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Spatiotemporal Dynamics of Blood Parasite Infections and Impacts on Avian Health and Reproduction

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ABSTRACT

Parasitic infections are ubiquitous in nature, but their consequences are often difficult to evaluate in wildlife. This is particularly the case for chronic infections for which fitness impacts can be subtle or confounded with other intrinsic and extrinsic factors. Infections by *Babesia* (Apicomplexa, Piroplasmidae) are widespread in wildlife, but little is known about their impacts on host health. Here, we evaluated the dynamics and possible fitness consequences of infection by *Babesia* sp. YLG, a recently described parasite infecting yellow-legged gulls (*Larus michahellis*). Using a qPCR assay developed for *Babesia* sp. YLG, we found that prevalence was much higher in chicks compared with adults. In chicks, infection depended on the colony and was tightly related to nest infestation by the tick vector *Ornithodoros* (*Alectorobius*) *maritimus*; this was not the case for adult birds. Infection intensities changed over time in chicks, increasing up to approximately 37 days old and then declining. Infection was associated with reduced body condition and fledging success for adults. In chicks, infection was significantly linked to reduced growth rates, even after accounting for the additive effect of ticks. However, no relationship was detected between infection and fledging date, migration strategy or flight distances measured on a subset of GPS-tagged juvenile birds. Through its subtle effects on fitness and reproductive success, infection by *Babesia* sp. YLG may have significant consequences for host demography and work focused on long-term dynamics is now called for to better understand how this parasite may shape the ecology and evolution of seabird populations.

1 | Introduction

Parasitic infections are ubiquitous in nature, but their consequences are often difficult to evaluate in wildlife. Indeed, if virulence is high (i.e., resulting in debilitating disease or death), the agent involved will be rapidly identified and the impact of infection can be readily quantified at individual, population and community levels. For example, the recent outbreak of highly pathogenic avian influenza (HPAIV) in seabirds was quickly discovered when unusual mortalities of Great skua (*Stercorarius skua*) were observed in British colonies (Banyard et al. 2022). The infectious agent then spread to a series of other species and

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locations over the next two breeding seasons (e.g., Grémillet et al. 2023; McPhail et al. 2024) and its population level consequences have now been estimated for several of the impacted species (e.g., Avery-Gomm et al. 2024). However, most wildlife infections are subclinical or chronic. Quantifying their impact is more challenging because the infectious agent may be unknown and difficult to detect in individuals without the development of specific tests. Similarly, the fitness impacts of such infections may be subtle or confounded with other intrinsic and extrinsic factors that can modify fitness (Dunn et al. 2021). Moreover, impacts may occur over long periods of time and require intensive population monitoring to be quantifiable (Asghar et al. 2015; Taylor et al. 2023). The presence of such infections can nonetheless be problematic because even mild impacts on host survival, fecundity, or feeding rate are known to affect host population size, with cascading effects on local ecosystems (Dunn et al. 2021; Hasik and Siepielski 2022; Koltz et al. 2022). Chronic infections can likewise alter movement and migratory patterns, leading to changes in species' interactions and potentially promoting parasite and pathogen dissemination (Teitelbaum et al. 2023). Considering the impact of chronic infections is therefore essential when trying to understand the factors that shape the ecology and evolution of natural populations.

Among chronic infections, the impact of blood parasites on their hosts has been the focus of some work, particularly in livestock (e.g., Schnittger et al. 2012), but also in wild birds (e.g., Asghar et al. 2015; Dufva 1996; Dunn et al. 2021; Hamilton and Zuk 1982; Risely et al. 2018; Valkiunas 2004). For example, severe population declines in native honeycreepers (Drepanidinae) of the Hawaiian Islands were caused by the introduction of avian malaria *Plasmodium relictum* and its mosquito vector *Culex quinquefasciatus* (Van Riper III et al. 1986). Since this early work, blood parasites have been more widely considered in ecological studies, particularly with the advent of PCR-based screening methods (Hellgren et al. 2004). These parasites are known to be ubiquitous, show high variability in virulence among hosts (Atkinson et al. 2000), and have diverse impacts on host fitness (e.g., Asghar et al. 2015; Marzal et al. 2005; Merino et al. 2000; Pigeault et al. 2018). However, most studies to date have focused on haemosporidians (Dunn et al. 2021; Valkiunas 2004), with work on other groups (e.g., piroplasms, coccidians, trypanosomas) largely restricted to describing their presence and taxonomic position (e.g., Cigler et al. 2024; Merino et al. 2014; Sgarioni et al. 2023; Yabsley et al. 2009). The impact of a parasite will depend on how it exploits its host, but also on both extrinsic factors, such as environmental conditions, availability of food resources, habitat quality and/or the presence of predators–competitors, and intrinsic host factors such as immunocompetence, age, sex or coinfection (Dunn et al. 2021; Khan et al. 2019). Understanding the fitness consequences of infection can therefore be complex and requires evaluations that consider the various additional stresses that may occur under natural conditions.

Among wildlife, seabirds represent particularly good study models to evaluate the impact of chronic infections. They are long-lived, relatively easy to monitor and form large, dense colonies that are predictable in time and space (McCoy et al. 2016). They can also host a range of parasites and pathogens (Khan et al. 2019; Quillfeldt et al. 2011), certain of which are known to have devastating immediate effects (e.g., avian cholera (Iverson et al. 2016); avian influenza (Avery-Gomm et al. 2024); avian pox virus (Tompkins et al. 2017)), whereas the effects of others are more difficult to evaluate (e.g., *Borrelia burgdorferi* sl (Chambert et al. 2012); *Plasmodium* (Cloutier et al. 2011)). The short-term fitness impacts of infection can be measured in these systems via population monitoring. However, the longer-term demographic effect is more challenging to estimate because seabirds have a delayed age of first reproduction, low seasonal fecundity and a tendency to skip reproduction if conditions are poor (Schreiber and Burger 2001). These traits also mean that seabird populations can be extremely sensitive to even slight modifications in certain demographic traits (Doherty Jr. et al. 2004; Weimerskirch 2001).

Although considered infrequent hosts to blood parasites, seabirds are known to harbour diverse haemospordians and microfilariae (Quillfeldt et al. 2011). Piroplasms of the genus *Babesia* have also been described in seabird blood (family Piroplasmidae) (Yabsley et al. 2017). *Babesia* are intraerythrocytic parasites that cause babesiosis in mammals, with symptoms resembling those of malaria (e.g., fever and hemolytic anaemia). Severe disease occurs with some species (e.g., *Babesia bigemina*, *B. bovis, B. divergens*), but varies greatly depending on the infected host species (Schnittger et al. 2012). Asymptomatic infections are also frequent, particularly in juvenile life stages (Schnittger et al. 2012). Transmission typically involves an obligate tick vector, which hosts the parasite's sexual reproductive phase (see Figure 1) and a specific vertebrate host species (Peirce 2000; Schnittger et al. 2012). Although the presence of *Babesia* in seabirds was first documented in the 1990s (see taxonomic references in Peirce 2000), the impact of these parasites on avian hosts remains largely unexamined (Peirce 2000, 2005; Yabsley et al. 2017).

Known avian *Babesia* species are classified into two lineages: the *Kiwiensis* group of clade X (*Babesia* sensu stricto) and the *Peircei* group of clade V (*Babesia* sensu lato) (Malandrin 2022). A recently described species, *Babesia* sp. *YLG*, was identified in the yellow-legged gull (*Larus michahellis*) and belongs to the *Peircei* group (Bonsergent et al. 2022); it is transmitted to seabirds by the tick *Ornithodoros* (*Alectorobius*) *martimus* (Family Argasidae) during the nesting period (Bonsergent et al. 2023). This parasite has been found to reach high prevalence in juveniles (up to 85% in chicks), but little to no information exists regarding infection in adult birds nor its spatiotemporal dynamics in these hosts (Bonsergent et al. 2022). No assessment of the potential impact of this blood parasite on individual health, breeding success and survival has been carried out to date.

