic population monitoring, including total counts, provides a more reliable
292 method for assessing demographic changes following outbreaks in wildlife (Leendertz et al.
293 2006). In our study, census data aligned with carcass counts, revealing abnormally high mortality
294 in the Temple, Selatan, and New Forest groups, while other groups exhibited stable or slightly
295 fluctuating growth rates probably attributable to demographic stochasticity (Lawler 2011). Slight

296 demographic variations in unaffected groups correspond with pre-epizootic population trends
297 observed from 2018 to 2021 (de Thier Nagelmackers 2021), further supporting the conclusion
298 that declines in the affected groups were outbreak-related.

299 Variation in mortality rates among affected groups may reflect differences in access to
300 antibiotic treatment provided by the management staff. During the outbreak, ciprofloxacin was
301 initially used, followed by amoxicillin delivered via provisioning with raw eggs, a highly
302 attractive food for macaques. Due to the intergroup competition characteristic of cercopithecine
303 species such as macaques (Wrangham 1980; Van Schaik 1989), dominant groups probably had
304 better access to treated eggs, potentially resulting in lower mortality rates compared to
305 subordinate groups. Another possible explanation is that delays in antibiotic intervention for
306 earlier-affected groups, due to the time required to identify the outbreak and respond, allowed the
307 disease to spread and result in higher mortality.

308 Transmission of SEZ among individuals probably occurred through direct contact (e.g.,
309 nasal secretions or respiratory droplets) or indirectly via contaminated food, water, or
310 environmental surfaces (Fulde and Valentin-Weigand 2012). High rates of social interactions in
311 macaques probably facilitated within-group and between-group spread of SEZ (Côté and Poulin
312 1995; Capitanio 2012; Griffin and Nunn 2012; Romano et al. 2016). Social behaviours such as
313 grooming contribute to pathogen transmission, including gastrointestinal parasites (MacIntosh et
314 al. 2012; Rimbach et al. 2015; Duboscq et al. 2016) and bacteria (Springer et al. 2016; Grassotti
315 et al. 2022). Overlapping ranges and shared feeding sites among affected groups (Fig. 1)
316 probably provided opportunities for intergroup transmission. Additionally, intergroup
317 interactions such as aggression or copulation, are known to facilitate infection spread among
318 primate populations (Gillespie et al. 2008; Howie and Pomiankowski 2016).

Mortality patterns by age and sex revealed that adult males and adult females were disproportionately affected, with mortality rates of 35% and 36%, respectively. Similarly, during the 2012 outbreak at the same site, adult females experienced the highest mortality rate (22%) (Brotcorne et al. 2015). Adult macaques may be more susceptible to infections compared to juveniles due to their greater involvement in social networks, higher stress levels, and the influence of hormonal status (Nunn and Altizer 2006). For epizootics associated with pathogens transmitted through physical contact or aerosols, risk factors include spatial proximity and proportion of time spent in social interactions. Social behaviors such as grooming, aggression, and sexual contacts (Peirera and Altmann 1985), coupled with physiological stress, may increase adults' exposure to infectious diseases and reduce their immune defences (Nunn and Altizer 2006; Bonier et al. 2009). These factors underscore the complex interplay between behaviour, social roles across age-sex classes, and disease transmission and susceptibility in primates.

The origin of the 2022 SEZ outbreak remains unclear (Fig.5). Potential sources of infection include a reintroduction of the bacterium through human activities. The sanctuary attracts thousands of tourists daily (Howells et al. 2022), providing opportunities for zoonotic and reverse zoonotic transmission (Fuentes 2006). Although direct human-to-macaque SEZ transmission has not yet been proven, it has been suspected in previous events involving other macaque species (Brack et al. 1997; Mätz-Rensing et al. 2009). Nearby pig farms could also represent a source of infection; pigs have been implicated as reservoirs during past outbreaks in Bali (Soedarmento et al. 1996). While no reports of infected pigs were documented in 2022, asymptomatic pigs shedding SEZ in feces could contaminate water or soil, or the bacterium could be transmitted indirectly by humans through contaminated clothing and footwear (Costa et al. 2022). Alternatively, previous research suggests that SEZ can persist in macaque populations

for years following an outbreak. A 2004 study in Bali detected SEZ strains in healthy macaques and pigs that were identical to those from the 1994 outbreak in the Ubud Monkey Forest (Salasia et al. 2004). During the 2022 epizootic, it is possible that the bacterium was reintroduced into the affected groups by macaque groups spared during the outbreak but infected during previous ones. This hypothesis is supported by the observation that the groups affected during the 2022 epizootic differed from those impacted in the 2012 event (Brotcorne and al. 2015). Asymptomatic carriers may harbor the SEZ bacterium for extended periods (Boyle 2016), while the rest of the population gradually becomes immunologically naïve. Temporary herd immunity probably develops after an epizootic but gradually wanes over time, leaving the population susceptible to subsequent outbreaks (Ashby and Best 2021). Similar cycles of waning immunity followed by renewed outbreaks have been observed in other species, such as wolves infected with canine distemper virus (Rosa et al. 2020) and wild boars infected with classical swine fever virus (Kramer-Schadt et al. 2007).

The scale of this SEZ epizootic highlighted the often underestimated risk of lethal bacterial infections in wild primates, as evidenced by other severe outbreaks affecting various populations. Lethal bacterial outbreaks, particularly those caused by gastrointestinal pathogens of the *Yersinia* genus, have been documented in both wild and captive settings. For instance, a *Yersinia sp.* outbreak among wild chacma baboons (*Papio ursinus*) in South Africa resulted in a mortality rate of up to 85% (Barrett and Henzi 1998). In captivity, *Yersinia* species frequently affect various primate species (*Macaca sp., Cercocebus sp., Callithrix sp.*), sometimes in co-infection with *Escherichia coli*, exacerbating disease severity and related mortality (Bronson et al. 1972; de Lemos et al. 2021). Another example is *Treponema pallidum ssp. pertenue* (TPE), the agent of yaws, a bacterial disease transmitted by direct contact with infectious lesions

