Social interactions do not affect mycoplasma infection in griffon vultures

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Running headline: Social interactions do not shape mycoplasma infection status

Supplementary information

Abstract: Uncovering the ways in which pathogens spread has important implications for population health and management. Pathogen transmission is influenced by various factors, including patterns of social interactions and shared use of space. We aim to understand how the social behavior of griffon vultures (*Gyps fulvus*), a species of conservation interest, influences the presence or absence of mycoplasma, a group of bacteria known to cause respiratory diseases in birds. We investigated how direct and indirect social interactions of griffon vultures in the wild, in different social situations, impacted the mycoplasma infection status. We inferred interactions from high-resolution Global Positioning system (GPS) tracking data. Specifically, we assessed how social behavior affects infection status when vultures share feeding and roosting locations, either at the same time (direct interactions) or subsequently, when

space use is asynchronous (indirect interactions). We did not detect a significant effect of any type of social interaction on infection status. However, we observed a high population prevalence of *Mycoplasma* spp., suggesting that other factors might be more important than social interactions in determining disease dynamics in this population. Uncovering the mechanisms that underlie infection status in wildlife is crucial for maintaining viable populations, designing containment management actions, and gaining insights into the ecological mechanisms that drive infectious disease dynamics.

Keywords: feeding, infectious disease, movement ecology, pathogen transmission, roosting, social interactions.

INTRODUCTION

Uncovering the ways in which pathogens spread through a population is crucial for mitigating the transmission of infectious disease, with implications for population health and management. Infectious disease transmission is influenced by many factors, including the transmission route, which may be facilitated by direct and/or indirect interactions among potential hosts (Sah et al., 2021; Gamble 2023). Traditional epidemiological studies utilize theoretical models and social network analysis to investigate pathogen spread (Lloyd-Smith et al., 2005; Bansal et al., 2007; Heesterbeek et al., 2015; Sah et al., 2021; Collier et al., 2022). While these studies can explicitly consider how host interactions mediate pathogen transmission, empirical studies testing these questions *in situ* are challenging because of the costs incurred by investigating pathogen spread throughout an entire population (Lloyd-Smith et al., 2005; Fefferman et al., 2007; VanderWaal et al., 2016; Silk et al., 2017; Dougherty et al., 2018; Albery et al., 2021; Collier et al., 2022). Understanding what factors influence host susceptibility to pathogens, including social behavior (such as proximity and social structure) and host attributes, is crucial for enhancing wildlife conservation efforts. Despite extensive investigations into pathogen spread and the development of sophisticated host–pathogen models, our understanding of the factors influencing infectious disease prevalence in wild animal populations remains limited.

These questions are particularly difficult to disentangle since transmission of pathogens is affected by the characteristics and biology of each infectious agent. Pathogens differ in their transmission modes (airborne, waterborne, vector-borne, foodborne, fecal-oral, etc.); therefore, it is important to investigate different social and ecological situations that may facilitate infectious disease transmission. For example, airborne pathogen, such as *Mycoplasma gallisepticum*, which infects birds, can be transmitted through airborne droplets when individuals are in close physical proximity and share airspace. In contrast, non-airborne pathogen transmission might

require the sharing of a feeder station, or drinking water contaminated with infectious agents (Dhondt et al., 2007; Hawley et al., 2007; Adelman et al., 2015). Thus, exploring pathogen spread is important for developing specific strategies to manage infectious disease dynamics in wild populations, such as periodic vaccination programs or interventions to reduce the risk of disease transmission at specific locations.

It is important to determine which attributes contribute to pathogen acquisition and spread, to inform effective disease management. Pathogen transmission can be influenced by host susceptibility and social behavior as well as host age and sex (Clark et al., 2017). Individuals often have different social roles in a population, which may impact how pathogens spread (Ezenwa 2004; Fenner et al., 2011; Fairbanks & Hawley 2012; Dizney & Dearing 2013; Johnson & Hoverman 2014; VanderWaal et al., 2016). For example, individuals that contact many others are more susceptible to infection (VanderWaal et al., 2016). Similarly, individuals with more unique social partners are more likely to become infected with an infectious disease that is transmitted through social interactions, due to increased exposure to infected individuals and their pathogens (VanderWaal et al., 2016). Furthermore, individuals that interact frequently with others might be more susceptible to infectious diseases that are transmitted through multiple exposures to a pathogen (Poisot et al., 2012; Heesterbeek et al., 2015). For instance, Japanese macaques (*Macaca fuscata fuscata* and *M. fuscata yakui*) that engage more frequently in grooming interactions are more likely to become infected with nematodes (Romano et al., 2016). In addition to social roles, host attributes such as age, can impact infectious status, for example because of the ontogeny of the immune system (Altizer et al., 2004; Lesser et al., 2006; Clark et al., 2017; Wren et al., 2021). For instance, in house finches, and raptors, the prevalence of *M. gallisepticum* is higher in juveniles than in adults (Altizer et al., 2004; Lierz et al., 2008a; Anglister et al., 2024). Uncovering how host attributes affect infectious disease dynamics can provide important information for managing the spread of pathogens; for example, by recommending the removal or vaccination of certain individuals that have potentially high impact on infectious disease transmission (Altizer 2004; Rushmore et al., 2014; Heesterbeek et al., 2015; Clark et al., 2017). Such understanding is also important for gaining knowledge about the ecological elements that drive the persistence of infectious diseases.

Griffon vultures (*Gyps fulvus*) (Hablizl, 1783) are social species that interact when feeding and roosting, and are exposed to a wide range of pathogens. Our study population in Israel is locally critically endangered (Anglister et al., 2023; BirdLife International 2024) and has been the target of many conservation efforts, including the implementation of GPS-tracking for the majority of the population. A reduction in flight distances has been observed in

mycoplasma-infected griffon vultures, particularly among sub-adults (Anglister et al., 2024). However, how *Mycoplasma* spp. or mycoplasma species spreads among griffon vultures remains poorly understood. Griffon vultures aggregate at communal roosts and around carcasses (Mundy et al., 1992). They use their night roosts to share information about the location of feeding sites (Harel et al., 2017), where they often feed together, exchanging bodily fluids through regurgitations. Additionally, territorial behavior during the breeding season (Bertran, 2002) can contribute to the exchange of bodily fluids and the potential transmission of pathogens. We know that griffon vultures differ in their social position across social situations (Sharma et al., 2023); therefore, each individual may have a different impact on disease spread dynamics. Because pathogens can persist in the environment, shared spaces, such as communal roosts or feeding sites, are potential sources for indirect pathogen transmission. The extent to which shared space use contributes to pathogen transmission and spread depends on the specific characteristics and biology of a pathogen.

