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Short title: Dual infection dynamics in a bat maternity

Summary

Bats are reservoirs for several zoonotic pathogens of medical importance; however infection dynamics of pathogens in wild bat populations remain poorly understood. Here, we examine the influence of host crowding and population age structure on pathogen transmission and diversity in bat populations. Focusing on two pathogen taxa of medical importance, *Leptospira* bacteria and paramyxoviruses, we monitored host population and pathogen shedding dynamics within a maternity colony of the tropical bat species *Mormopterus francoismoutoui*, endemic to Réunion Island. Our data reveal astonishingly similar infection dynamics for *Leptospira* and paramyxoviruses, with infection peaks during late-pregnancy and two-months after the initial birth pulse. Furthermore, though co-infection occurs frequently during the peaks of transmission, the patterns do not suggest any interaction between the two pathogens. Partial sequencing reveals a unique bat-specific *Leptospira* strain contrasting with the co-circulation of four separate Paramyxovirus lineages along the whole breeding period. Patterns of infection highlight the importance of host crowding in pathogen transmission and suggest that most bats developed immune response and stop excreting pathogens. Our results support that bat maternity colonies may represent hotspots of transmission for bacterial and viral infectious agents and highlight how seasonality can be an important determinant of host-parasite interactions and disease emergence.

Introduction

Seasonal changes can have important effects on infectious disease dynamics (Altizer et al., 2006). Of the many driving factors of infection that demonstrate seasonal cycles, annual reproduction has been identified as having a significant impact on the dynamics of various host-parasite interactions, particularly in animal species that are gregarious during the reproductive season (Altizer et al., 2006). Indeed, during this period, aggregation within colony, increased metabolic activity and the highly synchronized birth of newborns (i.e. birth pulse) lead to increased numbers of susceptible hosts and hence boost the potential for transmission of infectious agents (e.g. Hosseini *et al.* 2004). Investigating the infection dynamics during host breeding seasons may therefore help to better identify major mechanisms of pathogen maintenance within wild animal populations.

Bats play important ecological roles in prey and predator cycles, arthropod suppression, seed dispersal, pollination, as well as nutrient distribution and recycling (Kunz et al., 2011). Additionally, bats have been identified as natural reservoirs of many microbial agents, including pathogens of threat to human health (Wibbelt et al., 2010). Their ability to enter torpor or hibernation, the large sizes of their gregarious groups and possibly some peculiarities in their immune system, have been suggested to facilitate maintenance of infections in these unique long-lived flying mammals (Calisher et al., 2006). The role of seasonal breeding in bat infection dynamics has been the subject of several recent studies (Amengual et al., 2007; Turmelle et al., 2010; Drexler et al., 2011; Field et al., 2011; Mühldorfer et al., 2011), mostly focusing on viruses. For example, lyssaviruses and henipaviruses have been observed to exhibit robust and specific amplification in maternity colonies (Drexler et al., 2011; George et al., 2011). Furthermore, although multi-pathogen interactions have been shown to drive infection dynamics in other host-parasite models (e.g. Telfer *et al.* 2010), very few studies have considered the dynamics of multiple infections in

natural bat populations (Drexler et al., 2011; Mühldorfer et al., 2011).

Leptospirosis is considered as the most common bacterial zoonosis in the world and is caused by pathogenic spirochetes of the genus *Leptospira* (Adler and de la Peña Moctezuma, 2010). The incidence of human leptospirosis is high in tropical islands, particularly in the Indian Ocean Islands where some of the highest human incidences have been reported to date (Pappas et al., 2008). Chronic carrier animals are characterized by persistent leptospire colonization of renal tubules, which are then shed for months or years in urine and contaminate the environment. Human infection occurs either directly from contact with animal reservoirs or indirectly from contaminated soil or water (Adler and de la Peña Moctezuma, 2010). Rodents are classically recognized as the most significant reservoirs for the maintenance and dissemination of leptospires worldwide (Bharti et al., 2003). More recently, bats have also been identified as potential reservoirs of leptospires in a number of locations (e.g. Matthias *et al.* 2005; Tulsiani *et al.* 2011), with sometimes very high infection rates (Lagadec et al., 2012). Mathematical modeling has suggested seasonality of host reproduction as an important factor affecting *Leptospira* infection dynamics in rodent populations (Holt et al., 2006), however, no published information is currently available concerning *Leptospira* infection dynamics and maintenance in bat populations.