(Harper and Knauf 2013). In primates, TPE can cause severe genital ulcerations and may be lethal (Harper et al. 2012). Although long considered human-specific, TPE has been found in several wild African primate species (e.g. *Papio anubis* and *Cercocebus atys*), with strains closely related to those in humans, raising concerns about zoonotic transmission (Knauf et al. 2018). Airborne bacterial pathogens such as *Bacillus anthracis* (anthrax) and *Streptococcus pneumoniae* have also caused significant epizootics in primates. Anthrax has led to rapid and high mortality rates among chimpanzees in Taï National Park, Côte d'Ivoire (Leendertz et al. 2004; Hoffmann et al. 2017), while *S. pneumoniae*, often in co-infection with human-origin respiratory viruses, has been linked to infection rates as high as 92.2% in wild chimpanzees (Chi et al. 2007; Köndgen et al. 2017). Similarly, bacterial pathogens from the *Mycobacterium tuberculosis* complex (MTBC), particularly *Mycobacterium tuberculosis* and increasingly *M. bovis* (Garcia et al. 2004; Sapolsky and Share 2004), pose a significant threat to many primate species. In captive settings, tuberculosis causes severe pulmonary disease in primates, with high morbidity rates (42-60%; Mätz-Rensing et al. 2015; Gong et al. 2017). Although MTCB prevalence is high in some wild primate populations (e.g., up to 62% in *M. fascicularis* in Indonesia; Wilbur et al. 2012), large-scale mortality events are seldom reported, with notable exceptions such as the outbreak documented by Sapolsky and Share (2004) in a wild *Papio anubis* population. Moreover, some bacterial infections, such as tuberculosis, exhibit slow and chronic progression (Didier and Kondova-Perseng 2024), making them harder to detect than acute viral infections, which, due to their rapid transmission, short incubation periods, and acute symptoms, often cause sudden and dramatic mortality in wild primates, as seen with Ebola, yellow fever, and measles outbreaks (Devaux et al. 2019).

This detailed documentation of the SEZ epizootic provides valuable insights into high-mortality bacterial infections in wild primates and underscores the need for improved epidemiological surveillance to inform conservation strategies. Given their endangered status on the IUCN Red List (Hansen et al. 2022), synanthropic behavior, and frequent interactions with humans, long-tailed macaques, particularly this population, which has experienced recurrent SEZ epizootics (1994, 2012, and 2022) of uncertain but potentially human-associated origin, require sustained surveillance efforts. For instance, regular serological monitoring could provide insights into macaque immunity and the duration of acquired immunity in the study population (Howard and Fletcher 2012; Plowright et al. 2013; Farber et al. 2016). Although still unexplored in primates, SEZ infection has been shown to induce an adaptive immune response with IgG production in mice and horses (Causey et al. 2006; Wei et al. 2013; Tang et al. 2019). Since blood sampling is logistically challenging in wild populations, alternative matrices such as saliva or urine could be explored for antibody detection, an approach that has already been successfully applied to other pathogens (Eamudomkarn et al. 2018; Lyashchenko et al. 2021; Mohandas et al. 2022). Additionally, xenosurveillance using blood-fed mosquitoes is also an emerging non-invasive method that shows promise for pathogen monitoring (Grubaugh et al. 2015). Strengthening these analyses would enhance health management strategies and improve the ability to anticipate and mitigate future outbreaks.

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LITERATURE CITED

Abbott Y, Acke E, Khan S, Muldoon E, Markey B, Pinilla M, Leonard FC, Steward K, Waller A. 2010. Zoonotic transmission of *Streptococcus equi* subsp. *zooepidemicus* from a dog to a handler. *J Med Microbiol* 59:120–123.

Agustiningsih, Nugraha AA, Daryanto, Pawestri HA, Ikawati HD, Harianja H, Wibowo HA, Susanti I, Indalao IL, et al. 2020. *Pedoman: Pemeriksaan PCR SARS-CoV-2 bagi petugas laboratorium*. Lembaga Penerbit Badan Penelitian dan Pengembangan Kesehatan, Jakarta, Indonesia.

Ashby B, Best A. 2021. Herd immunity. *Curr Biol* 31:174-177.

Balasubramaniam KN, Sueur C, Huffman MA, MacIntosh AJJ. 2019. Primate infectious disease ecology: Insights and future directions at the human-macaque interface. In: *The behavioral ecology of the Tibetan macaque,* Li JH, Sun L, Kappeler PM, editors. Springer Open, Cham, Switzerland, pp 249-284.

433 Barrett L, Henzi SP. 1998. Epidemic deaths in a chacma baboon population. *S Afr J Sci* 94:441.

434 Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm
435 EJ, Arumugam M, et al. 2019. Reproducible, interactive, scalable and extensible
436 microbiome data science using QIIME 2. *Nat Biotechnol* 37:852-857.

437 Bonier F, Martin PR, Moore IT, Wingfield JC. 2009. Do baseline glucocorticoids predict fitness?
438 *Trends Ecol Evol* 24:634-642.

439 Boyle AG. 2016. Strangles and its complications. *Equine Vet Educ* 29:149-157.

440 Brack M, Günther E, Gilhaus H, Salzert W, Meuthen J. 1997. An outbreak of *Streptococcus equi*
441 ssp. *zooepidemicus* infection of probable human origin in wanderoos (*Macaca silenus*) –
442 case report. *Zentralbl Bakteriol* 286:441-446.

443 Bronson RT, May BD, Ruebner BH. 1972. An outbreak of infection by *Yersinia*
444 *pseudotuberculosis* in nonhuman primates. *Am J Pathol* 69:289-308.

445 Brotcorne F. 2014. *Behavioral ecology of commensal long-tailed macaque (*Macaca fascicularis*)*
446 *populations in Bali (Indonesia): Impact of anthropic factors*. PhD Dissertation,
447 University of Liege, Liege, Belgium, 281 pp.

448 Brotcorne F, Fuentes A, Wandia IN, Beudels-Jamar RC, Huynen MC. 2015. Changes in activity
449 patterns and intergroup relationships after a significant mortality event in commensal
450 long-tailed macaques (*Macaca fascicularis*) in Bali, Indonesia. *Int J Primatol* 36:548-
451 566.

452 Capitanio JP. 2012. Social processes and disease in nonhuman primates: Introduction to the
453 special section. *Am J Primatol* 74:491-496.