In this study, we evaluated the dynamics and potential fitness consequences of infection by *Babesia* sp. YLG in chicks and adults of the yellow-legged gull from two large breeding colonies over 3 years. We assessed *Babesia* infection using two approaches: classic microscopic examination of blood smears and via the development of a qPCR assay specific to *Babesia* sp. YLG. Once infection was characterised, we examined its spatiotemporal dynamics and the factors associated with infection probability (age, colony, sex, year and tick exposure). We then went a step further to evaluate the association between infection and key host traits: physiology, growth, survival and movement. If

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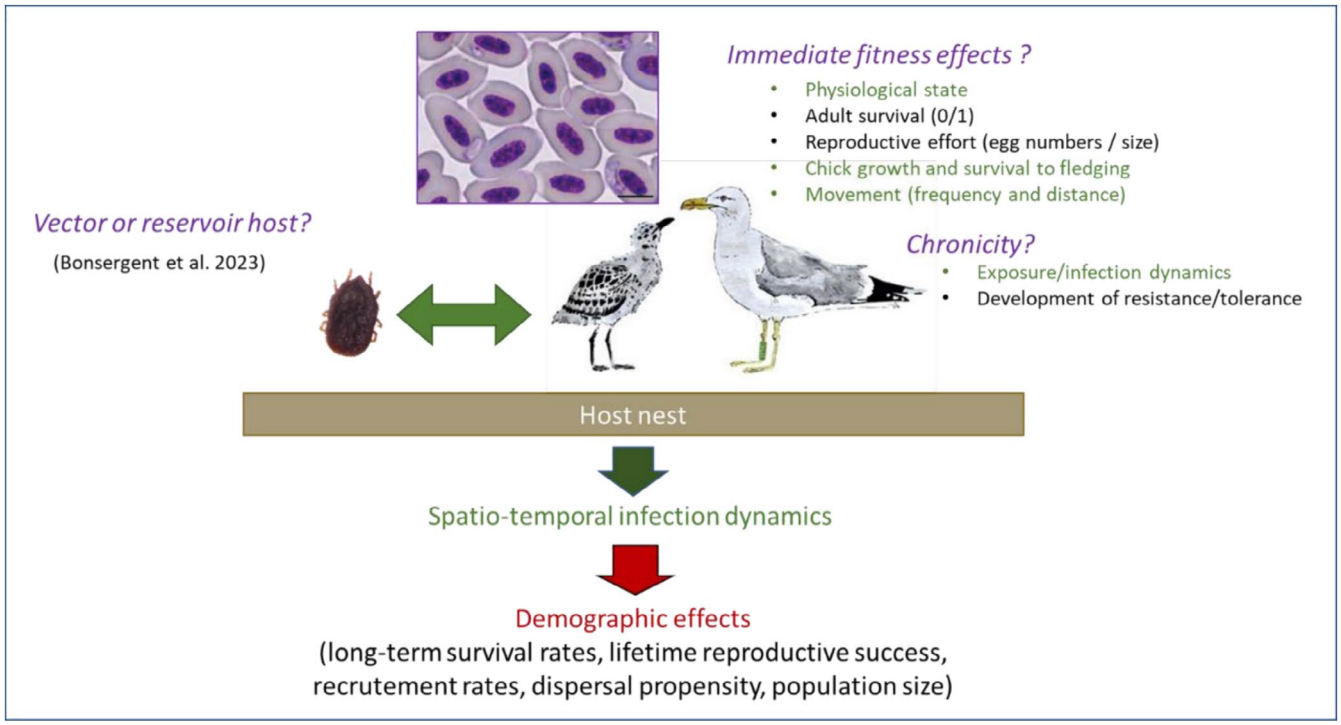


FIGURE 1 | Life cycle and pending questions in relation to the interaction between *Babesia* sp. YLG and its two host types, the tick *Ornithodoros* (*Alectorobius*) *maritimus* and yellow-legged gull (*Larus michahellis*). In purple, the main questions that require answers to better understand its infection dynamics. In green, the aspects addressed in the current study. Depending on the virulence of *Babesia* infections for the avian host, we could expect major changes in host demography (red). *Babesia* transmission occurs via ticks that live within and close to the nest of the seabird host. Ticks can infect a new host, or become infected, when they take short nocturnal blood meals on the host (see text for details). Photos: L. Malandrin, M. Dupraz; Sketch: C. van Weelden.

the interaction between this parasite and its seabird host is of ecological relevance, we expected to detect a negative association between infection and fitness components, and this despite variability due to other potential environmental stressors. In particular, we expected to see negative relationships between infection and host physiological condition (body condition, blood counts), growth, survival and movement due to the cost of infection.

2 | Materials and Methods

2.1 | Study Areas and Sampling

From 2022 to 2024, two yellow-legged gull colonies were surveyed: Carteau Island (GPS coordinates: 43°22′39″ N 4°51′28″ E) and Frioul Island (GPS coordinates: 43°17′8″ N 5°19′16″ E) in the Gulf of Lion, southern France. At each colony and in each year, 30 randomly selected nests were monitored from the end of March (egg-laying) to the end of June (start of fledging). Nests were visited weekly during the period. Early in the season (end of March to mid- April), adults were captured on the nest using clap nets (1 m × 1 m or 80 cm × 80 cm, Ecotone, Poland) when weather conditions were appropriate (i.e., with temperatures not exceeding 25°C and winds below 40 km/h). Standard biometric measurements were recorded for each captured individual and the bird was ringed for identification purposes (metal and PVC rings with individualised alpha-numeric codes, hereafter referred to as darvic rings). After hatching, chicks were caught directly by hand in or around the nest and were marked, first with a temporary plastic ring and then with permanent metal and darvic rings depending on their size. At each capture, chicks were weighed and the tarsus size was measured. To determine chick age, the hatching date of each nest was approximated based on field observations of eggs: If there were no signs of hatching at a visit, we used the median date between visits; if the egg was cracked, we considered the chick hatched 3 days later; if there was a small opening in the shell, we considered the chick hatched 2 days later. Blood samples were also collected, but differed according to age and body condition. For chicks weighing between 100 and 500 g, blood was drawn by capillary puncture using a 27G sterile needle (Terumo, France) and a 50-μL heparinised capillary tube (Hirschman Laborgerate, Germany) from a disinfected brachial vein. A 10-to-40-μL drop of blood was taken and used to prepare a blood smear (see below). For adults and chicks weighing over 500 g, blood was drawn by venipuncture from a disinfected brachial vein using a 25G heparinised needle attached to a 2.5 mL polypropylene syringe (Terumo, France). Birds were sampled only a single time using this method. For chicks, this corresponded to the day they received the darvic ring, at approximately 30 days old. The sampled blood was maintained in a cooler box until the return to the laboratory (see Table 1 for sample sizes). Blood residuals from the capillary tube or syringe were immediately conserved in 70° ethanol for later genetic analyses.

We equipped 47 chicks with GPS tags using a wing-loop harness attachment system for long-term monitoring (see Souc

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TABLE 1 | Primers and PCR conditions used in this study.