The *Paramyxoviridae* form a large virals family including the causative agents of many human and animal diseases, and some that have recently been linked to emerging and re-emerging epidemics (e.g. Hendra and Nipah viruses; Aguilar & Lee, 2011). Rodents and bats have been recently shown to host a broad spectrum of paramyxoviruses (Drexler et al., 2012; Wilkinson et al., 2012; Baker, Todd, et al., 2013). Phylogenetic reconstruction of host/virus associations suggests frequent spillover events between different hosts, with a predominance of host shifts from bats to other animal species (Drexler et al., 2012; Wilkinson et al., 2014), which has elsewhere been linked with the bat reproduction period (Plowright et al., 2008). For

instance, Plowright *et al.* (2008) have identified reproduction as a key factor in the risk of Hendra virus infection in Australian fruit bats and especially in the emergence of these viruses in horse populations.

In this study, we investigated the dynamics of two pathogens of medical importance, the bacteria *Leptospira* and Paramyxoviruses, which have previously been described in insectivorous bats (Lagadec et al., 2012; Wilkinson et al., 2012). The tropical and endemic bat species *Mormopterus francoismoutoui* is the most abundant bat species on Réunion Island, and roosts in large colonies in a variety of sites such as bridges, houses, churches and caves. We chose to study the largest known cave-based maternity colony of these bats in order to (i) determine whether the infection rate and/or the genetic diversity of infectious agents are influenced by host crowding and the succession of different developmental stages (adults, newborns and juveniles) over the breeding season, and (ii) compare the temporal infection profiles of both infectious agents. We hypothesize that transmission is host-density dependent favored by colony-induced crowding and fueled by the input of immunologically naïve juveniles (Drexler et al., 2011). Results are expected to reveal specific characteristics of the ecology and evolution of *Leptospira* and paramyxoviruses and to highlight general patterns in pathogen maintenance in bat populations.

Results

Bat colony dynamics

The bat population studied was strictly composed of adults at the beginning of the survey in late November (Fig. 1a). A large number of newborns were observed in early January, together with the first juveniles (~3 weeks old), indicating that the birth pulse occurred from mid-December to mid-January. We estimated that the parturition period lasted up to mid-February as the last observation of newborns was made on February 8th.

Over all outings, a total of 2652 urine droplets were counted on the 16 plastic trays. The spatial distribution of the urine droplets suggested that bats use a main route for exiting the cave: those trays facing the center of the entrance were more exposed to bat passage than the ones placed on each side of the main flyway ($\chi^2_{15} = 49.26$, $p < 0.001$; model 1 in Table S3). Trays lying directly underneath the main bat flyway (yellow-red in Fig. S1b) showed statistically significant variations in the number of urine droplets collected over time ($\chi^2_7 = 48.43$, $p < 0.001$; Fig. 1b and model 1 in Table S3). Indeed, at the first sampling session (i.e. November 20th), the number of exiting adult bats reached a maximum: 46 500 individuals as estimated by the video. Following this maximum, the number of exiting bats declined drastically to reach a minimum on January and remained very low for about three additional weeks. This corresponds to the parturition period where females gave birth and thus remained with newborns leading to this reduced pattern of exit. Then, the number of exiting bats increased to reach a second peak on mid-February, coinciding with the first flights of juvenile bats. Beyond that time point, the number of exiting bats declined progressively towards the end of the season corresponding to the definitive emptying of the cave; the number of exiting bats estimated by video analysis was still 13 400 individuals at the end of the sampling period (i.e. March 21st). In late May, a last visual inspection showed that the cave was unoccupied.