454 Causey RC, Weber JA, Emmans EE, Stephenson LA, Homola AD, Knapp KR, Crowley IF,
455 Pelletier DC, Wooley NA. 2006. The equine immune response to *Streptococcus equi*
456 subspecies *zooepidemicus* during uterine infection. *Vet J* 172:248-257.
457 Centers for Disease Control and Prevention. 2012. *Lesson 3: Measures of risk*, *Section 3:*
458 *Mortality Frequency Measures*, www.cdc.gov/csels/dsepd/ss1978/lesson3/section3.html.
459 Accessed March 2025.
460 Chalker VJ, Brooks HW, Brownlie J. 2003. The association of *Streptococcus equi subsp.*
461 *zooepidemicus* with canine infectious respiratory disease. *Vet Microbiol* 95:149-156.
462 Chi F, Leider M, Leendertz F, Bergmann C, Boesch C, Schenk S, Pauli G, Ellerbrok H,
463 Hakenbeck R. 2007. New *Streptococcus pneumoniae* clones in deceased wild
464 chimpanzees. *J Bacteriol* 189:6085-6088.
465 Cordoni G, Williams A, Durham A, Florio D, Zanoni RG, La Ragione RM. 2015. Rapid
466 diagnosis of strangles (*Streptococcus equi* subspecies *equi*) using PCR. *Res Vet Sci*
467 102:162-166.
468 Costa MO, Lage B. 2020. *Streptococcus equi* subspecies *zooepidemicus* and sudden deaths in
469 swine, Canada. *Emerg Infect Dis* 26:2522–2524.
470 Costa MO, Harding JCS, Huang Y, Nosach R. 2022. *Streptococcus equi* subsp. *zooepidemicus*
471 infection of pigs leads to shedding in faeces and a carrier state. *Transbound Emerg Dis*
472 69:e1503-e1509.
473 Côté IM, Poulin R. 1995. Parasitism and group size in social animals: A meta-analysis. *Behav*
474 *Ecol* 6:159-165.
475 Cowlishaw G, Dunbar RIM. 2000. *Primate conservation biology*. University of Chicago Press,
476 Chicago, Illinois, 498 pp.

de Lemos GAA, Pires BG, Mainardi RM, Chideroli RT, Pereira UP, Bracarense APFRL. 2021. Spontaneous outbreak of *Yersinia enterocolitica* infection and co-infection with *Escherichia coli* in black-tufted marmosets (*Callithrix penicillata*). *Braz J Vet Pathol* 14:173-179.

de Thier Nagelmackers F. 2021. *Refining the matrix population model of Balinese macaques under birth control: Current outcomes and future projections.* Masters thesis, Catholic University of Louvain, Louvain, Belgium, 89 pp.

Devaux CA, Mediannikov O, Medkour H, Raoult D. 2019. Infectious disease risk across the growing human-non human primate interface: A review of the evidence. *Front Public Health* 7:305.

Didier PJ, Kondova-Perseng II. 2024. Bacterial diseases in nonhuman primates. In: *Atlas of diagnostic pathology in nonhuman primates,* Kondova-Perseng II, Mansfield KG, Miller AD, editors. Springer International Publishing, Cham, Switzerland, pp.41-90.

Duboscq J, Romano V, Sueur C, MacIntosh AJ. 2016. Network centrality and seasonality interact to predict lice load in a social primate. *Sci Rep* 6:22095.

Eamudomkarn C, Sithithaworn P, Kamamia C, Yakovleva A, Sithithaworn J, Kaewkes S, Techasen A, Loilome W, Yongvanit P, et al. 2018. Diagnostic performance of urinary IgG antibody detection: A novel approach for population screening of strongyloidiasis. *PLoS One* 13:e0192598.

Eyre DW, Kenkre JS, Bowler IC, McBride SJ. 2010. *Streptococcus equi* subspecies *zooepidemicus* meningitis – a case report and review of the literature. *Eur J Clin Microbiol Infect Dis* 29:1459-1463.

499 Farber DL, Netea MG, Radbruch A, Rajewsky K, Zinkernagel RM. 2016. Immunological
500 memory: lessons from the past and a look to the future. *Nat Rev Immunol* 16:124-128.
501 Fuentes A. 2006. Human culture and monkey behavior: Assessing the contexts of potential
502 pathogen transmission between macaques and humans. *Am J Primatol* 68:880-896.
503 Fulde M, Valentin-Weigand P. 2012. Epidemiology and pathogenicity of zoonotic streptococci.
504 *Host-pathogen interactions in streptococcal diseases*, Chhatwal GS, editor, Springer,
505 Cham, Switzerland, pp. 49-81.
506 Garcia MA, Bouley DM, Larson MJ, Lifland B, Moorhead R, Simkins MD, Borie DC, Tolwani
507 R, Otto G. 2004. Outbreak of *Mycobacterium bovis* in a conditioned colony of rhesus
508 (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques. *Comp Med* 54:578-
509 584.
510 Garmyn A, Van de Velde N, Braeckmans D, Ronsmans S, Boyen F, Verlinden M. 2020. An
511 outbreak associated with *Streptococcus equi* subsp. *zooepidemicus* in layers: Evidence of
512 fecal transmission. *Avian Dis* 64:343-346.
513 Gillespie TR, Nunn CL, Leendertz FH. 2008. Integrative approaches to the study of primate
514 infectious disease: Implications for biodiversity conservation and global health. *Am J*
515 *Phys Anthropol* 137(Suppl47):53-69.
516 Giraud G. 2023. *Socio-behavioral implications of sterilization: Investigating changes in social*
517 *dynamics of free-ranging long-tailed macaques* (Macaca fascicularis) *under fertility*
518 *control*. PhD Dissertation, University of Liege, Liege, Belgium, 134 pp.
519 Gong W, Yang Y, Luo Y, Li N, Bai X, Liu Y, Zhang J, Chen M, Zhang C, Wu X. 2017. An alert
520 of *Mycobacterium tuberculosis* infection of rhesus macaques in a wild zoo in China. *Exp*
521 *Anim* 66:357-365.

522 Grassotti TT, Zvoboda DA, Costa LFX, Christiano FDP, Mann MB, Wagner PGC, Campos
523 AAS, Frazzon J, Frazzon APG. 2021. Intra and inter-monkey transmission of bacteria in
524 wild black capuchins monkeys (*Sapajus nigritus*): A preliminary study. *Braz J Biol*
525 82:e237460.
526 Griffin RH, Nunn CL. 2012. Community structure and the spread of infectious disease in primate
527 social networks. *Evol Ecol* 26:779-800.
528 Grubaugh ND, Sharma S, Krajacich BJ, Fakoli LS III, Bolay FK, Diclaro JW II, Johnson WE,
529 Ebel GD, Foy BD, Brackney DE. 2015. Xenosurveillance: A novel mosquito-based
530 approach for examining the human-pathogen landscape. *PLoS Negl Trop Dis*
531 9:e0003628.
532 Gruszynski K, Young A, Levine SJ, Garvin JP, Brown S, Turner L, Fritzinger A, Gertz RE Jr,
533 Murphy JM, et al. 2015. *Streptococcus equi* subsp. *zooepidemicus* infections associated
534 with guinea pigs. *Emerg Infect Dis* 21:156-158.
535 Hansen MF, Ang A, Trinh TTH, Sy E Paramasivam S, Dimalibot J, Jones-Engel L, Ruppert N,
536 Griffioen C, et al. 2022. *Macaca fascicularis* (amended version of 2022 assessment). The
537 IUCN Red List of Threatened Species.
538 Harper KN, Fyumagwa RD, Hoare R, Wambura PN, Coppenhaver DH, Sapolsky RM, Alberts
539 SC, Tung J, Rogers J, et al. 2012. *Treponema pallidum* infection in the wild baboons of
540 East Africa: Distribution and genetic characterization of the strains responsible. *PLoS*
541 *One* 7:e50882.
542 Harper KN, Knauf S. 2013. *Treponema pallidum* infection in primates: Clinical manifestations,
543 epidemiology, and evolution of a stealthy pathogen. In: *Primates, pathogens, and*
544 *evolution*, Brinkworth JF, Pechenkina K, editors. Springer, New York, pp. 189-219.