Gene	Hypothetical product		Primers (5′–3′)	Tm	Fragment size	References
CHD1	Chromodomain-helicase DNA-binding protein	2550F	GTTACTGATTCGTCTACGAGA	50°C	PCR assay: 2550F/2718R	Fridolfsson and Ellegren (1999)
		2718R	ATTGAAATGATCCAGTGCTTG			
18S rRNA (*Babesia* sp.YLG)	Small ribosomal unit	18SBab_qPCR_F	GTCAGAGGTGAAATTCTTAGA	60°C	qPCR assay: 18SBab_qPCR_F/BAG_GR2: 166 bp	Herwaldt et al. (2003) & this study
		BAG_GR2	CCAAAGACTTTGATTTCTCT			
18S rRNA (*Larus michahellis*)	Small ribosomal unit	18SLm_qPCR_F	ACGGCCGGGGGCATTCGTAT	60°C	qPCR assay: 18SLm_qPCR_F/18SLm_rev: 190 bp	Bonsergent et al. (2023) & this study
		18SLm_rev	AATAACGCCGCCGGATCGC			

et al. 2024 for details), including a breakable seam to accommodate chick growth. Forty birds were fitted with Ornitela (Lithuania) tags: 23 with the 'OT-20-4GC' model and 17 with the 'OT-20-4GEC' model. Seven individuals were equipped with Interrex (Poland) tags: six with 'DEBUT 15' tags and one with a 'LEGO 2G' tag. Of these 47 individuals, 44 were included in the analysis, as three tags malfunctioned between deployment and fledging. Among the 44 analysed birds, 31 originated from monitored nests. Including the attachment system, the tags represented, on average, 2.06% (range: 1.77%–2.36%) of the bird's body mass on the day of deployment.

Mediterranean yellow-legged gull colonies frequently harbour the soft tick *Ornithodoros* (*A.*) *maritimus* which feeds on the birds during the breeding season (Dupraz et al. 2016, 2017). We therefore evaluated the level of tick infestation in each monitored nest during egg incubation using a standardised technique (see Dupraz et al. 2017). This count data should represent a good index of tick exposure for adult birds during the incubation period, but is only an approximate index for chicks as they only remain in the nest for a few days after hatching; after this time, they move freely over the parental territory and exposure to ticks may vary.

2.2 | Blood Treatment

On return to the laboratory, haemoglobin levels were measured in blood samples using a Mission Hb Hemoglobinometer (ACON, USA) which provides a proxy for haemolytic anaemia. To detect the presence of *Babesia* spp. within erythrocytes, a 10-to-40-μL drop of blood was spread on a microscope slide, fixed and stained using the Diff-Quick HAEMA protocol (LABOR+TECHNIK, Germany). The remaining fresh blood was centrifuged to separate the red blood cell fraction from plasma and was then stored at −80°C until use.

2.3 | DNA Extraction

DNA from blood samples (i.e., red blood cell fraction or blood preserved in ethanol) was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Netherlands) following the manufacturer's instructions. A preliminary drying treatment was applied to the blood samples preserved in ethanol to prevent its interference in the extraction process. After centrifugation at 8000 rpm for 1 min, excess ethanol was removed and the blood pellet was spread along the tube and incubated at 56°C for 30 min with the cap open to allow any remaining ethanol to evaporate. The DNeasy Blood & Tissue protocol was then followed as per the red blood cell samples. Real-time quantitative polymerase chain reaction (qPCR) assays targeting a 190-bp fragment of the 18S sDNA gene of *L. michahellis* were performed on all extractions to assess DNA quality (Table 1 and see Section 2.6).

2.4 | Bird Sexing

To determine the sex of both adults and chicks, PCR assays targeting the CHD1 gene encoding a chromodomain-helicase

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DNA-binding protein were performed using primers 2550F and 2718R, as described by Fridolfsson and Ellegren (1999), (Table 1). The reactions were carried out in a 25-μL volume containing 5–50 ng of template DNA, 1X PCR buffer (Roche Diagnostics, USA), 0.5 mM of each dNTP (Thermo Scientific, France), 2 mM $MgCl_2$ (Roche Diagnostics, USA), 0.28 μM of each primer and 0.05 U of Taq DNA polymerase (Roche Diagnostics, USA). The thermocycling conditions were as follows: an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 1 min, with a final extension step at 72°C for 3 min. Individuals were then sexed by visualising the amplified bands on a 3% agarose gel run in 0.5X TAE buffer at 100 V for 30 min.

2.5 | Detection and Quantification of *Babesia* Using Blood Smears

Blood smears were initially observed under a light microscope at 100× (Nikon Instruments Inc., USA) and the presence of *Babesia* was determined by the complete examination of 20 random fields of vision, selected from homogeneous areas of the smear with a single layer of blood cells. The number of infected cells was counted to obtain a general estimate of infection intensity, assuming the same approximate number of cells per field. All blood smears were examined by the same person (MO).

2.6 | Detection and Quantification of *Babesia* Using qPCR Assays

qPCR assays were developed to check for the presence and intensity of *Babesia* sp. YLG in blood samples. DNA extracts were first tested using a *Babesia*-specific assay targeting a 166-bp fragment of the 18S rRNA gene (Table 1). Primers were designed based on reference sequences of *Babesia* sp. YLG and were tested for nonspecific matches against sequences of other *Babesia* and *Haemoproteus* species (see SM1 in Data S1 for details). Each DNA sample was tested in duplicate. Each detection run included two dilutions of a pEX-A2 vector (Eurofins, Germany) containing a single copy of the gene fragment (diluted to 3.57×10^5 and 3.57×10^2 copies/μL), which served as standards for identifying positive samples. The melting peak of the *Babesia* standard was 80.9°C. The cutoff CT value for a positive result was set at 40.

To assess infection intensity, positive samples were then further processed using two additional qPCR assays: one specific to the 18S rRNA gene of *Babesia*, and the second for the 18S rRNA of *L. michahellis* (Table 1). Each DNA template was analysed in triplicate for each gene. The comparison of these two assays enabled us to obtain a relative quantification of *Babesia* in each sample. Standard curves were plotted using dilutions of the pEX-A2 vector containing one copy of each gene fragment (a tenfold dilution from 3.57×10^7 to 3.57×10^1 copies/μL). The melting peak of the *Larus* standard was 84.6°C.

All assays were carried out in a 10-μL volume containing 5–50 ng of DNA, 1X SYBR Select Master Mix (Applied Biosystems, USA) and 2 μM of each primer, and under the following conditions: initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s, and a final dissociation step. Assays were performed and analysed using a LightCycler 480 system with associated software (Roche, Switzerland).

2.7 | Statistical Analyses

All analyses were carried out using R (vR4.4.2, R Core Team 2024) and RStudio (v 2024.12.0.467, Posit team 2024). As a general process, the distributions of all variables were examined using Shapiro tests to select the most appropriate statistical test. Response variables were log-transformed, if required. The method used for model selection depended on the complexity of the full model (i.e., that containing all explanatory variables identified according to our hypotheses) in relation to sample sizes. When possible, full models were successively simplified by eliminating nonsignificant variables in order to obtain adequate minimal models using a standard procedure of likelihood comparison (Stats package) and on the basis of AICc (MuMIn package), or using the dredge function (MuMIn package). When sample sizes were inadequate, we opted for testing simple variables individually compared to the null model and building up to the final model. Best-fit models were examined using the sjPlot package and the DHARMa package. The distribution of model residuals was tested using Shapiro tests. For each variable, model selection is detailed in the Supporting Informations (SM2 to SM6 in Data S1). All scripts, data sets and detailed analytical processes used in this study are available on Github in the repository https://github.com/mariebuysse/Babesia_avian_health/.