Leptospira and paramyxovirus infection dynamics

Altogether, 420 individual urine droplets were screened by PCR for *Leptospira* and paramyxoviruses during the 2012-2013 breeding season. The bacterial and viral excretion positively identified individual bats as being currently infected with either of the two infectious agents. Hereafter, 'infection rate' refers to the proportion of PCR-positive samples. Both *Leptospira* and paramyxovirus infection rates varied significantly over the breeding season (*Leptospira*: $\chi^2_7 = 25.209$, $p < 0.001$; paramyxovirus: $\chi^2_7 = 59.879$, $p < 0.001$, models 2

and 3 in Table S3) and revealed similar patterns of temporal variation (Fig. 1c). The relative intensity of *Leptospira* excretion also showed significant variation over time, despite the large variation in the Ct values ($\chi^2_7 = 148.06$, $p = 0.003$, fig. 1d and model 4 in Table S3). Overall, infection dynamics were characterized by two peaks, both in the rates of infection (Fig. 1c) and the intensity of *Leptospira* excretion (Fig. 1d). Notably, the first peak occurred at the beginning of the breeding season, during the late-pregnancy period of females, with infection rates (±95% confidence interval) reaching 45% (±12%) and 61% (±11%) for *Leptospira* and paramyxovirus, respectively. Later, during the parturition period, both infection rates (as well as the intensity of *Leptospira* excretion) decreased drastically down to a minimum of 6% (±6%) and 4% (±5%) for *Leptospira* and paramyxovirus respectively (Table S4). The second infection peak (as well as the intensity of *Leptospira* excretion) occurred roughly two months after the beginning of the parturition period. Towards the end of the season, infection rates slightly decreased, with *Leptospira* and paramyxovirus detection rates reaching 21% (±11%) and 31% (±13%), respectively. Infection rates were also monitored at the start of the following season in order to collect data on the early stages of colony formation. Infection rates were 28% (±16%) and 21% (±16%) on October 18th 2013 for *Leptospira* and paramyxovirus respectively, and increased within 2 weeks, to reach 62% (±18%) and 52% (±18%) respectively (Table S4).

Our data showed that bats were frequently co-infected (Fig. 2a). Statistically, the presence of one infectious agent was strongly correlated to the presence of the other agent ($\chi^2_1 = 22.170$, $p < 0.001$, models 2 and 3 in Table S3). Moreover, the rate of co-infection significantly varied over the breeding season ($\chi^2_7 = 41.761$, $p < 0.001$) and followed the same dynamics as mono-infections (Fig. 2b and model 5 in Table S3). However, the expected probability of being co-infected at random for each sampling date was not significantly different from the observed values (G-test: $p > 0.1$ for all tests; Fig. 2b). Finally, the intensity

of *Leptospira* excretion was not affected by co-infection with paramyxovirus ($\chi^2_1 = 0.020$, p = 0.957, model 4 in Table S3).

Genetic diversity of Leptospira and paramyxovirus

Phylogenetic analyses of *Leptospira* provided similar results for *secY* and *rrs2* genes (Fig. 3a and Fig. S4 respectively). Both phylogenetic trees revealed the circulation of a single haplotype in the maternity colony, closely related to *Leptospira borgpetersenii*. This haplotype was distinct from those previously described in wild animals and humans in the western Indian Ocean. In contrast, paramyxoviruses detected in the cave were much more diverse as 4 different genetic groups were observed (Fig. 3b). The first group corresponds to virus sequences associated with three different bat families of the western Indian Ocean region (Molossidae, Miniopteridae and Verspertilionidae). The second group includes virus sequences detected in the very closely related bat species *M. acetabulosus* (Mauritius) and *M. jugularis* (Madagascar). Virus sequence in the third group were initially identified in kidney tissue from a rat caught in Réunion Island near the studied cave (Wilkinson et al., 2014). Finally, one sequence grouped with viruses largely associated with rats in the region (group 4). There was no evidence of any correlation between the occurrence of the different Paramyxovirus genetic groups and sampling date (p = 0.467), suggesting that the 4 genetic groups were likely co-circulating within the colony.

Discussion

Previous studies have demonstrated that dynamics of bat-hosted infectious agents exhibit a strong seasonal pattern, with infection peaks being recorded during the breeding period (Turmelle et al., 2010; Drexler et al., 2011). Here, using a non-invasive sampling strategy in a natural maternity colony, we monitored the excretion of both a bacterial and a viral agent,

187 namely *Leptospira* and paramyxovirus, by a tropical insectivorous bat species endemic to
188 Réunion Island. Our data provide evidence that infection rates express strong temporal
189 fluctuations within the time scale of the breeding season. We find that *Leptospira* and
190 paramyxoviruses display remarkably comparable infection dynamics, a feature suggestive of
191 synchronized host susceptibility and a common transmission route within the colony, i.e.
192 urine. Data from two successive breeding seasons showed epidemic bursts occurring during
193 colony formation and two-months after the birth pulse. These findings strongly suggest the
194 occurrence of a vigorous horizontal transmission within the maternity colony and support that
195 seasonal coloniality and synchronized bat behavior is a major determinant of infection
196 dynamics at the population level.