Mycoplasmas are fastidious organisms of the class Mollicutes, which lack a cell wall (Razin & Naot 1998). The transmission of mycoplasmas is dependent on the species, and can be horizontally, through contact with infected individuals, contaminated surfaces, or airborne particles and/or vertically, from an infected mother to her offspring (Yoder 1991; Hartup et al., 1998; Levisohn & Kleven 2000; Faustino et al., 2004; Ley & Yoder 2008). More than 20 mycoplasma species have been found to infect birds (Lierz et al., 2008; Sawicka-Durkalec et al., 2021), including vultures (Lecis et al., 2010; Anglister et al., 2024). Nevertheless, due to the genetic heterogeneity of mycoplasma species, their impact on the host may vary (Sumithra et al., 2013; Xiao et al., 2022; Dawood et al., 2022). Some mycoplasma species are commensals, while others are pathogenic and their impact on the host will depend on the host body condition and presence of other pathogens (Poveda et al., 1990, 1990a; Lierz et al., 2000, 2002, 2008). Pathogenic mycoplasma species can cause acute or chronic conditions, including respiratory infections, conjunctivitis, arthritis, embryonic death, skeletal deformations, and reduced hatchling sizes, depending on the host species and the individuals they infect (Erdélyi et al.,1999; Razin & Naot 1998; Lierz et al., 2000a; Brown et al., 2002; Lierz et al., 2007, 2008a, 2008b, 2008c; Lierz & Hafez 2009; Grodio et al., 2013; Sumithra et al., 2013; Dhondt et al., 2014). Accordingly, high prevalence of mycoplasma often reduces host survival in the wild (Faustino et al., 2004; Sumithra et al., 2013; Sawicka et al., 2020). However, the effects of mycoplasma in non-passerines remains poorly understood, despite the high prevalence of the bacterium in some populations (Lierz et al., 2008a; Sumithra et al., 2013; Anglister et al., 2024).

Here, we investigate how the social behavior of wild griffon vultures affects whether individuals test positive for mycoplasma. We examine how direct and indirect social interactions, in different social situations (feeding and roosting), relate to mycoplasma infection status in a wild vulture population (**Figure 1**). We predicted that social interactions while feeding would have a greater impact on infection status than interactions while roosting because during feeding, individuals probably share more bodily fluids. Alternatively, interactions while roosting might be a better predictor of infection status compared to feeding interactions because vultures spend more time with one another overnight at the roost, resulting in potentially longer exposures to mycoplasma. Furthermore, we predicted that direct social interactions would have a greater impact on infection status than indirect interactions because direct contact between individuals may increase the likelihood of pathogen transmission through physical contact or exchange of bodily fluids. In contrast, indirect shared space use may involve contact only through the shared environment, reducing the chance of transmission due to factors such as environmental dilution and shorter exposure durations (Leung 2021).

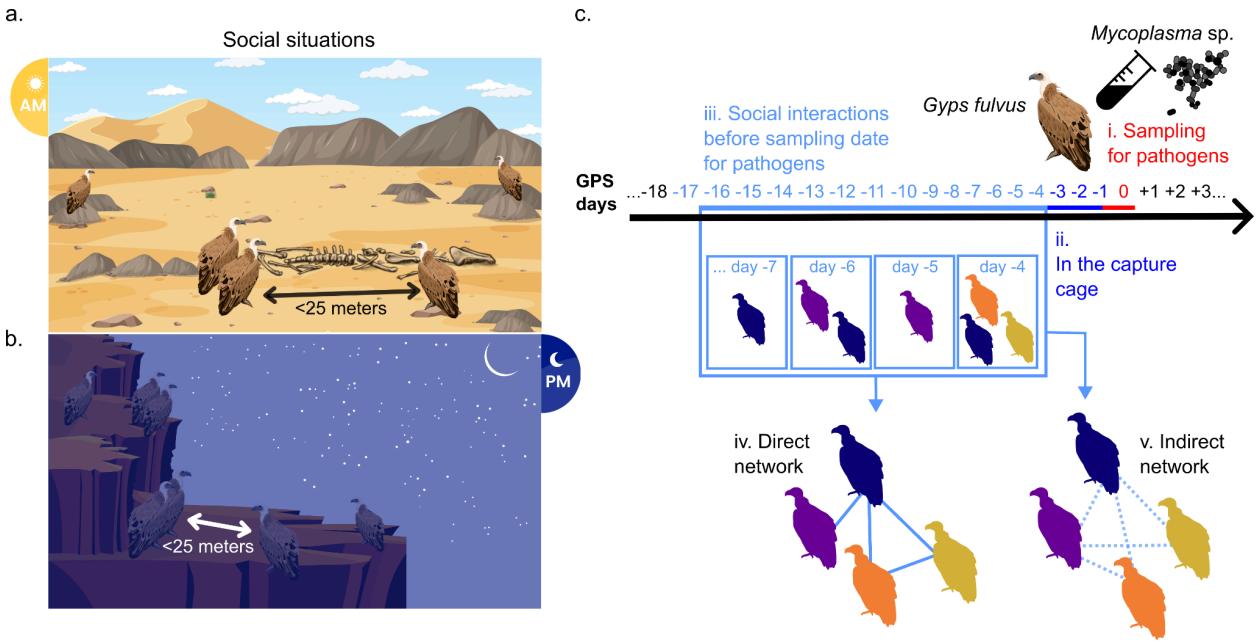

Figure 1. Constructing social networks to investigate the impact of social interactions when vultures are feeding (a) or roosting (b) on infection status (positive or negative) with mycoplasma. The timeline (c) illustrates when social interactions are considered before sampling for pathogens: (i) day on which vultures are sampled for pathogens; (ii) days when the vultures were in the capture cage (excluded from social interaction analysis); (iii) days used to examine social interactions; (iv) direct interactions occur within 30 minutes for co-feeding or over one night for co-roosting; (v) indirect interactions were recorded when more than 4 hours, for co-feeding, and more than one night, for co-roosting, elapsed between observations of vultures within 25 meters of each other.