2.7.1 | Parasite Detection and Quantification

To validate our qPCR detection technique for *Babesia*, we compared results with parasite detection and quantification from blood smears. We used a two-way contingency table and McNemar's test for paired nominal data to determine whether methods differed in detection sensitivity. We then performed a correlation test between measures of infection intensity estimated from each method (Spearman's Rank Correlation). Only values from 2024 were used for this correlation test. Based on results, only values from qPCR tests were included in the subsequent statistical analyses (see Results Section 3.1).

2.7.2 | Spatiotemporal Patterns of Infection

Using nest coordinates within each year of study, we tested if there was spatial autocorrelation in the probability of infection within each colony. We considered nest infection (binary 0/1) using data from both sampled chicks and adults (all) and for chicks alone (juv). We did not carry out this analysis using adult bird infection data alone because of the low number of infected adults (see Section 3.2). For each year and colony, we carried out joint count tests (spdep package) on nest infection to determine whether neighbouring nests were more likely to be infected

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than at random (i.e., that infected nests formed spatial clusters). The random distribution of infected nests was generated by 999 Monte Carlo simulations. Based on nest distributions and the relative mobility of infected ticks (Rataud et al. 2020), a maximum distance between neighbouring nests of 20 m was used.

In order to identify factors modifying the probability of infection, we used separate generalised linear mixed-effects models (GLMER) (binomial family) for adults and chicks with infection status as the response variable (0/1), sex (for adults only), colony, year and tick exposure as explicative variables; nest was included as a random factor. For chicks, we only considered the infection status on the day when the full blood sample was taken (approx. 30 days old, see Section 2.1). If individuals are able to clear infections over time and develop immunity, we expected chicks to have higher prevalence than adults. As males and females share nest incubation, we expected them to have similar levels of exposure to ticks in the nest and therefore a similar probability of being infected (i.e., no effect of sex). Finally, we assumed that greater exposure to ticks would increase the probability of being infected by *Babesia*.

To better understand the within-host dynamics of infection, we examined the relationship between parasitaemia and chick age using a Generalised Additive Model (GAM) with days since hatching as a fixed effect, and with colony and individual as random effects. We expected infection intensity to increase with age post infection, with a general decline once the chick's own immunity started to develop. However, as chicks may have maternally transferred antibodies that can protect them for some time after hatching (Staszewski et al. 2008), initial infection dynamics could vary strongly among individuals.

2.7.3 | Infection and Individual Physiology

To examine the effects of infection on gull health, we examined two physiological variables that should be associated with overall individual health: circulating concentration of haemoglobin (Hb) and body condition. Body condition was measured as the scaled mass index (SMI), a body condition indicator that standardises individual body mass to a reference body length (here, tarsus length) using an allometric scaling relationship (Peig and Green 2009). For both variables, we carried out linear mixed-effects models (LMER), including infection status (binary) or parasitaemia (continuous), SMI on the day of sampling (for the Hb model) and tick infestation level (continuous) as fixed effects, with sex, colony and year as random factors. For tests including parasitaemia, Pearson Correlation tests were used if sample sizes were too low for modelling. As *Babesia* destroys red blood cells during within-host replication, we expected a strong effect of infection intensity on Hb. Similarly, as infection should rob energy that can be invested in other physiological states, we expected to see impacts on body condition in the case of strong parasitaemia, with additive effects of increasing tick infestation levels. Given that Hb concentrations (Ad_{avg} (±SE) = 11.86 ± 0.19, $Chick_{avg}$ = 8.03 ± 0.10; Wilcoxon test, W = 528.5, p value < 2.2e-16) and SMI (Ad_{avg} (±SE) = 1046.032 ± 9.085, $Chick_{avg}$ = 742.73 ± 10.668; Wilcoxon test, W = 232, p value < 2.2e-16) differ strongly between adult birds and chicks, separate models were run for each age group.

2.7.4 | Infection and Reproductive Success (Chick Growth and Survival)

To estimate chick growth rates, we employed two methods.

First, we used a generalised additive mixed model (GAMM) to examine the relationship between tarsus length and the time since hatching. The model included a smoothing term to capture the nonlinear variation in tarsus length, as well as a random effect for each individual:

$$\text{tarsus} \sim s(\text{days since hatching}) + (\text{days since hatching} | \text{individual}).$$

Following this, a normalised sum of residuals for each individual was extracted. This indicator reflects the cumulative deviations between observed values and model predictions, thereby capturing individual growth dynamics.

Second, we estimated the growth slopes of each chick during their first 19 days by fitting a linear model (tarsus~days since hatching). This period was selected because, according to the GAMM, it corresponds to the linear phase of tarsus growth.

We then modelled growth using a LMER for each growth rate estimate, considering infection status, tick infestation level and sex as fixed effects, with colony and year as random effects. As for the physiological measures, we also tested for a relationship between growth and parasitaemia, with the expectation that higher infection intensities should reduce growth rates. In this case, parasitaemia was considered at the time of the blood sample, that is, when chicks were approx. 30 days old. For this reason, we did not examine the relationship between parasitaemia and growth measured by the slope method.

To understand the impact of infection on fledging success (or survival), we examined two relationships. First, we evaluated the probability that chicks reached the final ringing age (when they received the darvic ring) according to the infectious state of their parents (here only one of the two parents was of known infection status) and the tick infestation level of the nest using GLMER (binomial family) and including colony and year as random effects. If infection has a cost for parents, they may invest less in chick rearing than uninfected parents, resulting in a reduced probability of chicks fledging. The same prediction holds for exploitation by ticks. Second, we directly examined juvenile survival probability for 1 month postfledging for those chicks that were equipped with a GPS device (n = 41 chicks, as survival was undetermined for three chicks). We tested for a significant relationship using a Fisher's exact test. As nest infestation levels by ticks were not always known for these birds, this variable was not considered.

2.7.5 | Infection and Movement

Using the GPS data from 44 chicks, we examined the relationship between infection and movement. First, we investigated whether infection status influenced fledging age, the migration strategy (migratory or nonmigratory), and the age of departure on migration (for individuals that migrated). Next, we tested whether infection status modified daily flight

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distances between fledging and September 1st (i.e., over the first 1–2 months postfledging). To minimise errors caused by localisation inaccuracies, fledging age was defined as the date on which a bird recorded 10 consecutive positions at more than 200 m away from the natal colony during a period of at least 1.5 h. Migration age and strategy were determined using a threshold distance of 80 km from the Mediterranean coastline, as birds that cross this threshold are those that migrate north from their breeding area, while sedentary individuals remain within the Mediterranean region (Souc et al. 2023). If *Babesia* infection has a cost, we expected birds to invest less energy in movement, resulting in delayed fledging age, reduced migratory behaviours, older migration ages and shorter daily flight distances compared to healthy individuals. We did not treat parasitaemia data for these birds due to low sample sizes of infected birds ($n = 15$). We also did not consider tick infestation level for the same reason as stated above.

We tested for differences in fledging age in relation to infection status using a LMER model, considering sex, colony and year as random variables. To determine whether infection was associated with a chick's migration strategy (migrant or resident), we considered only those chicks that successfully fledged ($n = 37$) and performed a GLMER model (binomial family), with sex, colony and year as random effects. For those young birds that migrated, we also tested whether infected individuals delayed migration. As the variable 'age at migration' was not normally distributed, we examined the effect of chick infection status using a nonparametric Wilcoxon–Mann–Whitney test. Finally, we tested for an impact of infection on daily distances covered by the juvenile birds that migrated. We evaluated two movement metrics: average daily distances and daily distance covered by each individual. For the first, we used a nonparametric Wilcoxon rank sum exact test. For the second, we employed a LMER model, log transforming daily distance to improve normality and including colony, year and individual as random effects.