545 Hoffmann C, Zimmermann F, Biek R, Kuehl H, Nowak K, Mundry R, Agbor A, Angedakin S,
546 Arandjelovic M, et al. 2017. Persistent anthrax as a major driver of wildlife mortality in a
547 tropical rainforest. *Nature* 548:82-86.
548 Hopkins ME, Nunn CL. 2007. A global gap analysis of infectious agents in wild primates.
549 *Divers Distrib* 13:561-572.
550 Howard CR, Fletcher NF. 2012. Emerging virus diseases: Can we ever expect the unexpected?
551 *Emerg Microbes Infect* 1:e46.
552 Howells ME, Loudon JE, Brotcorne F, Petterson JV, Wandia IN, Putra IA, Fuentes A. 2022.
553 Primates and primatologists: Reflecting on two decades of primatological and
554 ethnoprimatological research, tourism, and conservation at the Ubud Monkey Forest. In:
555 *Ecotourism and Indonesia's primates*, Gursky SL, Supriatna J, Achorn A, editors.
556 Springer, Cham, Switzerland, pp. 153-178.
557 Howie JM, Pomiankowski A. 2016. Female sneak copulation. In: *Encyclopedia of Evolutionary*
558 *Psychological Science*, Weekes-Shackelford V, Shackelford T, Weekes-Shackelford V,
559 editors. Springer, Cham, Switzerland, pp. 3036-3040.
560 Johansen MD, Irving A, Montagutelli X, Tate MD, Rudloff I, Nold MF, Hansbro NG, Kim RY,
561 Donovan C, et al. 2020. Animal and translational models of SARS-CoV-2 infection and
562 COVID-19. *Mucosal Immunol* 13:877-891.
563 Jones EE, Alford PL, Reingold AL, Russell H, Keeling ME, Broome CV. 1984. Predisposition to
564 invasive pneumococcal illness following parainfluenza type 3 virus infection in
565 chimpanzees. *J Am Vet Med Assoc* 185:1351-1353.
566 Jovanović M, Stevanović G, Tošić T, Stošović B, Zervos MJ. 2008. *Streptococcus equi* subsp.
567 *zooepidemicus* meningitis. *J Med Microbiol* 57:373-375.

568 Knauf S, Gogarten JF, Schuenemann VJ, De Nys HM, Düx A, Strouhal M, Mikalová L, Bos KI,
569 Armstrong R, et al. 2018. Nonhuman primates across sub-Saharan Africa are infected
570 with the yaws bacterium *Treponema pallidum* subsp. *pertenue*. *Emerg Microbes Infect*
571 7:157.
572 Köndgen S, Calvignac-Spencer S, Grützmacher K, Keil V, Mätz-Rensing K, Nowak K, Metzger
573 S, Kiyang J, Lübke-Becker A, et al. 2017. Evidence for human *Streptococcus*
574 *pneumoniae* in wild and captive chimpanzees: A potential threat to wild populations. *Sci*
575 *Rep* 7:14581.
576 Köndgen S, Kühl H, N'Goran PK, Walsh PD, Schenk S, Ernst N, Biek R, Formenty P, Mätz-
577 Rensing K, et al. 2008. Pandemic human viruses cause decline of endangered great apes.
578 *Curr Biol* 18:260-264.
579 Kramer-Schadt S, Fernández N, Thulke HH. 2007. Potential ecological and epidemiological
580 factors affecting the persistence of classical swine fever in wild boar *Sus scrofa*
581 populations. *Mammal Rev* 37:1–20.
582 Kuusi M, Lahti E, Virolainen A, Hatakka M, Vuento R, Rantala L, Vuopio-Varkila J, Seuna E,
583 Karppelin M, et al. 2006. An outbreak of *Streptococcus equi* subspecies *zooepidemicus*
584 associated with consumption of fresh goat cheese. *BMC Infect Dis* 6:36.
585 Lappan S, Malaivijitnond S, Radhakrishna S, Riley EP, Ruppert N. 2020. The human–primate
586 interface in the New Normal: Challenges and opportunities for primatologists in the
587 COVID-19 era and beyond. *Am J Primatol* 82:e23176.
588 Lawler RR. 2011. Demographic concepts and research pertaining to the study of wild primate
589 populations. *Am J Phys Anthropol* 146:63-85.

590 Leendertz FH, Ellerbrok H, Boesch C, Couacy-Hymann E, Mätz-Rensing K, Hakenbeck R,
591 Bergmann C, Abaza P, Junglen S, et al. 2004. Anthrax kills wild chimpanzees in a
592 tropical rainforest. *Nature* 430:451-452.
593 Leendertz FH, Pauli G, Maetz-Rensing K, Boardman W, Nunn C, Ellerbrok H, Jensen SA,
594 Junglen S, Boesch C. 2006. Pathogens as drivers of population declines: The importance
595 of systematic monitoring in great apes and other threatened mammals. *Biol Conserv*
596 131:325-337.
597 Lowenstine LJ, Osborn KG. 2012. Respiratory system diseases of nonhuman primates. In:
598 *Nonhuman Primates in Biomededical Research*, 2nd Ed., Abee CR, Mansfield K, Tardif
599 S, Morris T, editors. Academic Press, London, UK, pp. 413-481.
600 Lyashchenko KP, Sikar-Gang A, Sridhara AA, Johnathan-Lee A, Elahi R, Greenwald R,
601 Lambotte P, Esfandiari J, Roos EO, et al. 2021. Use of blood matrices and alternative
602 biological fluids for antibody detection in animal tuberculosis. V*et Immunol*
603 *Immunopathol* 239:110303.
604 MacIntosh AJJ, Jacobs A, Garcia C, Shimizu K, Mouri K, Huffman MA, Hernandez AD. 2012.
605 Monkeys in the middle: Parasite transmission through the social network of a wild
606 primate. *PLoS One* 7:e51144.
607 Mätz-Rensing K, Hartmann T, Wendel GM, Frick JS, Homolka S, Richter E, Munk MH, Kaup
608 FJ. 2015. Outbreak of tuberculosis in a colony of rhesus monkeys (*Macaca mulatta*) after
609 possible indirect contact with a human TB patient. *J Comp Pathol* 153:81-91.
610 Mätz-Rensing K, Winkelmann J, Becker T, Burckhardt I, van der Linden M, Köndgen S,
611 Leendertz F, Kaup FJ. 2009. Outbreak of *Streptococcus equi* subsp. *zooepidemicus*
612 infection in a group of rhesus monkeys (*Macaca mulatta*). *J Med Primatol* 38:328-334.