RESULTS

We examined direct and indirect social interactions of griffon vultures when feeding and roosting (**Figure 1a-b**, **Table 1-3**). To infer the four interaction types (co-feeding direct/indirect and co-roosting direct/indirect) we used GPS-tracking data from the 14-day period preceding the capture and sampling of vultures for mycoplasma (**Figure 1c**). During the two years of the study (2021-2022), there were seven capture events in which vultures were sampled for mycoplasma, resulting in 28 social networks (**Table 1**). In our tracking dataset, based on the criteria we applied, we observed a total of 106 individuals interacting while feeding and 114 individuals interacting while roosting. Of these, 76 unique individuals were sampled for pathogens. We examined the relationship between social behavior and infection status, considering mycoplasma identification at the genus level. Subsequently, mycoplasma samples were identified to species (for more details see Table S2; Anglister et al., 2024), however, only one species (*M.* sp. strain 005V) had high enough prevalence to warrant further examination of the relationship between social behavior and infection status with a particular mycoplasma species.

Table 1. Sampling date, social network size (i.e., the number of the griffon vultures tracked within a 14-day period leading up to pathogen sampling), and prevalence of mycoplasma at each sample day.

Sampling date	Social network size				Individuals sampled for mycoplasma			
	Direct interactions		Indirect interactions		Number sampled	Negative	Positive	Prevalence
	Feeding	Roosting	Feeding	Roosting				
1 (2021-09-13)	27	28	27	27	2	0	2	100%
2 (2021-09-29)	46	48	39	49	2	0	2	100%
3 (2021-10-07)	60	71	46	65	24	4	20	83%
4 (2021-10-22)	67	69	69	70	2	0	2	100%
5 (2021-11-09)	58	58	42	59	17	4	13	76%
6 (2022-09-03)	66	79	58	79	5	0	5	100%
7 (2022-11-03)	70	79	69	80	24	12	12	50%
Average:	56.285	61.714	50	61.285	10.85	2.85	8	-

In contrast to our expectations, vulture infection with mycoplasma was not related to social position in any type of interaction network (**Figure 2**, **Table 2**). This was the case even

after combining direct interactions when feeding and roosting into a single network (**Figure 3a-c**, **Table 2**), and when combining indirect interactions when feeding and roosting into a single network (**Figure 3d-f**, **Table 2**). We further did not find a significant relationship between infection status and age, although juveniles were slightly, but not statistically significantly, more likely to be infected than adults (**Table 2**). When considering infection with the most prevalent mycoplasma species in the tested population, *M.* sp. strain 005V*,* we still did not find an impact of social position in any type of interaction on the infection status (**Figure 4**, **Table 3**).

In most statistical models examining infection with the mycoplasma genus, we found that some variation in infection status was attributed to the sampling date. The random effect "sampling date" accounted for 45% (sd± 0.671) of the variance in the model for co-feeding direct interactions. For models of indirect co-feeding interactions, the random effect "sampling date" accounted for 0.01% (sd± <0.0001) model variance. For models of direct co-roosting interactions, the random effect "sampling date" accounted for 35.9% (sd± 0.599) model variance. For models of indirect co-roosting interactions, the random effect "sampling date" accounted for 47.1% (sd± 0.686) model variance. In the models of the aggregated network (co-feeding + co-roosting) the random effect "sampling date" accounted for 49.1% (sd± 0.701) of the variance in the model for direct interactions, and 99.1% (sd± 0.995) of the variance in the model for indirect interactions. However, the models that considered only *M*. sp. strain 005V infection status the random effect "sampling date" exhibited zero variance and standard deviation for all types of interactions, both direct and indirect, in both co-feeding and co-roosting interactions.