3 | Results

3.1 | Parasite Detection and Quantification

Detection of *Babesia* infection differed between the two methods, with the qPCR assay being more sensitive than observations of blood smears (Table 2, McNemar's test: $\chi^2 = 6.353$, df = 1, p value = 0.0117). There was a significant positive correlation between infection intensity measures using the two techniques ($r = 0.599$, p value = 1.693e-15).

3.2 | Spatiotemporal Patterns of Infection

Overall, prevalence was much higher in chicks compared to adult birds ($\text{Preval}_{\text{Ad}} = 9.62 \pm 4.66$; $\text{Preval}_{\text{Chick}}$ ($\pm$SE) $= 41.25 \pm 33.19$; Table 2), as was parasitaemia ($\text{Intensity}_{\text{Ad}}$ ($\pm$SE) $= 7.99 \times 10^{-5} \pm 4.93 \times 10^{-5}$; $\text{Intensity}_{\text{Chick}}$ ($\pm$SE) $= 1.14 \times 10^{-3} \pm 6.39 \times 10^{-4}$).

There was little evidence for spatial autocorrelation in the probability of infection by *Babesia* at the within-colony scale (Figure 2, Table S2, SM3 in Data S1). Few infected nests occurred on Frioul, reducing the robustness of these analyses. On Carteau, there was some evidence for spatial autocorrelation in 2023 when classifying nest infection using data for both adult and juvenile birds; this autocorrelation disappeared when considering only nests with infected chicks.

For adults, no included factors could explain infection by *Babesia*, that is, the probability of infection was independent of sex, colony, year and tick infestation levels in the nest (final model = null, see Table S3, SM3 in Data S1). This was not the case for chicks, where the probability of infection was related to both the colony and the level of nest infestation by ticks (final model qPCR_bino ~ Site + Ticks_by_nest + (1|Nest), see Table S4, SM3 in Data S1). Breeding on Frioul reduced the odds of being qPCR positive by 91% compared to Carteau (IRR Site[Frioul] = 0.09). However, across both colonies, every additional tick per nest increased the odds of being infected by *Babesia* by 9% (IRR Ticks_by_nest = 1.09).

We found that parasitaemia was dynamic over early chick development. Indeed, parasitaemia increased up until approximately 37 days old and then started to decline (Figure 3; log_ratio-qPCR ~ s(Days) + s(Sample, bs = "re") + s(Site, bs = "re"); p value (Days) = 0.000628 with R-sq.(adj) = 0.493, see SM3 in Data S1). There was no effect of chick sex on this dynamic (see Table S5, SM3 in Data S1).

3.3 | Infection and Individual Physiology

3.3.1 | Haemoglobin Concentrations

For adult birds, the null model best explained haemoglobin concentrations, although infection status was retained in the second best model (ΔAIC < 2) suggesting a possible weak effect of infection (see Table S6, SM4 in Data S1). There was no suggestion of an effect of tick infestation levels or SMI on haemoglobin concentrations for these birds. Infection prevalence was too low in adults to use the same type of analysis with parasitaemia data. However, we found no evidence for a correlation between Hb concentrations and parasitaemia in infected adults (Pearson correlation $r = 0.311$ df = 8, p value = 0.382). Results were similar in chicks. Neither infection status, nor parasitaemia seemed to impact Hb concentrations (see Tables S7 and S8, SM4 in Data S1). Similarly, there was no indication of an effect of ticks or SMI.

3.3.2 | Body Condition (SMI)

For adult birds, the best model included infection status, tick infestation level and an interaction between these factors (SMI~Statut_qPCR*Tick_by_nest + (1|Site) + (1|Sex) + (1|Year)). However, two other models were within a ΔAICc of 2, both of which included infection status (see Table S9, SM4 in Data S1). No one factor was significant when considering the IRR estimates for these three top models, but infection was suggested to reduce body condition in all cases (i.e., top model: IRR Status[uninfected] = 71.16, p value = 0.185). No correlation was found between parasitaemia and SMI in

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TABLE 2 | Number of sampled individuals of each life stage in each study colony and breeding year that were tested for *Babesia* infection. N_{tot} refers to the total number of samples tested and N_i to the number of individuals found infected, with the percent prevalence indicated.

			"Blood smear" dataset			"qPCR" dataset		
Sampling site	**Sampling year**	**Stage**	***N*(tot)**	***N*(i)**	**% infected**	***N*(tot)**	***N*(i)**	**% infected**
Carteau	2022	Chick	20	16	80	15	9	60
		Adult	20	1	5	17	2	11.77
		All	40	17	42.5	32	11	34.38
	2023	Chick	25	9	36	25	15	60
		Adult	20	0	0	19	1	5.263
		All	45	9	20	44	16	36.36
	2024	Chick	15	14	93.33	17	15	88.24
		Adult	16	1	6.25	17	3	17.65
		All	31	15	48.39	34	18	52.94
	All	Chick	60	39	65	57	39	68.42
		Adult	56	2	3.57	53	6	11.32
		All	116	41	35.34	110	45	40.91
Frioul	2022	Chick	21	0	0	18	1	5.56
		Adult	17	0	0	14	1	7.14
		All	38	0	0	32	2	6.25
	2023	Chick	22	3	13.64	23	2	8.70
		Adult	17	1	5.88	17	1	5.88
		All	39	4	10.26	40	3	7.5
	2024	Chick	19	7	36.84	20	5	25
		Adult	20	3	15	20	2	10
		All	39	10	25.64	40	7	17.5
	All	Chick	62	10	16.13	61	8	13.11
		Adult	54	4	7.41	51	4	7.84
		All	116	14	12.07	112	12	10.71
All	All	Chick	122	49	40.16	118	47	39.83
		Adult	110	6	5.45	104	10	9.62
		All	232	55	23.71	222	57	25.68

infected adult birds (Pearson correlation $r = -0.133$, df = 8, p value = 0.715). In chicks, only the null models were selected, both when considering infection status and parasitaemia, and no other variables seemed to contribute to this body condition score (see Tables S10 and S11, SM4 in Data S1). Therefore, if infection affects body condition, its impact may only be evident during chronic infections (as suggested in adults).

3.4 | Infection and Reproductive Success

With both measures of chick growth, a negative impact of infection was evident (Figure 4). For the residual growth measure, the best-fit model included infection status, sex and tick infestation level in the nest, and an interaction between infection status and sex (residuals ~ Statut_qPCR + Sex + Tick_by_nest + Status qPCR:sex + (1|Site) + (1|Year), see Table S12, SM5 in Data S1). However, no significant difference was found among the top three models which included the main effects only or the main effects with an interaction between infection status and tick infestation. Therefore, although an impact of infection on growth based on the residual measure is suggested (Figure 4A), growth with this measure cannot easily be explained by a single factor (see Figure S1, SM5 in Data S1). Results were more evident when considering parasitaemia. The best model (residuals ~ log_ratio_qPCR + (1|Site) + (1|Year), see Table S13, SM5 in Data S1) suggested a negative relationship between parasitaemia and residual