613 Meesawat S, Warit S, Hamada Y, Malaivijitnond S. 2023. Prevalence of *Mycobacterium*
614 *tuberculosis* complex among wild rhesus macaques and 2 subspecies of long-tailed
615 macaques, Thailand, 2018–2022. *Emerg Infect Dis* 29:551-560.
616 Mohandas S, Balan S, Mourya DT. 2022. Urinary immunoglobulins in viral diagnosis: An
617 overview. *Indian J Med Res* 155:11-21.
618 Napit R, Manandhar P, Poudel A, Rajbhandari PG, Watson S, Shakya S, Pradhan SM, Sharma
619 AN, Chaudhary A, et al. 2023. Novel strains of *Campylobacter* cause diarrheal outbreak
620 in rhesus macaques (*Macaca mulatta*) of Kathmandu Valley. *PLoS One* 18:e0270778
621 Newton JR, Laxton R, Wood JLN, Chanter N. 2008. Molecular epidemiology of *Streptococcus*
622 *zooepidemicus* infection in naturally occurring equine respiratory disease. *Vet J* 175:338-
623 345.
624 Nunn C, Altizer S. 2006. *Infectious diseases in primates: Behavior, ecology and evolution*.
625 Oxford University Press, Oxford, UK, 384 pp.
626 Nunn CL, Gillespie TR. 2016. Infectious disease and primate conservation. In: *An introduction*
627 *to primate conservation*, Wich SA, Marshall AJ, editors. Oxford University Press,
628 Oxford, UK, pp. 157-173.
629 Pal A, Kumara HN, Velankar AD, Mishra PS, Singh M. 2018. Demography and birth seasonality
630 in the Nicobar long-tailed macaque (*Macaca fascicularis umbrosus*). *Curr Sci* 114:1732-
631 1737.
632 Palmiere C, Egger C, Prod'Hom G, Greub G. 2016. Bacterial translocation and sample
633 contamination in postmortem microbiological analyses. *J Forensic Sci* 61:367-374

634 Patouillat L, Hambuckers A, Adi Subrata S, Garigliany M, Brotcorne F. 2024. Zoonotic
635 pathogens in wild Asian primates: A systematic review highlighting research gaps. *Front*
636 *Vet Sci* 11:1386180
637 Pereira ME, Altmann J. 1985. Development of social behavior in free-living nonhuman primates.
638 In: *Nonhuman primate models for human growth and development*, Watts ES, editor.
639 Alan R Liss, New York, pp. 217-309.
640 Pelkonen S, Lindahl SB, Suomala P, Karhukorpi J, Vuorinen S, Koivula I, Väisänen T,
641 Pentikäinen J, Autio T, Tuuminen T. 2013. Transmission of *Streptococcus equi*
642 subspecies *zooepidemicus* infection from horses to humans. *Emerg Infect Dis* 19:1041-
643 1048.
644 Plowright RK, Manlove K, Cassirer EF, Cross PC, Besser TE, Hudson PJ. 2013. Use of exposure
645 history to identify patterns of immunity to pneumonia in bighorn sheep (*Ovis*
646 *canadensis*). *PLoS One* 8:e61919.
647 Priestnall S, Erles K. 2011. *Streptococcus zooepidemicus*: An emerging canine pathogen. *Vet J*
648 188:142-148.
649 Rimbach R, Bisanzio D, Galvis N, Link A, Di Fiore A, Gillespie TR. 2015. Brown spider
650 monkeys (*Ateles hybridus*): A model for differentiating the role of social networks and
651 physical contact on parasite transmission dynamics. *Philos Trans R Soc B* 370:20140110.
652 Romano V, Duboscq J, Sarabian C, Thomas E, Sueur C, MacIntosh AJJ. 2016. Modeling
653 infection transmission in primate networks to predict centrality-based risk. *Am J Primatol*
654 78:767-779.
655 Rosa GM, Santos N, Grøndahl-Rosado R, Fonseca FP, Tavares L, Neto I, Cartaxeiro C, Duarte
656 A. 2020. Unveiling patterns of viral pathogen infection in free-ranging carnivores of

northern Portugal using a complementary methodological approach. *Comp Immunol Microbiol Infect Dis* 69:101432.

Salasia SIO, Wibawan IWT, Pasaribu FH, Abdulmawjood A, Lammler C. 2004. Persistent occurrence of a single *Streptococcus equi* subsp. *zooepidemicus* clone in the pig and monkey population in Indonesia. *J Vet Sci* 5:263-265.

Sapolsky RM, Share LJ. 2004. A pacific culture among wild baboons: Its emergence and transmission. *PLoS Biol* 2:e106

Schiller CA, Wolff MJ, Munson L, Montali RJ. 1989. *Streptococcus zooepidemicus* infections of possible horsemeat source in red-bellied tamarins and Goeldi's monkeys. *J Zoo Wildl Med* 20:322-327.

Soedarmanto I, Pasaribu FH, Wibawan IW, Lämmler C. 1996. Identification and molecular characterization of serological group C streptococci isolated from diseased pigs and monkeys in Indonesia. *J Clin Microbiol* 34:2201-2204.

Springer A, Mellmann A, Fichtel C, Kappeler PM. 2016. Social structure and *Escherichia coli* sharing in a group-living wild primate, Verreaux's sifaka. *BMC Ecol* 16:6.

Sweeney CR, Timoney JF, Newton JR, Hines MT. 2005. *Streptococcus equi* infections in horses: Guidelines for treatment, control, and prevention of strangles. *J Vet Intern Med* 19:123-134.

Szentiks CA, Köndgen S, Silinski S, Speck S, Leendertz FH. 2009. Lethal pneumonia in a captive juvenile chimpanzee (*Pan troglodytes*) due to human-transmitted human respiratory syncytial virus (HRSV) and infection with *Streptococcus pneumoniae*. *J Med Primatol* 38:236-240.