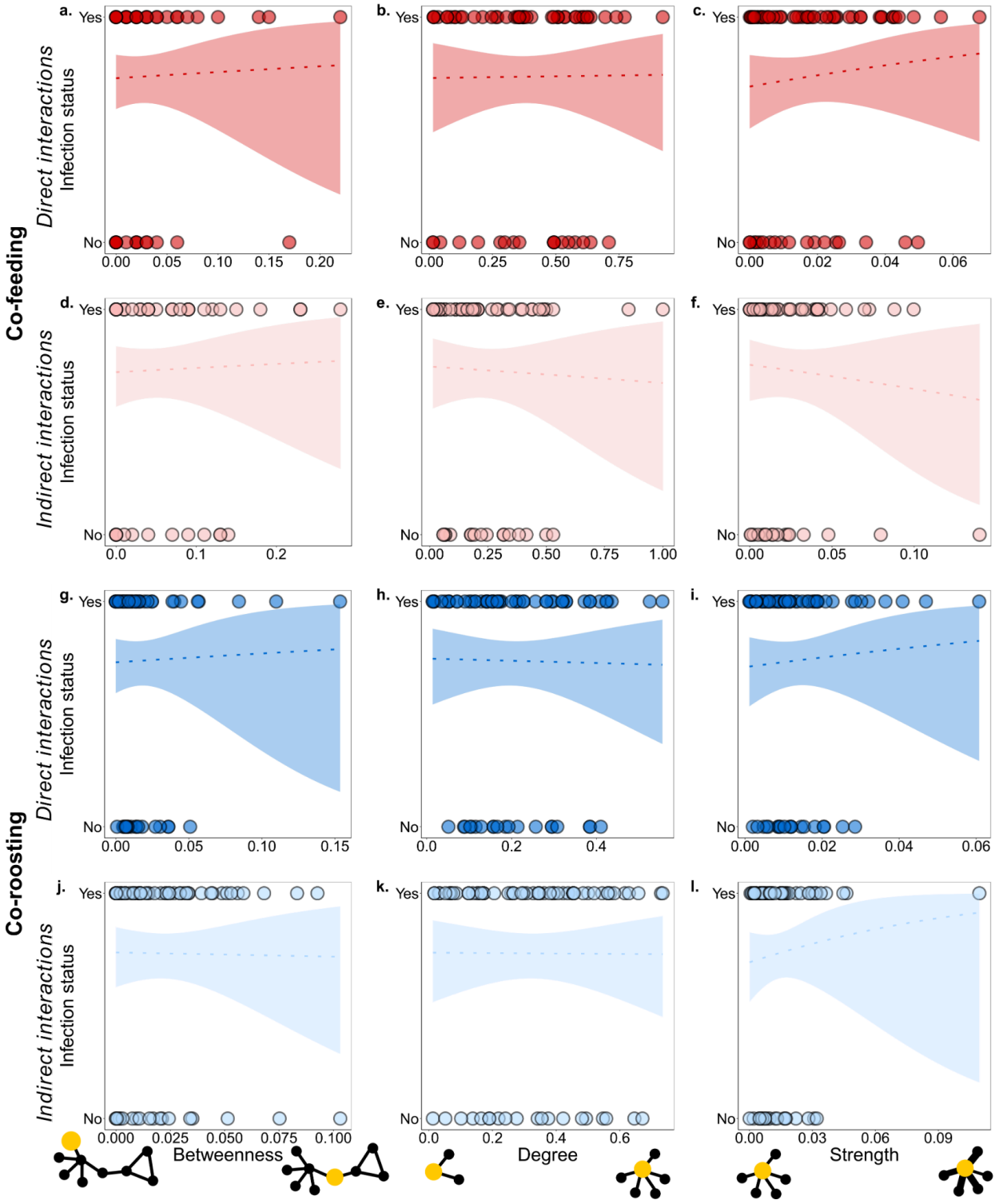

Figure 2. Relationship between social position (betweenness (a, d, g ,j), degree (b, e, h, k), and strength (c, f, i, l)) of griffon vultures (*Gyps fulvus*) and infection with mycoplasma. We examined both direct (a-c, g-i) and indirect (d-f, j-l) interactions when vultures were co-feeding (a-f) or co-roosting (g-l) during the 14 days before they were sampled for mycoplasma.

Table 2. Results of the binomial generalized linear mixed model (GLMM) testing the relationship between mycoplasma infection status and social position (degree, betweenness, and strength) of griffon vultures.

Social situation	Type of interaction (sample size)	Fixed effect	Estimate	Standard error	z-values	p-value
Co-feeding	Direct (N= 68)	Intercept	0.582	0.769	0.757	0.449
		Degree	0.431	2.047	0.211	0.833
		Strength	10.323	26.500	0.390	0.697
		Betweenness	-1.150	7.322	-0.157	0.875
		Age (Juvenile)	1.047	0.632	1.657	0.097
	Indirect (N= 63)	Intercept	0.530	0.582	0.911	0.362
		Degree	0.392	1.670	0.235	0.815
		Strength	-4.540	10.910	-0.416	0.677
		Betweenness	0.967	4.495	0.215	0.830
		Age (Juvenile)	1.057	0.626	1.689	0.091
Co-roosting	Direct (N=76)	Intercept	0.697	0.680	1.025	0.305
		Degree	-2.787	5.054	-0.551	0.581
		Strength	55.968	59.377	0.943	0.346
		Betweenness	-0.105	16.182	-0.007	0.995
		Age (Juvenile)	1.193	0.632	1.888	0.059
	Indirect (N=75)	Intercept	0.844	0.725	1.164	0.245
		Degree	-1.068	2.923	-0.365	0.715
		Strength	44.370	43.687	1.016	0.310

		Betweenness	-9.777	15.671	-0.624	0.533
		Age (Juvenile)	1.201	0.641	1.873	0.061
Aggregate networks	Direct (N=76)	Intercept	0.805	0.742	1.085	0.278
		Degree	0.050	1.555	0.032	0.974
		Strength	6.839	26.177	0.261	0.794
		Betweenness	4.200	17.410	0.241	0.809
		Age (Juvenile)	1.095	0.614	1.784	0.074
	Indirect (N=76)	Intercept	1.085	0.873	1.243	0.214
		Degree	1.890	1.800	1.050	0.294
		Strength	-20.539	17.170	-1.196	0.232
		Betweenness	-18.167	18.286	-0.994	0.320
		Age (Juvenile)	1.027	0.631	1.627	0.104

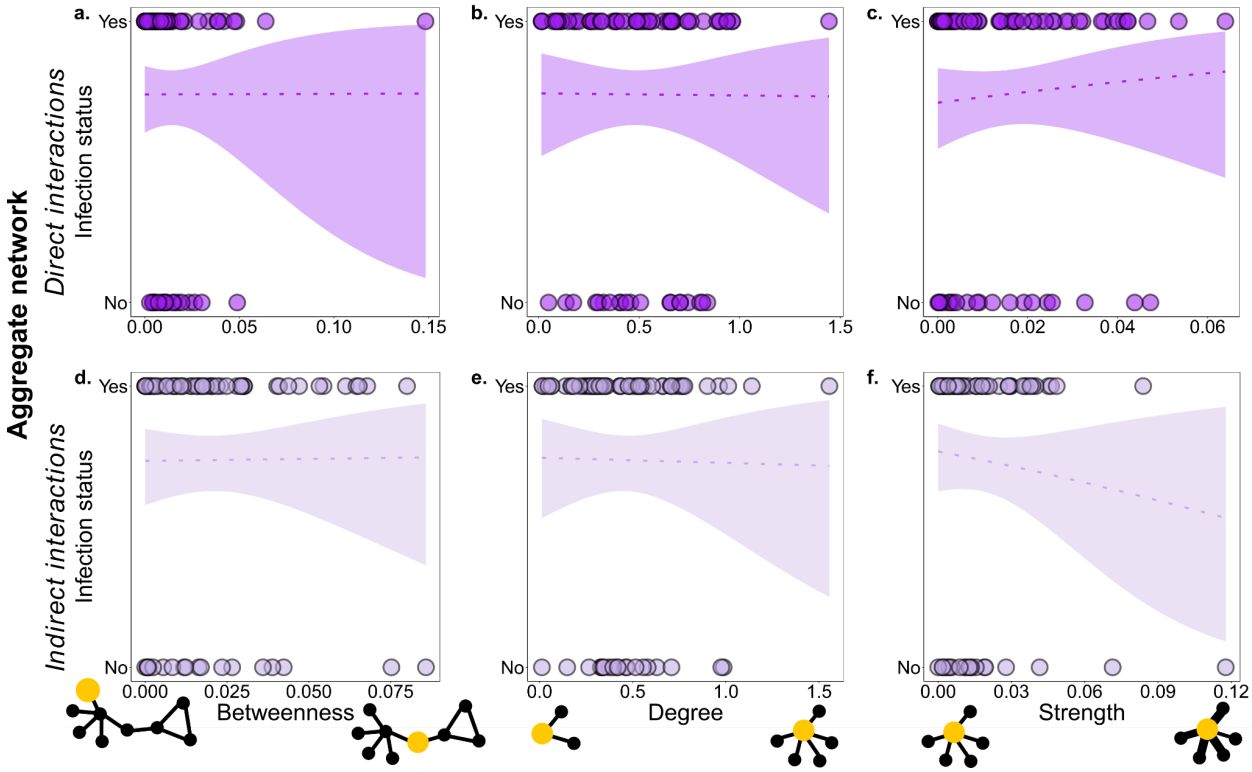

Figure 3. Relationship between social position (betweenness (a, d), degree (b, e), and strength (c, f)) of griffon vultures and infection with mycoplasma. We examined both direct (a-c) and indirect (d-f) interactions when co-feeding and co-roosting interactions were aggregated into a single network.