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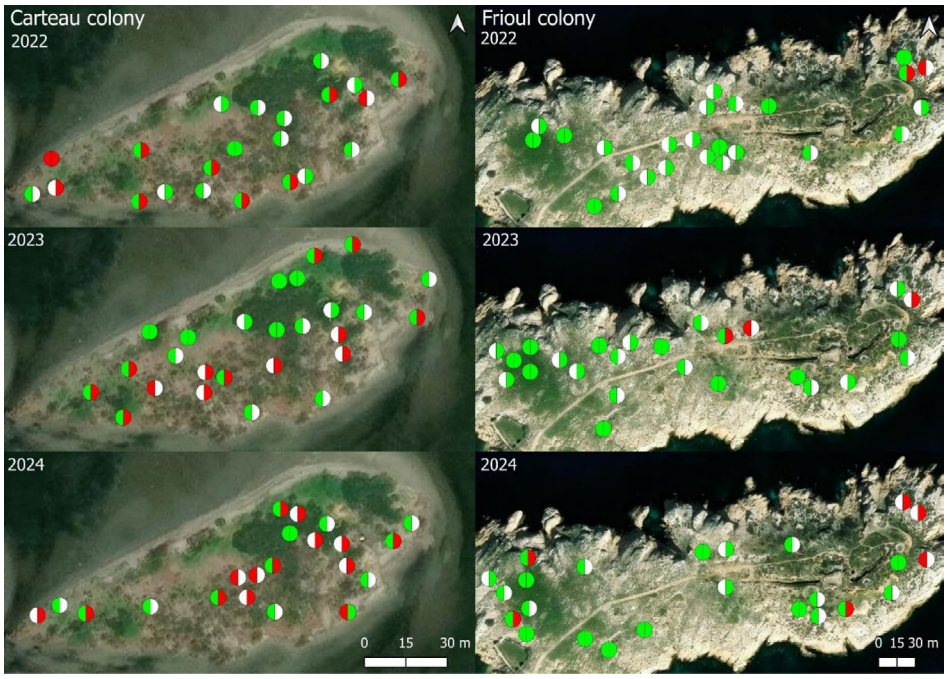


FIGURE 2 | Maps of the two studied YLG colonies, Carteau (left) and Frioul (right) over the study years. Each circle indicates a monitored nest. White indicates the absence of tests, green that an individual was tested by qPCR and found uninfected by *Babesia* sp. YLG, and red that an individual was tested and found infected. The left half of the circle refers to an infected adult and the right to at least one if its chicks. It should be noted that it was not always possible to test adults and chicks from the same nest and that other nests were present on the island, but do not appear in the figure. The number of monitored nests varied per year due to early breeding failures.

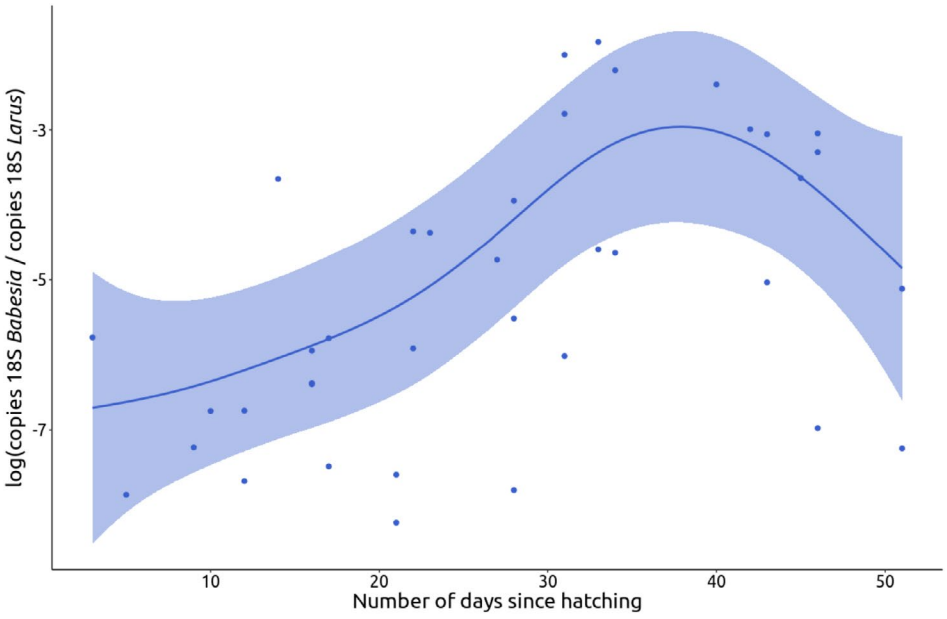


FIGURE 3 | Temporal dynamics of *Babesia* sp. YLG infection over chick rearing based on the GAM model. Parasitemia, measured as the standardised number of 18S gene copies, started to decline when chicks reached approximately 37 days old.

growth (IRR = −1.21, $p = 0.003$; Figure 5), with no significant impact of sex or tick infestation.

For growth measured by the slope, infection status and tick infestation level were retained in the top model (slope ~ Statut_qPCR + Ticks_by_nest + (1|Site) + (1|Year)), suggesting that both factors play a role (see Table S14, SM5 in Data S1). The IRR estimates for this model show a growth rate slope that is 0.17 units higher for uninfected individuals and that declines by 0.01 units for each added tick in the nest (Figure 4B).

Fledging success, measured as the probability that a chick reached the age to receive a Darvic ring, was significantly related to the infection status of their parent (final model

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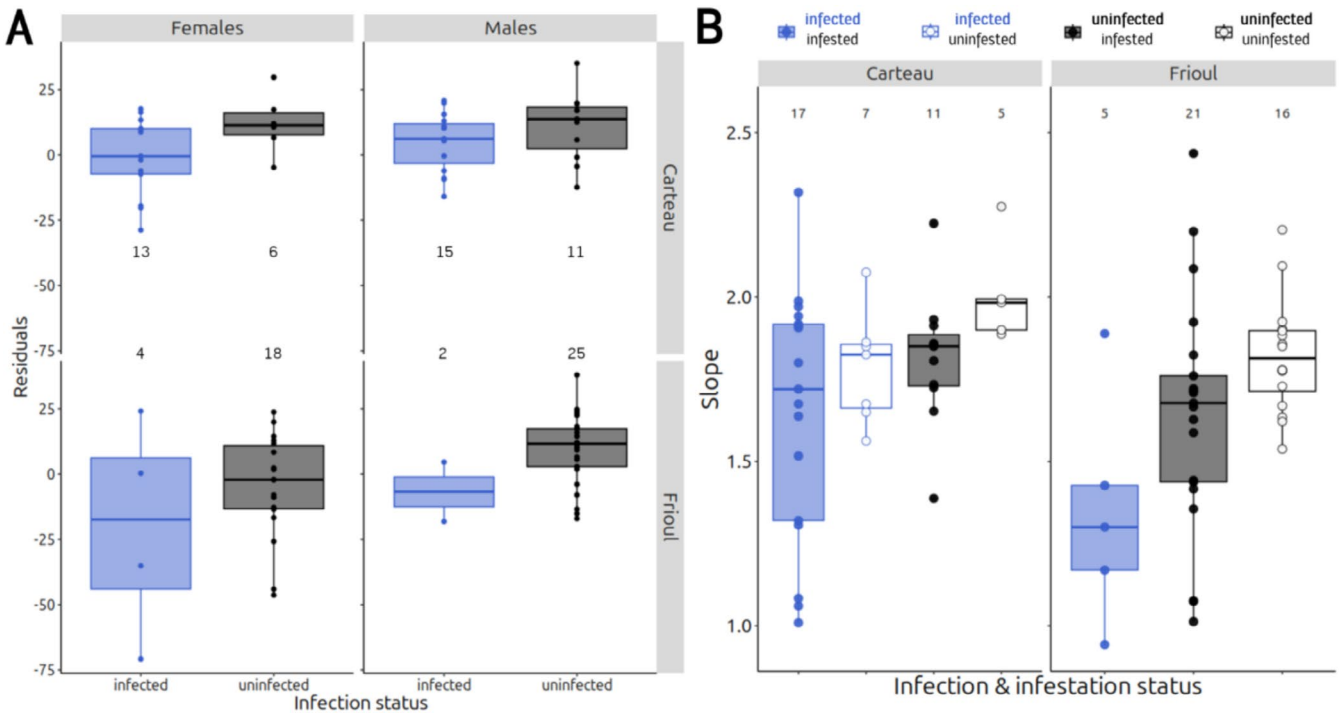


FIGURE 4 | (A) Average growth rate (as measured by the residuals of individual growth compared to the standard growth curve) for infected and uninfected chicks (see text for more details). Here, growth differences have been broken down by colony and sex. (B) Average growth rate (as measured by the slope of tarsus growth) for infected and uninfected chicks in each colony in relation to the tick infestation status of the nest. There is no data for chicks that were infected with *Babesia* sp. YLG in uninfested nests on Frioul, because this state did not occur in this colony. The sample sizes are indicated on each graph.