197 Synchronized host susceptibility in females may have resulted from a transient
198 pregnancy-related depressed immunity. Increased susceptibility to infectious agents during
199 pregnancy has been demonstrated in different mammalian systems (e.g. Sheldon & Verhulst
200 1996; Cattadori *et al.* 2005) and short-term trade-offs between immune activity and
201 reproduction have been suggested to explain such patterns. In the greater mouse-eared bat
202 (*Myotis myotis*), for instance, weaker cell-mediated responses and heavier ectoparasite
203 infestations have been observed in pregnant females compared to non-reproductive females
204 from the same roost (Christe et al., 2000). Our sampling design does not give access to the
205 immunological status of the population, and thus does not allow verification of this
206 hypothesis. However, such undulating patterns of immunomodulation and viral replication in
207 females during late-pregnancy have already been reported in other field studies investigating
208 Hendra or filo-viruses dynamics in frugivorous bats (e.g. Plowright *et al.* 2008; Breed *et al.*
209 2011).

210 Following the infection burst that occurs early-on in colony formation, a rapid and
211 highly synchronous decline in *Leptospira* and paramyxovirus excretion was observed,

212 suggesting that a large majority of adult bats rapidly control the acute infection and that
213 excretion of infectious agents is only transitory. This pattern is most likely the consequence of
214 the synchronous development of a robust immune response in bats against the two infectious
215 agents. Such an immune response would not only protect parturient females, but also
216 newborns through the passive transfer of specific antibodies from mother to offspring as
217 demonstrated experimentally in frugivorous bat species (Baker, Suu-Ire, et al., 2013; Epstein
218 et al., 2013), and largely suggested by several field studies (Plowright et al., 2008; Breed et
219 al., 2011), including one carried out on insectivorous bats (Drexler et al., 2011). Though
220 transitory, the conferred passive protection may shape age related patterns of infection
221 (Boulinier and Staszewski, 2008) and has been suggested to last between one and six months
222 after maternal severing in insectivorous (Drexler et al., 2011) and frugivorous bats (Baker,
223 Suu-Ire, et al., 2013; Epstein et al., 2013), respectively. Thus, the influx of juveniles in the
224 group of susceptible bats is delayed until the disappearance of passively transferred maternal
225 antibodies. Indeed, the two-months delay observed before appearance of the second infection
226 peak is consistent with such waning of passively transferred maternal antibodies, as
227 previously suggested by a field study on *Myotis* bats (Drexler et al., 2011). In addition to an
228 immunization of the mothers, the emergence of uninfected juveniles may contribute to the
229 prevalence decrease measured in February. However, considering that females give birth to a
230 single pup, this decrease should be at maximum of 50%, which is consistently lower than the
231 actual observed drop (i.e. xx% for *Leptospira* and xx% for paramyxovirus, respectively).
232 Thus we propose that the dramatic decrease in prevalence results from both immunization of
233 the mothers and the emergence of transiently immunized juveniles. Interestingly, some of the
234 expected positive relationship between host density and infection prevalence (Kallio et al.,
235 2010) may be lacking at the end of the season when host density has increased by twice with
236 the birth of newborns. The number of immune females may explain why infection rates, and

especially that of paramyxovirus, did not return to the peak levels measured at the beginning of the season. Further investigations addressing the serological status of pregnant mothers, newborns and juveniles towards *Leptospira* and paramyxovirus need to be carried out in order to properly address these major points.

Seasonality of reproduction might play a role in the evolution of infectious agents by causing alternating periods of potential mixing of different lineages with high transmission and population bottlenecks that simultaneously limit strain diversity and cause rapid genetic shifts (e.g. Ferguson *et al.*, 2003). In the present study, we observed that one single haplotype of *Leptospira* co-circulated with multiple paramyxovirus lineages, though no apparent evolution of the viral diversity could be recorded over the timescale of our observations. Multiple circulating virus strains may affect within-host and within population dynamics by conferring cross-immunity (Hayman et al., 2013). Interestingly, we also found evidence of paramyxovirus sharing between bat and rodent populations, suggesting that host shift between rats and bats may contribute to the paramyxovirus diversity observed within the maternity colony.