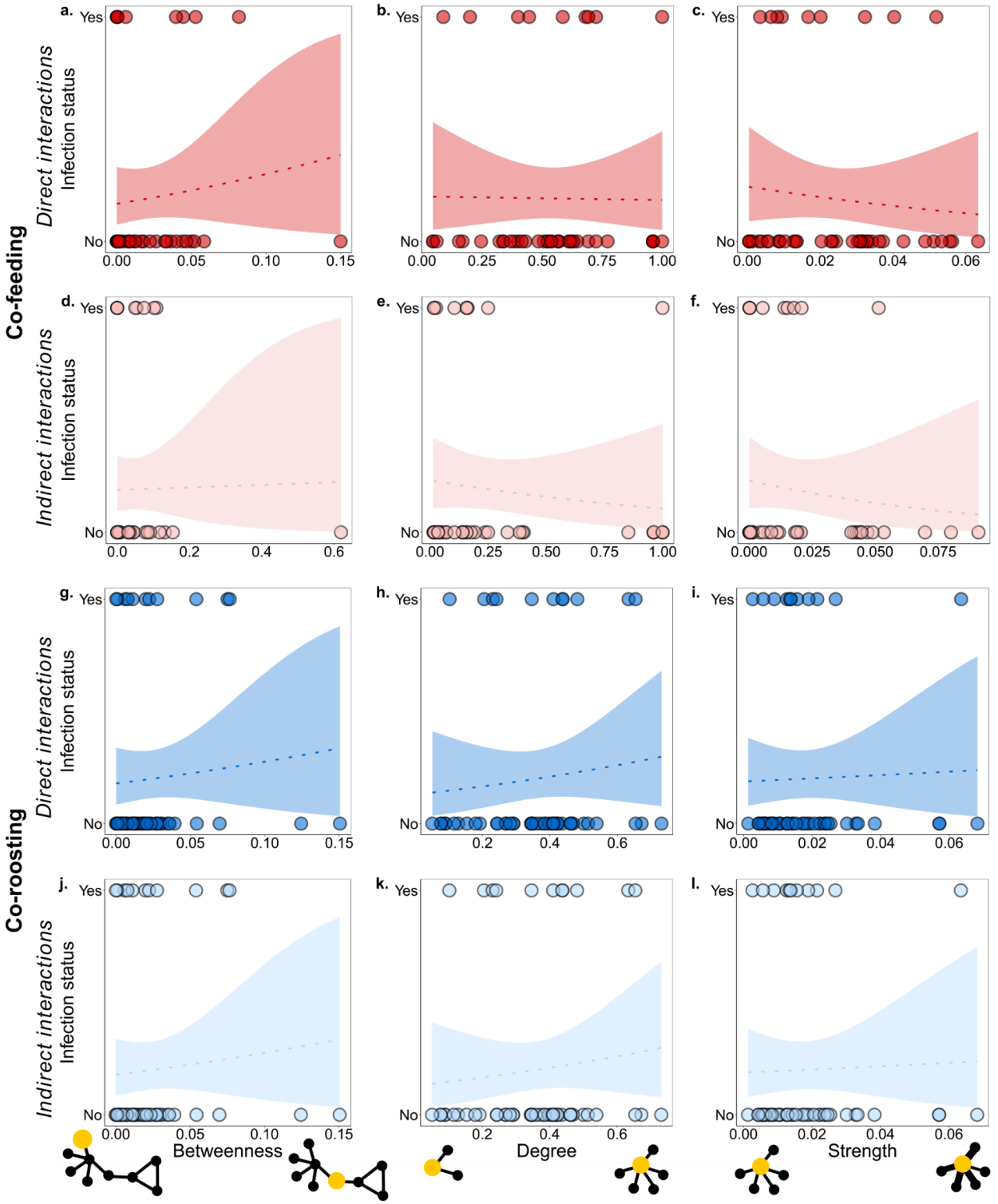

Figure 4. Relationship between social position (betweenness (a, d, g ,j), degree (b, e, h, k), and strength (c, f, i, l)) of griffon vultures and infection with *Mycoplasma* sp. strain 005V. We examined both direct (a-c, g-i) and indirect (d-f, j-l) interactions when vultures were co-feeding (a-f) or co-roosting (g-l) during the 14 days before they were tested for the presence of *M.* sp. strain 005V.

Table 3. Results of the binomial generalized linear mixed model (GLMM) testing the relationship between *Mycoplasma* sp. strain 005V infection status and social position (degree, betweenness, and strength) of griffon vultures.

Social situation	Type of interaction (sample size)	Fixed effect	Estimate	Standard error	z-values	p-value
Co-feeding	Direct (N=47)	Intercept	-1.418	0.946	-1.499	0.134
		Degree	1.210	2.095	0.578	0.564
		Strength	-24.929	33.941	-0.734	0.463
		Betweenness	5.851	12.100	0.484	0.629
		Age (Juvenile)	-0.442	0.765	-0.578	0.563
	Indirect (N=47)	Intercept	-0.945	0.609	-1.553	0.120
		Degree	-0.474	1.618	-0.293	0.770
		Strength	-9.916	23.236	-0.427	0.670
		Betweenness	0.701	3.805	0.184	0.854
		Age (Juvenile)	-0.470	0.770	-0.610	0.542
Co-roosting	Direct (N=55)	Intercept	-1.721	0.915	-1.882	0.060
		Degree	3.120	3.444	0.906	0.365
		Strength	-23.807	37.561	-0.634	0.526
		Betweenness	1.050	12.957	0.081	0.935
		Age (Juvenile)	-0.860	0.726	-1.184	0.236
	Indirect (N=55)	Intercept	-1.026	0.448	-2.289	0.022
		Degree	0.511	0.564	0.906	0.365
		Strength	-0.359	0.566	-0.634	0.526

	Betweenness	0.031	0.376	0.081	0.935
	Age (Juvenile)	-0.860	0.726	-1.184	0.236

DISCUSSION

Contrary to our predictions, we found that social behavior did not affect vultures' mycoplasma infection status. This finding held regardless of the type of interactions (direct or indirect), the social context (feeding or roosting), and the taxonomic level of analysis (mycoplasma genus level or the most prevalent strain, *M*. sp. strain 005V). This lack of relationship is likely not due to low statistical power because we sampled 60% griffon vulture population in the south region of Israel for mycoplasma (Anglister et al., 2024). Our inability to detect an effect of social interactions on infection status is likely due to the very high prevalence of mycoplasma in the population, because when a large proportion of the population is positive, it is difficult to determine how individuals become infected through social interactions and who became infected first. It is possible that the high prevalence of mycoplasma in the population is influenced by factors we did not examine, such as contaminated commonly used food or water sources, climatic conditions, or chronic carrying of the bacteria without pathology, as we discuss in more detail below.