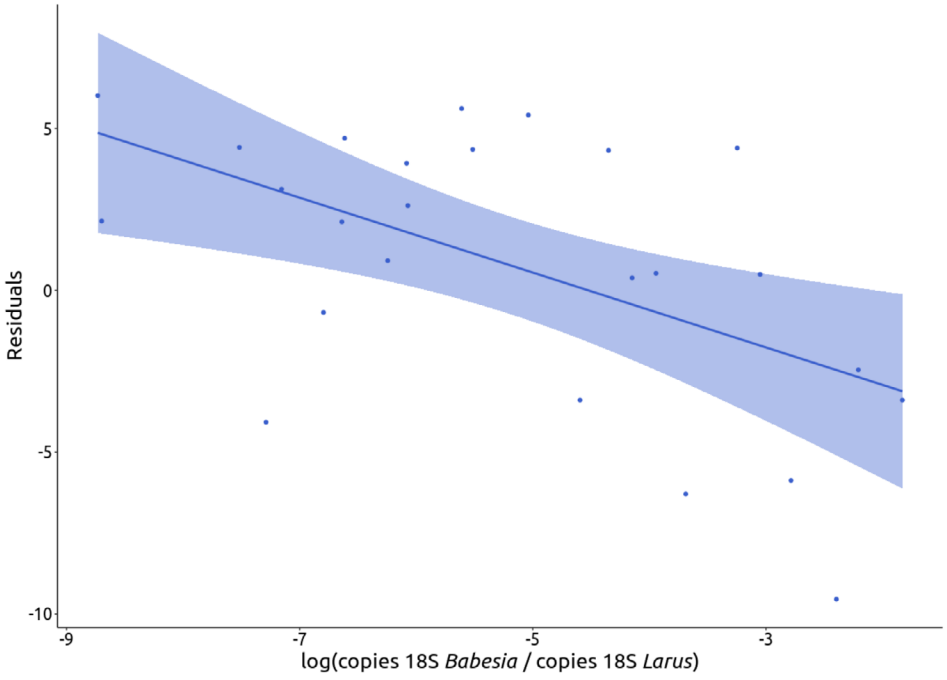


FIGURE 5 | Relationship between the intensity of infection by *Babesia* sp. YLG as measured by qPCR and the residual growth of chicks.

Repro_success ~ Statut_ad + (1|Site) + (1|Year), see Table S15, SM5 in Data S1), with the odds of a chick receiving a Darvic ring being 8.17 times higher when the parent was uninfected. Indeed, adults that successfully reared chicks up to fledging tended to have lower parasitaemia than those that failed (effect size = 0.683), but given the low sample size of infected adult birds for which parasitaemia was quantifiable ($n = 9$), the significance of the model was marginal (Wilcoxon test, $W = 14$, p value = 0.0556). When looking at fledging survival using young birds equipped with GPS tags, we found no indication that infected chicks had a lower survival rate 1-month postfledging (Fisher's Exact test, p value = 1; $n_{\text{infected}} = 15$, $n_{\text{noninfected}} = 26$).

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3.5 | Infection and Movement

We found no significant influence of infection status on fledging age (Fledging_age~1+(1|Site)+(1|Year)+(1|Sex), see Table S16, SM6 in Data S1; average number of days to fledging for infected (±SE) = 67.80 ± 3.16 and uninfected (±SE) = 68.35 ± 1.92), nor on juvenile migration strategies (Migration ~1+(1|Site)+(1|Year)+(1|Sex), see Table S17, SM6 in Data S1). Similarly, for those individuals that migrated, infection did not delay departure (Exact Wilcoxon–Mann–Whitney test: $Z = -0.0503$, p value = 0.981). Finally, there was no obvious effect of infection on flight distances. The average daily distance (in km) covered by each group of juvenile birds was similar ($W = 70$, p value = 0.6883; avg. $\text{distance}_{\text{infected}}$ (±SE) = 80.01 ± 14.48, avg. $\text{distance}_{\text{uninfected}}$ (±SE) = 78.69 ± 16.04), as was the daily distance covered by infected and uninfected individuals (log_distance_day ~1+(1 | Site) + (1 | Year) + (1 | Individual), see Table S18, SM6 in Data S1).

4 | Discussion

The impact of chronic infections on wildlife population dynamics is difficult to quantify due to the practical limits of characterising infection, the logistics associated with evaluating fitness consequences, and the ability to disentangle these effects from other sources of environmental variation. Here, we developed a highly sensitive qPCR method to detect infection and quantify parasite loads (parasitaemia) of the blood parasite *Babesia* sp. YLG. We combined these analyses with data from field monitoring to describe the spatiotemporal dynamics of infection, and to evaluate its possible short-term impacts on reproductive success and survival in its Mediterranean host, the yellow-legged gull *Larus michahellis*. Our results demonstrate the significant impact chronic infections may have on individual fitness, with broader consequences for wildlife population dynamics. These findings also stress the importance of carrying out longitudinal studies in combination with the development of appropriate tools to evaluate these effects.

Our qPCR assay was generally more sensitive than classical blood smear analyses, particularly when parasites were at low intensities (see Supporting Informations). These results could be explained by several nonmutually exclusive factors. First, the quality of blood smears can be variable and may add error in quantifying infected cells. At low parasitaemia, the probability of seeing infected blood cells may require high search times, and thus the 20 randomly selected microscope fields we used here may not have been enough to conclude noninfection. However, there were eight cases where parasites were seen in blood smears, but not detected by qPCR. These cases should not be due to the quality of the DNA extraction, which was controlled, but could represent infection by some other blood parasite that was mistakenly identified as *Babesia*. In this system, other parasites can be present (*Haemoproteus, Plasmodium*) which is why we designed our qPCR assay to be as specific as possible for *Babesia* sp. YLG. Finally, human error could result in the misidentification of an infected cell, particularly given the variable quality of some smears. Given these different factors, we consider that the qPCR method we developed is more reliable for detecting and quantifying infection.

The prevalence of infection was much lower in adult birds compared to chicks and we were unable to link the probability of infection in adults to any of the considered factors. This suggests that infection took place under different conditions than those measured during the study. One can therefore wonder how long these birds carried infection and when initial exposure occurred. Infection was suggested to affect adult body condition in combination with other factors (i.e., tick infestation), but the exact nature of these relationships was difficult to estimate due to the low number of infected adult birds. However, the probability of chick survival to fledging age was significantly lower when at least one of the parents was infected. These results suggest that infection may be costly, and that infected adult birds may trade off chick provisioning with the maintenance of their own health, a general strategy in long-lived species such as seabirds (Schreiber and Burger 2001). Although only low levels of parasitaemia were detected in infected adult birds, work on mammals has shown that according to the *Babesia* species, parasites may accumulate in organs other than blood (Schnittger et al. 2012). Our quantification of *Babesia* sp. YLG in adult birds may therefore underestimate true infection intensities in these individuals, if this parasite shows a specific tissue tropism. More detailed work on within-host infection dynamics will now be needed to better understand the relationship between circulating parasites in blood and infection loads in adult birds.