The presence of multiple infectious agents within a host, i.e. co-infection, can critically impact infection dynamics (Telfer et al., 2010). Interactions may be synergistic or antagonistic and infectious agents may interact either directly or indirectly via 'bottom-up' (e.g. competition for shared host resources) or 'top-down' processes (e.g. immune-mediated competition or facilitation) (Pedersen and Fenton, 2007). Here, although bats were frequently co-infected with *Leptospira* and paramyxovirus, the observed rate of co-infection was not different from what is expected at random and we found that the presence of paramyxovirus infection had no effect on the intensity of *Leptospira* excretion. The possibility that the intensity of paramyxovirus infection could be positively influenced by *Leptospira* infection could not be tested with our experimental setup and, thus, cannot be ruled out. The large

confidence intervals for pathogen prevalence and intensity of *Leptospira* infection preclude any solid conclusion but our study suggest that co-infection most likely appears as the mere consequence of the temporal conjunction between the infection dynamics and did not result in any apparent interaction between the two pathogens.

In conclusion, our study has focused on infection dynamics within the time frame of the bat breeding season. Although it is difficult to distinguish between episodic shedding from persistently infected bats and transient epidemics (Plowright et al., 2015), our result suggest that following epidemic bursts, the vast majority of infected bats likely develop immunity efficiently and stop excreting pathogens; however, the question of infection maintenance beyond the breeding season has not been addressed. A recent theoretical study, using stochastic epidemiological models with a seasonal birth pulse, suggests that pre-existing immunity is critical for pathogen maintenance in bat populations (Peel et al., 2014). Here, rates of infection at the beginning and end of the breeding season were roughly similar (~25%) implying that the bat maternity *in fine* serves as an ecological "vaccination center", providing potential antibody immunity to large proportions of the population outside of the breeding season. Transmission outside of maternity colonies may thus be maintained by a small fraction of individuals chronically excreting the two pathogens, waning immunity and/or immigration events between colonies (Breed et al., 2011; Field et al., 2011; Sohayati et al., 2011; Peel et al., 2012). As fission–fusion social structures are being increasingly recognized in bats (Kerth et al., 2011), and because the bat species studied herein tends to split into small populations during non-reproductive period, it is possible that a complex meta-population structure will influence seasonal infection dynamics within the community (Plowright et al., 2011). Multidisciplinary research on the interplay between population structures and temporal dynamics in the context of their associated pathogens are important for the prediction of possible emergence events in specific geographic regions (Restif et al.,

2012), such as the western Indian Ocean, where leptospirosis and paramyxoviruses are widely prevalent (Bourhy et al., 2010; Lagadec et al., 2012; Wilkinson et al., 2012; Desvars et al., 2013; Dietrich et al., 2014).

Experimental procedures

Urine collection

A colony of the insectivorous bat *Mormopterus francoismoutoui* was monitored during the 2012-2013 breeding season, in Réunion Island, a tropical oceanic Island located 800km East of Madagascar. The maternity colony is sited in a natural cave of approximately $30m^3$ and is occupied by one single bat species, i.e. *M. francoismoutoui*, from October to May. The maternity colony is mostly composed of adult females at the early stages of the breeding season. A total of eight field outings were carried out from November 2012 to April 2013, a timing that did not allow monitoring the first weeks of the colony formation. We hence conducted additional monitoring in the same cave during the next season in the very early stages of colony formation, with four field outings during October 2013. A non-invasive sampling strategy was implemented in order to avoid direct manipulation of bats thus limiting disturbance of the colony. This strategy is based on the demonstration that infected bats excrete both *Leptospira* and paramyxovirus in their urine (Baker et al., 2012; Desvars et al., 2013). In addition, urine droplets are spontaneously shed by flying bats during their daily emergence at dusk and could then be collected on plastic films layered on the ground. For each sampling date, 16 plastic film (Saran wrap) -covered cardboard rectangles (20x15cm) were placed inside larger plastic trays and positioned at the entrance of the cave following a referenced map. This allowed positioning each tray at the exact same place throughout the whole survey. As shown on the overview of the sampling design provided in Fig. S1 trays were separated by at least 1 meter in order to minimize the possibility of sampling several