Mycoplasma infection status was not related to social behavior during feeding or roosting, suggesting that the social interactions of griffon vultures do not impact mycoplasma infection. Our findings contrast with previous studies in which birds that were more social were also more likely to be infected with mycoplasma (Dhondt et al., 2007; Adelman et al., 2013; Adelman et al., 2015; Sawicka et al., 2020; Hawley et al., 2021; Briard & Ezenwa 2021; Langager et al., 2023). Thus, social behaviors may have different implications for mycoplasma spread across different bird and bacteria species (Sumithra et al., 2013; Sawicka et al., 2020). While Adelman et al. (2015) found that songbirds feeding with more conspecifics exhibit a higher likelihood of transmitting mycoplasma, we did not observe a relationship between the number of conspecifics with which a vulture feeds and mycoplasma infection. This difference could be explained by behavioral differences among the two host species. Species of birds differ in their social behaviors, immune responses, susceptibility to infections, and may experience different environmental conditions, all of which can influence disease prevalence and transmission. One way in which griffon vultures are different from songbirds is their robust immune system, which was likely shaped by their scavenging behavior. The physiological and

immunological characteristics of vultures (López-Rull et al., 2015) may make them less prone to pathological impacts of mycoplasma, particularly compared to other bird species, like songbirds.

There are many possible explanations for the high prevalence of mycoplasma observed in our study. Mycoplasma bacteria can be commensal and/or pathogenic. Mycoplasma can act as a commensal in the respiratory tract without causing diseases, allowing it to persist in the host population without eliciting clinical signs or causing harm (Sawicka-Durkalec et al., 2021). Such persistence in a non-harmful state can lead to chronic infection in which the bacteria is present in a large portion of the population. Indeed, several mycoplasma species associated with respiratory diseases in birds are known to cause chronic conditions (Grodio et al., 2013; Hamzah et al., 2022). Certain species of mycoplasma can be pathogenic, causing respiratory diseases, especially under certain conditions, such as compromised host immune system and hot weather (Gelfand 1993; Blount et al., 2003; Verbisck-Bucker et al., 2008; Gangoso et al., 2009). It is possible that in our study system some species of mycoplasma are commensal, while others are pathogenic (or become pathogenic at some point). This is suggested by the observation that while some griffon vultures exhibit clinical signs of respiratory diseases caused by mycoplasma, the majority do not (Anglister et al., 2024).

Pathogenic bacteria elicit the production of antibodies by the immune system, which can also explain high population prevalence of mycoplasma. Vultures are exposed to many pathogens because they consume carcasses and roost communally; therefore, they have strong immune systems (Blount et al., 2003; López-Rull et al., 2015). Strong immune systems can establish robust defense mechanisms and provide protection against mycoplasma infections. Thus, it is possible that immunity to mycoplasma is high in vultures, allowing even a pathogenic bacteria to be prevalent in the population while exhibiting only low levels of pathology. Indeed, mycoplasma prevalence is generally very high in other raptor species, for instance, it reaches 91% in nest sites of *Circus aeroginosus* and *Milvus milvus*, as well as 94% in adult birds, as reported by Lierz et al. (2008a). Additionally, in *G. fulvus*, the prevalence of mycoplasma was recorded at 47% and 70% by Blass et al. (2012) and Anglister et al., (2024), respectively. Finally, some of the high prevalence values in our study come from sampling days on which sample sizes are low - for example with two out of two sampled individuals being positive (**Table 1**), which is often a challenge in studies of infectious diseases in wildlife (Jovani & Tella 2006). Still, on the sampling dates when we had large sample sizes, we also observed high prevalence (**Table 1**), indicating that small sample sizes are not the main driver for the high observed prevalence. Further investigations into the causes underlying the high prevalence of

mycoplasma bacteria in griffon vultures might provide important information on whether there is need to manage its spread and what such management might entail.

When examining infection with bacteria at the genus level, it is not always possible to determine transmission directly, because infected individuals might be carrying different species of the bacteria. Indeed, species of mycoplasma in this system differ in their origin; e.g., some arrive with translocated individuals from the Iberian peninsula (Anglister et al., 2024). It is further possible that transmission dynamics differ among bacteria species. Our analysis primarily focused on the mycoplasma genus because the prevalence at each identified species level was too low to allow for separate analyses. If mycoplasma species differ in how social interactions impact their transmission, we would not be able to distinguish those differences in our analysis. Still, we did not find an effect of social interactions on infection with the most prevalent mycoplasma species in our study (**Figure 4** and **Table 3)**. Future work on transmission dynamics of mycoplasma in this system should focus on specific species of the bacteria.

Previous analyses showed that age can affect infection with mycoplasma in griffon vultures, with juveniles having higher mycoplasma infection rates than adults (Anglister et al., 2024). However, our analysis did not reveal such an effect of age. This difference between the two studies that examine the same population of griffon vultures can be explained by the difference in sample sizes. Anglister et al., 2024 considered a larger sample sizes, including samples of mycoplasma taken over a longer duration (2019-2022) of both captive and wild vultures, and included repeated samples of some individuals (N = 167 individuals and 244 mycoplasma samples; Anglister et al., 2024). In our study, we considered a shorter period of mycoplasma sampling (2021-2022) because only that period had sufficient information on social behavior. Furthermore, we included data only from wild individuals and considered a single bacterial sample (the first one taken) from each griffon vulture (N = 114 individuals, and 76 mycoplasma samples). Despite the smaller sample size in our study, juveniles still tended to have higher (but not statistically significant) infection rates than adults (**Table 2**). This higher prevalence in juveniles can be caused by fewer antibodies for mycoplasma compared with adults, resulting in higher detection rate of the bacteria with PCR (Anglister et al., 2024).