In chicks, infection prevalence was generally high and varied strongly according to colony. As expected, there was also a significant relationship with the intensity of nest infestation by the tick, *Ornithodoros* (*Alectorobius*) *maritimus*. As this tick is the only known arthropod vector of *Babesia* sp. YLG (Bonsergent et al. 2022, 2023), we expected that increased exposure to tick bites would increase the probability of infection. We found a 9% increase in the probability of infection by *Babesia* with every additional tick in a nest. Colony effects are likely related to this aspect. Indeed, tick infestation was much higher in the Carteau colony compared with the Frioul colony and model results suggested a 90% higher probability of exposure to *Babesia* at Carteau.

We found a significant effect of infection on early chick growth, with infected chicks showing lower growth rates, regardless of the measure of growth that we used. This effect was combined with an added impact of tick infestation levels in the nests during early development. However, in contrast to our predictions, we found no obvious physiological effects based on our measures of body condition or Hb concentrations. It may be that these instantaneous measures, considered on the date a chick received a darvic ring (i.e., approx. 30 days old), were not in sync with their infection dynamic. As seen in the within-individual dynamics of infection, parasitaemia varies greatly over development and its dynamic and impact will likely depend on the age at which a chick is exposed to an infected tick. Indeed, a significant effect of infection was suggested in adult birds, indicating that when dealing with chronic infections, the ability to maintain body condition may depend on the time since infection. Other factors likely also play a strong role in determining the physiological condition of chicks, and in particular food availability, adult investment in chick rearing and local climatic conditions (e.g., Oro et al. 1995). Other measures of physiological state may be required to reveal the more subtle consequences of infection (e.g., immunity measures; (Pais De Faria et al. 2023)). If energetic demands are targeted at maintaining a stable physiological state, growth rate

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measures, as we used here, may be better able to capture the subtle effects of parasite infection than physiological traits.

In contrast to predictions, we found no relationship between infection and short-term survival, fledging date and flight distances in the subset of chicks that were equipped with GPS tags. However, sample sizes were very small in this case ($n = 15$ infected chicks), and there was an inherent bias in the chicks chosen for this subset. Indeed, only chicks that weighed close to 1 kg at capture were considered for GPS deployment. This means that infected chicks may have been eliminated and that only those birds who had already managed infection were selected. We also only considered flight metrics for 1 month postfledging. It would now be interesting to evaluate longer-term movement dynamics in infected individuals.

As *Babesia* infection is strongly related to tick exposure, the effects of the two types of parasites can be difficult to disentangle. Indeed, high tick infestations are known to alter reproductive success (Militão et al. 2024). This is generally assumed to be due to the disturbance and blood loss caused by repeat biting. Here, we found that infection was related to a reduction in chick growth, and this, after accounting for the effect of tick infestation. Similarly, in adult birds, body condition was related to both *Babesia* infection and tick infestation levels, but fledging success was only associated with parasite infection. This suggests that it is not biting ticks that are provoking failure, but rather the cost of chronic infection by *Babesia* sp. YLG.

After exposure, we found that infection intensity in chicks continued to increase up until approximately 37 days old, after which intensity started to decline. The different components of the avian immune system are not fully functional at hatching and continue to develop with chick growth (e.g., Song et al. 2021). We therefore suspect that the within-host dynamic that we found for *Babesia* sp. YLG is likely linked to this development. Indeed, Bonsergent et al. (2023) suggested that babesiosis may be a 'childhood' disease for gulls. Young birds are exposed to the parasite during early life. This exposure may impact their growth, but if they survive this early stage, their own immunity may either clear the infection, or keep it under tolerable levels (Valkiunas 2004). This observation falls in line with our results on adult birds, in which *Babesia* infections were infrequent, of low intensity and unrelated to extrinsic factors measured during monitoring (see above). Interestingly, if adult birds are rarely infected and carry low parasitaemia in their blood, one can ask how this parasite is maintained locally. Our results suggest that the maintenance host for *Babesia* sp. YLG is likely the tick, *O. maritimus*, with young birds acting as the vector between ticks. Bonsergent et al. (2023) found that *Babesia* sp. YLG was present in numerous tick organs and could be transmitted via several alternative pathways, including transstadial transmission, an essential pathway for maintaining infection within a local population in the absence of a vertebrate reservoir host. Ticks can harbour high levels of infection (avg prevalence = 31.63 ± 13.15 on Carteau between 2019 and 2022; Bonsergent et al. 2023) and are very long-lived (Vial 2009), although the number of years a tick can maintain an active infection has not yet been examined. More information on the mechanisms of *Babesia* reproduction in alternative host types is now required to understand to what point this parasite requires its gull host and the potential risks of exposure to other vertebrate hosts if gulls do not occupy zones where ticks are present.

Here, we found that chronic infections by *Babesia* sp. YLG can be of ecological relevance for its seabird host. Via longitudinal monitoring and the development of specific molecular assays, we were able to detect potential consequences for several fitness components, and this despite the inherent variability in field samples due to other environmental factors. Not all of our predictions held, suggesting that the costs of infection can likely be compensated for if environmental conditions are good. However, the relative pathogenicity of this parasite if other environmental stressors are present (reduced food availability, poor climatic conditions, coinfections) may increase substantially. In their analyses of *Babesia* sp. YLG, Bonsergent et al. (2023) found the presence of at least four parasite variants at the Cox1 gene in ticks from the Carteau colony. Future work will now need to examine the genetic diversity present in *Babesia* sp. YLG, the spatial distribution of this variation across colonies and potential strain-specific differences in pathogenicity.

Recent meta-analyses have shown that chronic parasite infections that reduce host fitness can affect both the population dynamics of the affected species and the larger community, via cascading effects (Koltz et al. 2022). On a longer time scale, these infections can also alter patterns of natural selection, increasing population variation in vital rates (Hasik and Siepielski 2022). Our results suggest that infection by *Babesia* sp. YLG may have subtle impacts on the reproductive success of yellow-legged gulls. Future work will now need to focus on the longer-term consequences of infection for both population and community dynamics, in addition to examining its impacts on the evolution of these populations in a rapidly changing world.

Author Contributions

Marie Buysse: conceptualisation, field work, supervision, molecular methodology, data generation, data curation, statistical analyses, writing. **Mathilde Ollagnier:** field work, methodology, data generation, writing. **Charly Souc:** field work, data generation, data curation, statistical analyses, writing. **Marjorie Bruley:** field work, data generation. **Carole Leray:** conceptualisation, field work, supervision, data generation, data curation, administration. **Thomas Blanchon:** field work, data generation, administration. **Marion Vittecoq:** conceptualisation, field work, supervision, writing, funding acquisition, project administration. **Karen D. McCoy:** conceptualisation, field work, methodology, supervision, statistical analyses, writing, funding acquisition, project administration.

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Disclosure

Benefit-sharing statement: The research described in this study complies with relevant national laws implementing the Convention on Biological Diversity and Nagoya Protocol agreements (ABSCH permit number: TREL2417485S/834).

Ethics Statement

Authorisations to capture, sample and tag birds were provided by the Ministere de l'Enseignement Superieur, de la Recherche et de l'Innovation—France (no. AFIS#25183–2 020 090 713 423 689). Ringing was carried out under programme no. 990 of the CRBPO (MNHN, France).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All scripts, datasets, and detailed analytical processes used in this study are available on Github in the repository https://github.com/mariebuysse/Babesia_avian_health/.

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Supporting Information

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