urine droplets from a single bat specimen. After the emergence of bats, up to 5 distant (about 5cm) urine droplets assumed to correspond to 5 independent bat specimens, were collected from each of the 16 plastic trays and buffered in micro-tubes containing 300 µL of Minimum Essential Medium Eagle buffer. Samples were then kept in the field in a cool box filled with ice packs and brought back to the lab where they were immediately stored at -80°C until tested.

Bat colony dynamics

The colony was visually monitored throughout the whole study in order to estimate the population age structure and timing of parturition. The presence of adults (brown), newborns (pink colored) and juveniles (dark grey) was thus checked by visual inspection at each sampling date (see photos in Fig. S2).

We attempted to characterize changes in bat behavior during the breeding season by monitoring the numbers of bats at emergence. Relative estimates were obtained by counting the number of individual urine droplets landing on the plastic films, as we showed that the number of urine droplets correlates with the number of exiting bats (see details in Fig. S3). Thus, collected figures allowed comparative analysis between sampling field missions.

Video analyses were also used to estimate the absolute size of the exiting population. When lightness and weather conditions permitted, videos of bats leaving the cave were acquired using the sky as a backdrop. Custom MATLAB-encoded algorithms were then used to define a line within the video-images and video frames were segmented based on the contrast of each image (dark bats on a clear sky background). A binary image was then constructed using mask data along the defined line (X-dimension), and for each frame of the video (Y-dimension). Non-touching objects were then counted in the obtained image mask to generate a minimum-number estimate of the number of bats that had left the cave.

Screening and genetic diversity of Leptospira and Paramyxovirus

Paramyxoviruses are single stranded negative sense RNA viruses, and thus their nucleic acids require reverse transcription before detection via PCR. Therefore, detection was carried out on a single cDNA preparation for both bacterial and viral detection. Using QIAamp Mini spin columns (Qiagen, Valencia, CA), total nucleic acids were extracted following manufacturer's instructions from 140µl of each buffered urine sample. A negative control was added for each extraction run. Following extraction, 10 µL of total nucleic acids were submitted to reverse transcription using the Promega cDNA kit (Promega, Madison, USA) with 1.25 µL of Hexamers (Promega, Madison, USA), following manufacturer's instructions.

Five microliters of cDNA were then used as templates for *Leptospira* and paramyxovirus detections using previously described detection methods (Smythe et al., 2002; Tong et al., 2008). Briefly, the presence of *Leptospira* was detected using a probe-specific real-time PCR, targeting a fragment of the *16S rRNA* gene, specific of all known pathogenic *Leptospira* species. The threshold cycle (Ct) of positive samples was noted to infer the relative bacterial load and thus the relative intensity of *Leptospira* excretion. In order to compare Ct values among different PCR runs, we verified the consistency of the Ct value for the positive control in each run. For positive samples, a partial fragment of the *16S rRNA* and *secY* genes was then amplified, as described in Dietrich *et al.* (2014), and sequenced (Genoscreen, Lille, France) for subsequent phylogenetic analyses. For the detection of paramyxoviruses, a semi-nested PCR targeting the polymerase-encoding gene was performed as previously described (Wilkinson et al., 2012). After electrophoresis on a 2% agarose gel stained with GelRed (Biotium Inc.), PCR products of the approximate anticipated size (450-500 bp) were purified using the QIAGEN PCR purification kit, cloned into the pGEMt vector system (Promega), and submitted to direct Sanger sequencing (Genoscreen, Lille, France). DNA sequences of

paramyxoviruses obtained from independent bacterial clones were aligned to generate consensus sequences for each sample in order to correct for the majority of sequencing or PCR-introduced errors. These sequence data have been submitted GenBank database under accession number KJ607934-KJ607957 for *Leptospira* and KJ748495-KJ748520 for paramyxoviruses

Nucleotide sequences were assembled and edited manually using ChromasLite 2.01 (Technelysium Pty Ltd, South Brisbane, Australia), and aligned using CLC Sequence Viewer 6.8 (CLC bio, Aarhus, Denmark) with references sequences including samples from bats and terrestrial small-mammals of the western Indian Ocean Islands (see details in Tables S1 and S2). Phylogenetic trees were constructed using PHYML (Guindon and Gascuel, 2003) under the AIC best-fitted model of evolution (*Leptospira*: TPM3uf+I and HKY+I for *16S rRNA* and *secY*; Paramyxoviruses: TIM3+I+G) selected by JMODELTEST v.0.1.1 (Posada, 2008), a BIONJ as starting tree and NNI tree search. The robustness of branches was evaluated performing bootstrap analysis with 1000 repetitions.