679 Tang B, Liang H, Gao X, Yan L, Deng M, Zhai P, Yang H, Wei Z. 2019. Identification of a
680 surface protective antigen, MAP of *Streptococcus equi* subspecies *zooepidemicus*. *Res*
681 *Vet Sci* 124:387-392.
682 Unwin S, Chatterton J, Chantrey J. 2013. Management of severe respiratory tract disease caused
683 by human respiratory syncytial virus and *Streptococcus pneumoniae* in captive
684 chimpanzees (*Pan troglodytes*). *J Zoo Wildl Med* 44:105-115.
685 Van Schaik CP. 1989. The ecology of social relationships amongst female primates. In:
686 *Comparative socioecology: The behavioural ecology of humans and other mammals*,
687 Standen V, Foley RA, editors. Blackwell, Oxford, UK, pp. 195-218.
688 Wei Z, Fu Q, Chen Y, Li M, Cong P, Mo D, Liu X. 2013. *Streptococcus equi* ssp. *zooepidemicus*
689 C5a peptidase, a putative invasin, induces protective immune response in mice. *Res Vet*
690 *Sci* 95:444-450.
691 Wilbur AK, Engel GA, Rompis A, Putra IGAA, Lee BPYH, Aggimarangsee N, Chalise M,
692 Shaw E, Oh G, et al. 2012. From the mouths of monkeys: Detection of *Mycobacterium*
693 *tuberculosis* complex DNA from buccal swabs of synanthropic macaques. *Am J Primatol*
694 4:676-686.
695 Wrangham RW. 1980. An ecological model of female-bonded primate groups. *Behavior* 75:262-
696 300.
697

Table 1. Mortality rates per group (affected groups are italicized) during the spring 2022 epizootic event in long-tailed macaques (*Macaca fascicularis*) inhabiting an urban mosaic habitat in central Bali. Mortality rates with 95% confidence intervals (CI) were calculated based on the number of carcasses found in each group relative to the pre-outbreak group size in 2021.

Macaque groups	**Census 2021**	**Census 2022**	**Number of carcasses**	**Mortality rate (%)**	**95% CI for mortality rate**
Temple	193	105	52	27	21-34
Selatan	178	67	94	53	45-60
New Forest	180	141	18	10	6-15
Central	232	259	0	0	0-0
East	159	158	0	0	0-0
Michelin	169	161	0	0	0-0
Utara	41	NA	0	0	0-0
Cemetery	71	NA	0	0	0-0
Atap	37	NA	0	0	0-0

Table 2. Mortality rates per age-sex class in affected groups during the epizootic event in spring 2022 in long-tailed macaques (*Macaca fascicularis*) inhabiting an urban mosaic habitat in central Bali. Mortality rates (with 95% confidence intervals, CI) were calculated based on the number of carcasses discovered across the three affected groups, relative to the age-sex class size in 2021, before the epizootic.

Age-Sex classes	**Census 2021**	**Census 2022**	**Number of carcasses**	**Mortality rates (%)**	**95% CI for mortality rates**
Adult male	60	44	21	35	24-48
Adult female	211	101	75	36	29-42
Immature	207	120	53	26	20-32
Infant	73	48	12	16	10-27
Unknown	0	0	9	0	0-0

Table 3. Results of SARS-CoV-2 RT-PCR and *Streptococcus equi zooepidemicus* (SEZ) quantitative PCR analyses of lung and blood samples from four long-tailed macaques (*Macaca fascicularis*) autopsied during the epizootic event in 2022 in an urban mosaic habitat in central Bali.

Case	SARS-CoV-2 RT-PCR (lung RNA)	SEZ qPCR Ct[a] value (blood DNA)	SEZ qPCR Ct value (lung DNA)	SEZ interpretation
Case 1	Negative	30.3	33.2	Positive
Case 2	Negative	29.6	NT	Positive
Case 3	Negative	NT[b]	34.4	Positive
Case 4	Negative	NT	31.9	Positive

[a]Ct= cycle threshold

[b]NT=not tested.

Figure 1. Land cover map of the study site (central Bali), showing the preliminary spatial distribution of the nine social groups of long-tailed macaques (*Macaca fascicularis*), based on a visual estimation made during group encounters throughout the study period. The outlined ellipses in the southwestern area correspond to the zones occupied by (i.e. known ranges) of the three groups affected by the 2022 epizootic. [Alt-Text: Map of the monkey forest study site in central Bali showing nine macaque groups, with the three groups affected by the 2022 outbreak highlighted in the southwest.]