In conclusion, the social behavior of wild griffons does not appear to influence mycoplasma infection. Identifying the reasons behind the high prevalence of mycoplasma in the population is crucial for guiding appropriate management strategies and protecting griffon vulture. Future use of theoretical models could help explore the potential dynamics of this bacteria to develop effective control strategies to mitigate its impact. Pathogens and infectious diseases have been identified as potential contributors to population decline and species

extinction, and vaccination has been a utilized and recommended strategy to reduce the impact of infectious diseases in threatened wildlife populations (McCallum & Dobson 1995; Haydon et al., 2006; Ishfaq et al., 2020). Thus, it is essential to consider ecological and social contexts when examining disease prevalence due to their potential impact on disease spread in the population. While the social behaviors of hosts are often studied to understand the spread of pathogens, there is a research gap, especially in the perspective of pathogens, which is often neglected, primarily considering pathogen conditions (e.g., commensals becoming pathogenic, pathogens causing chronic diseases). Understanding both host social interactions and pathogen biology is crucial for developing effective control strategies.

METHODS

Study system

The Eurasian griffon vulture (*G. fulvus*) is a social scavenger that engages in frequent social interactions when feeding, roosting, resting, and flying. Over the past two decades, the species has experienced a rapid population decline in Israel, from over 500 to fewer than 180 individuals (Hatzofe 2020). To combat the population decline, the Israel Nature and Parks Authority (INPA) maintains a management program that includes food provisioning at feeding stations (e.g., goats or cow carcasses), annual population counts, captures, tracking of individuals, and pathogen sampling. In September - November, when vultures are not breeding, cages are baited with large mammal carcasses, resulting in captures of ~100 unique griffons yearly, as well as many recaptures.

Among the captured individuals, a total of 114 vultures (87 individuals in 2021 and 93 in 2022) were fitted with GPS-GSM-Accelerometer tags (Ornitrack-50 3G transmitters) using a Teflon harness in a leg-loop configuration (for more details see Nemtzov et al., 2021; Acácio et al., 2023). The GPS tags provide information on vulture location approximately every 10 minutes during the day. Vultures are active during the day and, to preserve battery, the solar-powered GPS tags operate only during daylight hours, providing one or two locations at night (for more details see Sharma et al., 2023). The high spatial and temporal resolution of the GPS information allow us to infer social interactions in different social situations based on temporal and spatial proximity (Sharma et al., 2023) (for more details see the ‘Script S2a-b’ in supplementary information S2). When vultures were captured for tagging, they were inspected for injuries or diseases and sampled for mycoplasma (76 unique vultures). Individuals are often recaptured during the season, but are usually not sampled again for mycoplasma to minimize stress. If repeated measures were conducted in a single year, we used only information about

infection status from the first sample for the year. Vulture age is determined based on the plumage molting stage. Individuals aged 0 to 4 years, characterized by a light brown neck plumage, are classified as juveniles (Duriez et al., 2011); individuals aged more than 4 years have white head and neck feathers, light brown bodies, and dark flight feathers, and are categorized as adults (Duriez et al., 2011).

Characterizing social networks from spatial and temporal data

We examined interactions only of vultures that had been GPS-tracked during the 14 days prior to sampling for mycoplasma. We included only individuals that stayed within the local geographic region of southern Israel, specifically within a 400 km radius of their tagging location. After applying these temporal and geographic filters, we retained high-quality ecological movement data for 114 vultures, representing at least 65% of Israel's vulture population and nearly all griffon vultures in the south of the country. Simulation studies show that tracking 20% of the effective population provides approximately 75% accuracy of network measures (Silk et al., 2015), thus our data likely provided very high accuracy for the network measures we quantified. We excluded from the social interaction analysis the three days during which the vultures were in the capture cage (**Figure 1c**) to account for any potential influence (e.g., social interactions imposed by cage confinement and their impact on mycoplasma transmission inside the cage) on our results. Our analysis focused on interactions that occurred during the 14 days preceding pathogen sampling and cage confinement because the incubation period of mycoplasma can range from 2 to 23 days. We took 14-days as a midpoint of this range and show in the supplementary information that our results are not sensitive to using slightly longer or shorter periods (Table S3-S4 and Figure S1-S4). Seven sampling events were included in our analysis and we constructed different interaction networks for each sampling event (see **Table 1** and S1 for information on each of these networks).

We constructed social networks for two social situations: feeding and roosting (**Figure 1a-b**). An interaction was recorded when two vultures were within 25 meters of one another, when not flying (i.e., moving at a speed of less than 5m/s), during the day for feeding interactions (**Figure 1a**) and during the night for roosting interactions (**Figure 1b**). We used a 25 meters distance threshold based on biological considerations of vulture behavior, and we show in the supplementary information that our results are not affected by using slightly different distance thresholds (Table S5-S6 and Figure S5-S8). Roosting interactions were only considered if they occurred within a known roost site, during the night, as defined in Sharma et

al., (2023). For feeding interactions, we excluded daytime interactions that occurred within known roost sites.

To distinguish between direct and indirect interactions, we used different time thresholds (**Figure 1a-c**). We considered direct co-feeding interactions if vultures were feeding within 25 meters of each other within 0-30 minutes, and considered indirect co-feeding interactions if vultures were feeding within 14 days but at least 4 hours apart (**Figure 1a**). Because vultures may stay near a feeding station for a long period (up to 4 hours), if vultures were within 25m of each other within 30 minutes and 4 hours, we did not consider those interactions to ensure that there is no ambiguity between direct and indirect co-feeding interactions. Similarly, direct co-roosting interactions were recorded if vultures roosted within 25 meters (distance threshold) of each other on the same night. Indirect co-roosting interactions were recorded if vultures roosted within 25 meters of each other more than one night apart but less than 14 nights apart (**Figure 1b**). To quantify the edge weight between pairs of vultures (strength of associations) we used a simple ratio index, defined as the number of occasions two vultures were observed together divided by the total number of occasions when both birds had a recorded GPS location (Ginsberg & Young, 1992).

To examine interactions in both social situations together (co-feeding and co-roosting combined) we created an aggregate network (Finn et al., 2019). The weight of each interaction in the aggregate network was the sum of the weights of interactions in the co-feeding and co-roosting situations. For example, consider two vultures, *i* and *j*, with an edge weight of 2 when co-feeding and an edge weight of 3 when co-roosting. In the aggregate network, the edge connecting *i* and *j* would have a weight of 5 representing the cumulative interactions when both feeding and roosting.