Statistical analyses

We examined changes in the number of urine droplets (as a correlate of the number of bats emerging at dusk) over the breeding season and among the trays by using a Generalized Linear Model (GLM) with a Poisson distribution and a log link function. “Sampling date” and “tray number” were included as explanatory variables.

We investigated the dynamic of infections (both mono- and co-infections) by using GLMs with binomial error and a logit link function, with the “sampling date” as an explanatory variable. We aimed to determine whether the presence of one infectious agent was associated with the presence of the alternative infectious agent, adding “infection status” for *Leptospira* and paramyxoviruses as explanatory variables in models testing mono-

infections. A G-test was used to test differences between the co-infection rates, as observed or as expected at random. Expected co-infection rates at each sampling date were calculated as the product of the rates of infection with each single pathogen measured at the same date (Nieto and Foley, 2009).

The temporal variation in the relative intensity of pathogen excretion in urine was analyzed for *Leptospira* only, as no accurate quantitative information on viral load could be obtained from the semi-nested PCR used for paramyxovirus detection. We used a GLM with a Gaussian distribution and a logit link function, using the threshold cycle (Ct) as a proxy for the relative intensity of *Leptospira* excretion and "sampling date", "tray number" and "paramyxovirus infection status" as explanatory variables.

All starting models were simplified by backward stepwise elimination of non-significant terms ($p > 0.05$) beginning with interactions, to obtain the minimum adequate model (Crawley, 2007) (see models in Table S3). In models using a binomial error structure, we systematically checked for over dispersion by calculating that the ratio of *residual deviance* over *residual degrees of freedom* was <2. Curves were produced using a loess smoother. Evolution of pathogen genetic diversity (proportion of the different lineages over time) was analyzed using a Fisher's exact test. All analyses were carried out using the R software package v.3.0.2.

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Figure legends

Fig. 1. Seasonal dynamics of (a, b) the bat population and (c, d) *Leptospira* and paramyxovirus infections in the maternity colony of *M. francoismoutoui*. (a) Estimated presence of adults, newborns and juveniles within the colony (black rectangles). (b) Emergence of bats at dusk. The continuous line represents the number of urine droplets lying along the main flight-path of bats exiting the cave at dusk, and the shaded area the standard deviation. (c) *Leptospira* and paramyxovirus infection rates. The continuous lines represent the proportion of PCR-positive samples for *Leptospira* (green) and paramyxovirus (purple), and the shaded area the 95% CI. (d) Intensity of *Leptospira* excretion. The dashed line represents the mean Ct values for positive *Leptospira* samples (represented in its negative form) and the shaded area the standard deviation. In each panel, the x-axis depicts the seasonal time scale in month and the white dots correspond to sampling dates.

Fig. 2. (a) Proportion of mono- and co-infections among infected individuals and (b) seasonal dynamics of co-infections in the *M. francoismoutoui* maternity colony. (a) White bars represent mono-infected individuals and grey bars individuals with co-infection. Error bars represent 95% confidence intervals. (b) The continuous line represents the observed rate of co-infection, while the dashed line corresponds to the expected probability at random. 95% CI are represented by shaded areas. The x-axis depicts the seasonal time scale in month and the white dots correspond to sampling dates.

Fig. 3. Phylogenetic relationships of (a) Leptospira and (b) paramyxovirus detected in the maternity colony of *M. francoismoutoui*. Samples from this study are represented in green for *Leptospira* and purple for paramyxovirus and are coded with sample ID and date (month-day-year). Reference sequence labels refer to the host species and geographic location. Genbank

accession numbers are indicated in parentheses. Bootstrap values higher than 80% are represented by a dark circle.