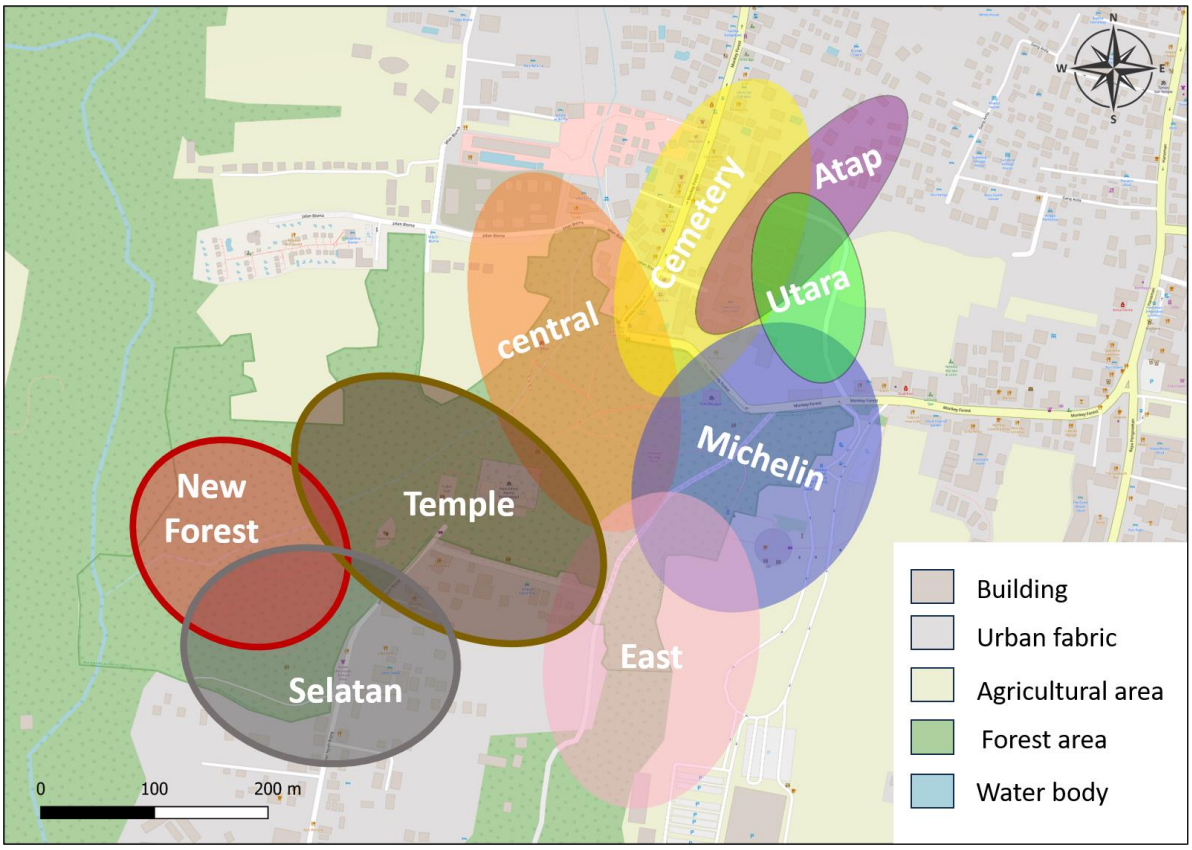

Figure 2. Number of carcasses of long-tailed macaques (*Macaca fascicularis*) found per day in each infected group within inhabiting an urban mosaic habitat in central Bali, and the cumulative death curve in the population from 5 March to 10 May 10 2022. [Alt-Text: Bar chart showing daily deaths in three macaque groups and a line indicating cumulative mortality during the 2022 outbreak in a monkey forest, central Bali]

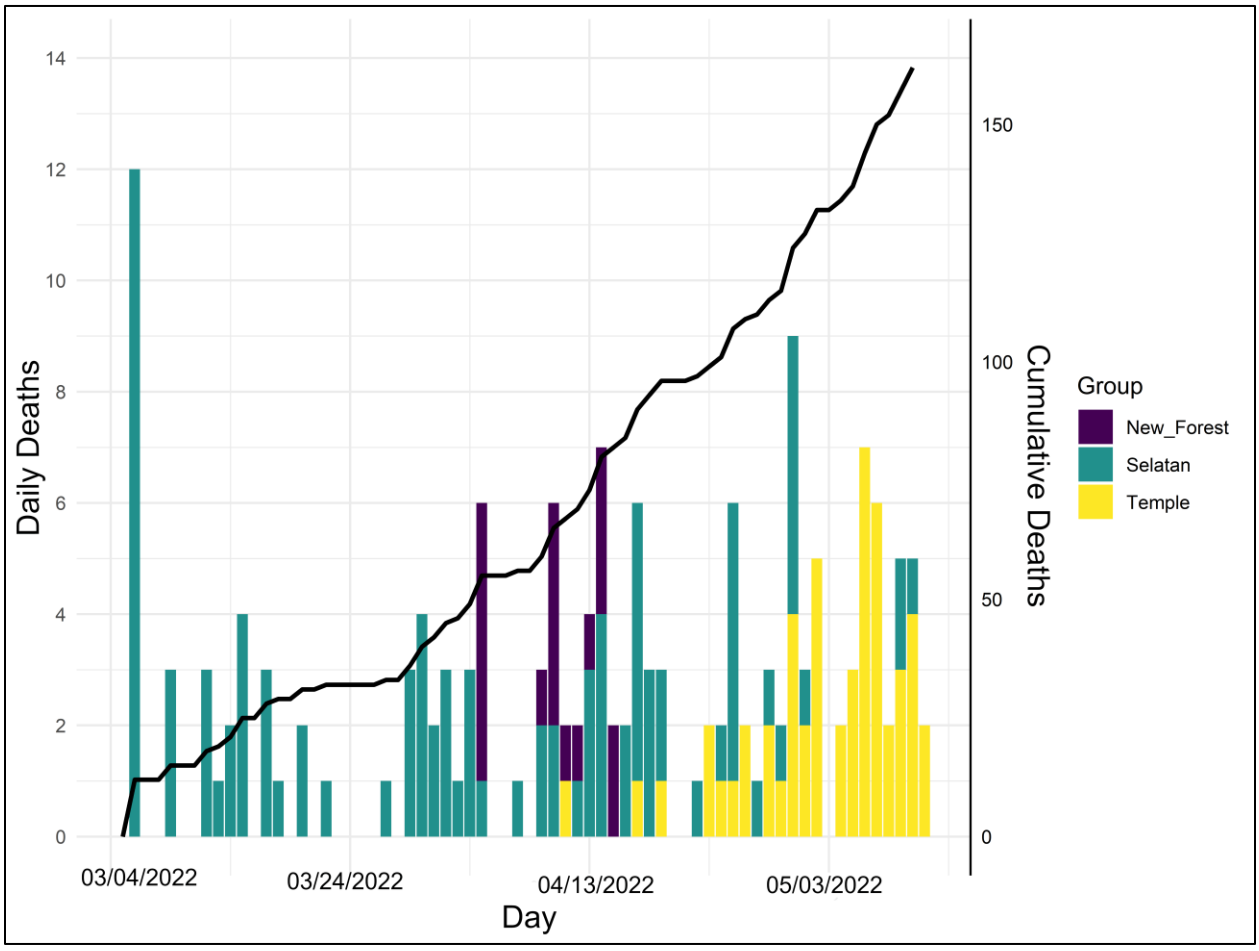

Figure 3. Demographic trends of six long-tailed macaque (*Macaca fascicularis*) groups in the study population in an urban mosaic habitat in central Bali from 2021-22, based on the abundance in 2021 (pre-outbreak period) and 2022 (post-outbreak period). Trends are presented for each age-sex class (AM = adult male; AF = adult female; IMA = immature), showing absolute numbers of individuals and percentage changes between 2021 and 2022. Groups affected by the epizootic are highlighted in blue, and unaffected groups are presented in grey (the remaining three unaffected groups of the population are not represented due to the absence of demographic data in 2022). [Alt-Text: Dot plots showing changes in age-sex classes and group sizes for six macaque groups in a monkey forest, central Bali, comparing 2021 and 2022; sharp declines are seen in outbreak-affected groups.]