Quantifying social role of individuals

To determine the social position of individuals within the social network, we used individual-level centrality measures (Wey et al., 2008; Pinter-Wollman et al., 2014). We used *betweenness* to quantify the extent to which a vulture serves as a bridge or intermediary between other individuals (Freeman 1991). An individual with high betweenness is likely to facilitate the rapid spread of a pathogen (Wasserman & Faus 1994; Perkins et al., 2009). We used *degree* to quantify the number of unique individuals that a vulture interacted with (Krapivsky et al., 2001). A vulture with high degree is exposed to more individuals, and their pathogens. We used *strength* to describe the frequency of interactions of each vulture (Poisot et al., 2012). An individual with high strength has more social interactions and therefore potentially

more pathogen exposure opportunities. To account for different network sizes in the 7 different sampling days, we normalized the centrality measures by using the "normalize" argument for betweenness and degree in the respective functions in '*igraph*'. This normalization divides degree or betweenness by the number of individuals in the network minus one. To normalize strength we divided individual strength by the total strength of all edge weights in each network. Network analysis was conducted using the "*igraph*" R package (Csardi & Nepusz 2006).

Mycoplasma data

We sampled 76 griffon vultures (out of the 114 GPS-tracked individuals used to analyze social interactions) for the presence and absence of mycoplasma (**Table 1**, S1, S2). We collected samples from the vultures' choanal or tracheal mucosa using a sterile swab and stored them at -20°C until DNA extraction. The DNA was extracted directly from individual choanal/tracheal swabs by agitating them vigorously in 1 ml of PBS (Sigma, Rehovot, Israel). Genomic DNA was then extracted from 400 µl of PBS solution using the Maxwell DNA Isolation Kit for Cell/Tissue and the Maxwell® 16 apparatus (Promega), following the manufacturer's instructions.

The extracted DNA was amplified using the forward GPF primer (5' GCT GGC TGT GTG CCT AAT ACA 3'; Lierz et al., 2007) and the reverse MGSO primer (5' TGC ACC ATC TGT CAC TCT GTT AAC CTC 3'; Van Kuppeveld et al., 1992). The PCR reactions were performed in 25 µl volumes, consisting of 0.5 µl of Phire Hot Start II DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA), ×5 Phire reaction buffer, 1 µl of 10 mM dNTPs, 0.4 µM of each primer, and 5 µl of DNA. The PCR amplifications were carried out using a C1000 Touch™ Thermal Cycler (Bio-Rad, Hercules, CA, USA).

The amplification was conducted procedure as outlined by Lierz et al., (2007) with a slight modification: initiating incubation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 66°C for 30 seconds, and synthesis at 72°C for 1 minute. The process concluded with a final extension at 72°C for 5 minutes. DNA of *M. falconis* was used as a positive control while nuclease free water (Sigma, Rehovot, Israel) served as a negative control.

The amplified PCR products were separated in a 1% agarose gel and visualized using ethidium bromide staining and ultraviolet transillumination. A biomarker (bp-100 Bio-Rad, Hercules, CA, USA) was used to determine the size of DNA fragments. The positive PCR samples were purified using the MEGAquick-spinTM -spin PCR & Agarose Gel DNA Extraction System (iNtRON Biotechnology) and subjected to Sanger sequencing (Hylab Ltd, Rehovot,

Israel) using the Applied Biosystems DNA sequencer and the ABI BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA). The sequence editing, consensus generation, and alignment construction were conducted using Lasergene software (version 5.06/5.51, 2003, DNAStar, Inc., Madison, WI), and Geneious software version R9 (https://www.geneious.com/academic/). Additionally, we compared the nucleotide sequences of the resulting amplicons with data deposited in GenBank (for more details see S1, Table S2 and Anglister et al., 2024). Finally, we measured the prevalence of mycoplasma (genus and species) on each sampling date. The prevalence was calculated by dividing the number of individuals infected with mycoplasma by the total number of sampled individuals, and then multiplying the result by 100 to express it as a percentage (Bush et al., 1997).

Statistical analysis

To determine the relationship between social position and infection status we used generalized linear mixed models (GLMMs) with a binomial distribution of errors (Bates et al., 2014; Zuur et al., 2009). We ran a separate model for each type of interaction (co-feeding direct, co-feeding indirect, co-roosting direct, co-roosting indirect, aggregate direct, and aggregate indirect) resulting in 6 statistical models when examining infection with bacteria from the mycoplasma genus. We further ran 4 additional models for the first 4 interactions types listed above to examine infection with the species *M*. sp. strain 005V. Infection status (yes/no) was the response variable, and the centrality measures betweenness, degree, and strength were the fixed effects. We incorporated age (juvenile/adult) as a fixed effect in the model to account for the impact that age might have on infection status, which has been observed in other studies (Anglister et al., 2024). We included the sampling date as a random effect in all models to account for variation that might be introduced by sampling vultures on different days. We determined if the underlying model assumptions were met by examining collinearity of fixed effects, random effects distribution, homoscedasticity, independence, and normality of residuals (Zuur et al., 2009). Before analyses, we tested all of the variables and did not find collinearity using a variance inflation factor test (VIF <3). For more details about the GLMM analysis see **Tables 2-3** and S3-S4. In addition, we applied the Bonferroni correction to the GLMMs models to account for multiple comparisons - we ran 6 models so we considered only p-values smaller than 0.0083 to be statistically significant. We conducted all statistical analysis in R version 4.3.1 (R Core Team, 2021) using the '*DHARMa*' (Hartig & Hartig 2017), '*lmer4*' (Bates et al., 2014), '*Performance*' (Lüdecke, et al., 2020), and '*Stats*' (R Core Team, 2018)) packages. Data and analysis code can be found at https://github.com/elviradbastiani/MycoplasmaProject_2023.

Ethical statement

This research was conducted with permissions from Israel Nature and Parks Authority (permit #2020/42529). All samples were collected as part of annual health inspection, tagging, and telemetry tracking for monitoring and management, and no special designated captures were made for this study.

DATA AVAILABILITY STATEMENT

Data is provided as part of the supplementary information, and analysis code is available on GitHub (https://github.com/elviradbastiani/MycoplasmaProject_2023).

Author contributions

Elvira D'Bastiani: conceptualization; data collection; formal analysis and interpretation of data; visualization; writing - original draft, editing and review.
Nili Anglister: conceptualization; data collection; methodology; and review.
Inna Lysnyansky: methodology; and review.
Inna Mikula: methodology.
Marta Acácio: data collection; methodology; and review.
Gideon Vaadia: data collection.
Kaija Gahm: methodology; and review.
Orr Spiegel: conceptualization; interpretation of data; funding acquisition; methodology; project administration; resources; and review.
Noa Pinter-Wollman: conceptualization; formal analysis and interpretation of data; funding acquisition; methodology; project administration; resources; supervision; writing - editing and review.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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