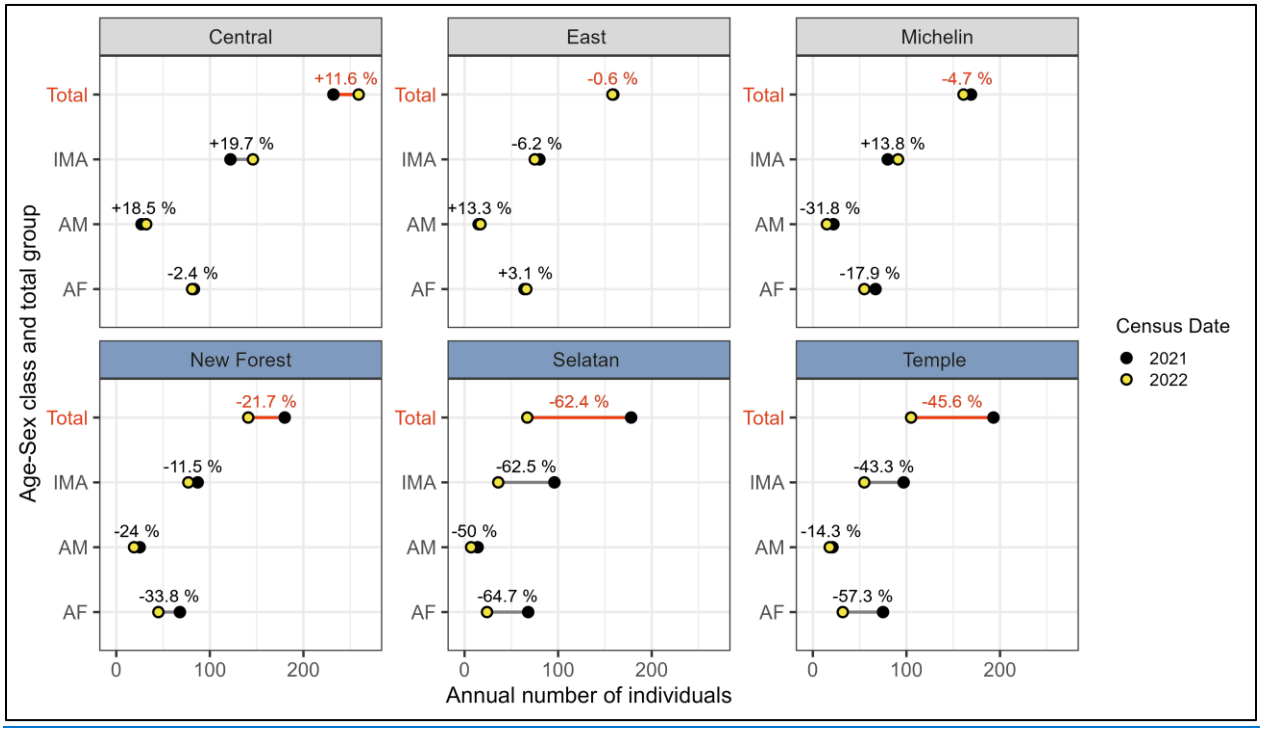

Figure 4. Relative abundance of the most prevalent bacterial species in the blood (Case 2) and lung tissue samples (Case 1, 3, and 4) of long-tailed macaques (*Macaca fascicularis*) found dead during an in an urban mosaic habitat in central Bali, March to May 2022, based on metabarcoding 16S rRNA analysis of samples. [Alt-Text: Stacked bar chart showing bacterial composition in four macaque samples; *Streptococcus equi* dominates most cases.]

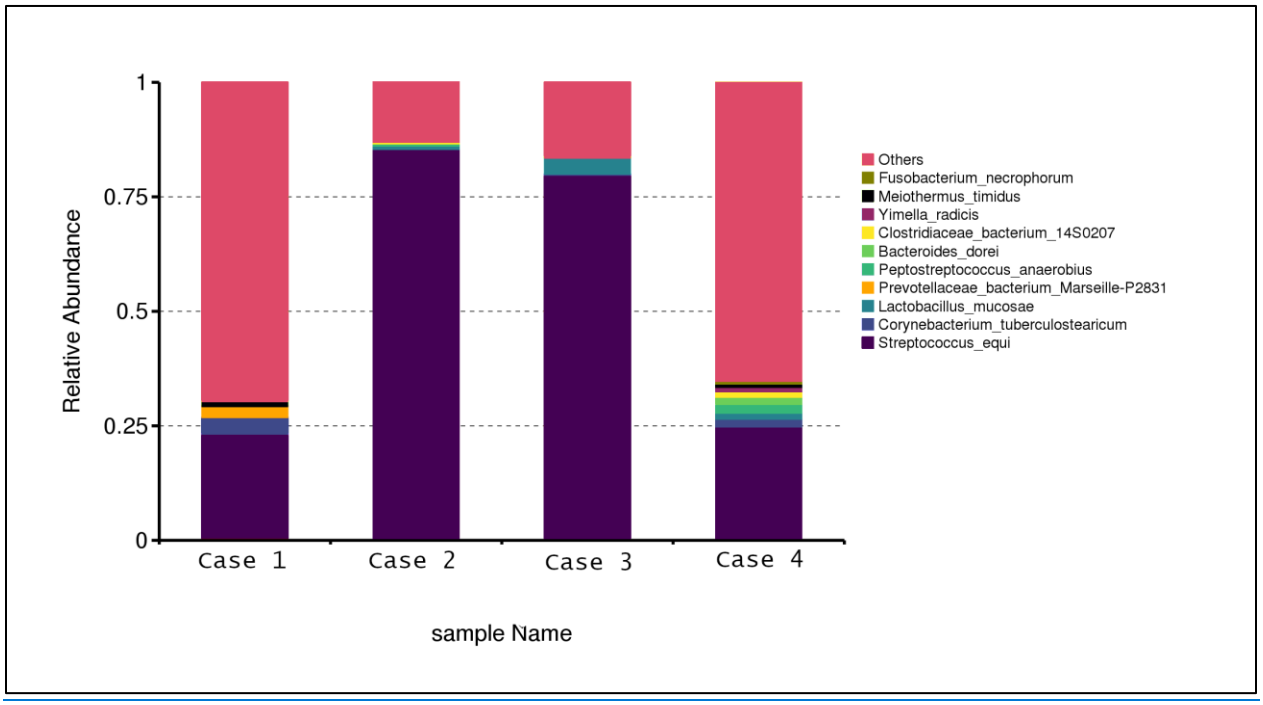

Figure 5. Schematic representation of potential transmission routes of *Streptococcus equi subsp. zooepidemicus* (SEZ) during the 2022 epizootic outbreak affecting Balinese long-tailed macaques (*Macaca fascicularis*) in an urban mosaic habitat in central Bali [Alt-Text: Diagram showing possible transmission routes of *Streptococcus equi zooepidemicus* during the 2022 outbreak, including social contact, contaminated environment, and potential human or animal reservoirs.]