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Brief report

Prevalence of blood parasites in two western-Mediterranean local populations of the Yellow-legged Gull *Larus cachinnans michahellis*

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1. Introduction

Historical information on haematozoan parasites of gulls is scarce. Peirce (1981a) reported on the absence of blood parasites in small samples of several gull species (Black-backed Gull *Larus fuscus*, Herring Gull *L. argentatus*, Great Black-backed Gull *L. marinus*, Common Gull *L. canus* and Kittiwake *Rissa tridactyla*) and a low prevalence of *Haemoproteus* sp. in the Black-headed Gull *Larus ridibundus* (1 individual out of 22 sampled). More recently Ruiz *et al*. (1995, 1998) reported on the prevalence of *Haemoproteus lari* in Audouin's Gull *Larus audouinii* and Yellow-legged Gull *Larus cachinnans michahellis* in the Ebro Delta and Chafarinas Islands colonies. Both host species had higher prevalences and intensities of parasitism at the Chafarinas Islands (4.5 km off the Moroccan Mediterranean coast) than at the Ebro Delta (on the mainland of eastern Spain), which was attributed to higher densities or activity of Haemoproteus vectors (ceratopogonid flies of the genus Culicoides) in the former location (op. cit.). Bosch *et al*. (1997) also found a high prevalence and a high intensity of a *H. lari* parasitemia in the Medes Islands, located 0.9 km off the coast (NE Spain).

Here we compare data on the prevalence and intensity of a *H. lari* parasitemia infecting the sedentary Yellow-legged gull, during one breeding season in two small colonies located at different distances from the Mediterranean coast of Spain.

2. Material and Methods

2.1. Study areas

The Columbretes archipelago is formed by a 19 hectares volcanic outcrop (comprising 3 major islet groups) located ca. 57 km off the coast of Castellón (39°54´N, 0°41´E) close to the edge of the wide continental shelf of Castellón, eastern Spain. Gulls breed mainly on the largest of the islands (Columbrete Grande) with an area of about 13 ha. Mean annual rainfall is 265 mm and mean annual temperature is 16.8 °C. The vegetation is mainly composed of small shrubby species like *Suaeda vera* (J. F. Gmelin), *Lycium intrincatum* (Boiss.) and *Asparagus horridus* (L.). On the main island, vegetation has been severely altered in the past by human activities (fires, agriculture, livestock) from a climax composed of *Pistacia lentiscus* (L.), *Chamaerops humilis* (L.), *Smilax aspera* (L.), *Lavatera arborea* (L.) and *Medicago citrina* (Bolòs & Vigo). The only sources of freshwater on the island are two water tanks that collect water from rainfall.

The island of Benidorm (6.5 ha.) is a limestone outcrop located approx. 3 km off the coast of Benidorm (38°30´N, 0°08´E), Alicante, 165 km South of the Columbretes Islands. Mean annual rainfall is approximately 300 mm and annual temperature fluctuates from 12 °C in January–February to 27 °C in July–August. The vegetation is dominated by small shrubs like *Lycium intrincatum* (L.), *Whitania frutescens* (L.), *Ephedra fragilis* (Desf.), *Salsola oppositifolia* (Desf.) and *Atriplex prostrata* (Boucher *ex* DC) with some scattered wild olives *Olea europaea* (L.) and abundant exotic vegetation like *Opuntia maxima* (Miller). There are no sources of freshwater on the island.

2.2. Parasite sampling procedure

All gulls were captured by placing traps on the nests. The gulls approaching their nests triggered traps. Trapping took place during the incubation period of 2000. A total of 22 gulls were captured and sampled at the island of Benidorm and 13 gulls were trapped at the Columbretes Islands. In both cases the sex ratio of the captured birds was approximately 1:1. All gulls captured had fully developed adult plumage (i.e. they were all at least 4 years old).

For all captured birds measurements of the length of wing, tarsus, tail, head and bill as well as body mass were taken (Bosch 1996). Birds were immediately released after measurements and samples were taken. A drop of blood was obtained from the ulnar vein and immediately smeared, air dried and fixed in absolute ethanol for one minute within the next few hours. Samples were stained with Giemsa solution in the laboratory fifteen days after collection.

Parasite presence was established by inspecting 100 fields containing about 100 erythrocytes each. Intensity was established by counting the number of infected red cells in 40 fields that is on the basis of approximately 4000 red cells. All smears were scanned twice by one observer (AM) and once by two other observers (SM, BE). Blood parasites were screened microscopically using 1000× oil-immersion magnification. One of the observers (SM) followed a different screening method (see Merino & Potti 1995, Merino *et al.* 1997) but results were consistent between methods.

2.3. Vector sampling procedure

During the first fortnight of September 2000 vectors were sampled by means of light traps (CDC or Communicable Disease Center style) baited with carbonic ice (Reisen *et al.* 1999). Traps were placed at Columbretes (main island), Benidorm Island and the garbage dump of the city of Benidorm (during its final stage of sealing). One trap was placed at each location. Traps were active during three consecutive nights at Columbretes and during one night at the other two sampling sites.

2.4. Sex, body condition and mean egg volume

Sex was determined according to the table of mean body measurements for males and females compiled by Bosch (1996) for birds from the Medes Islands (NE, Spain). Body condition was estimated by using the residuals of a tarsus length on body mass as well as the ratio of body mass to tarsus length. Mean egg volume was calculated as the arithmetic mean of the volume of all eggs in a clutch.

2.5 Clutch size

Mean clutch size could not be calculated based on the overall census of the colonies because dates of Columbretes surveys were too late in relation to the breeding season (20–25 April 2000) and many eggs had already hatched. Hence, we obtained mean clutch size from a random sample of nests(n = 51 at Benidorm and n = 22 at Columbretes)which were monitored throughout the laying and incubation periods every 2–3 days. We used the data collected on a date centered within the laying period (16 April 2000). At that time, the peak of mean clutch size is reached (i.e. most nests have full clutches and hatching is about to start).

2.6. Statistics

The independence of prevalence and location was tested by means of a 2 × 2 contingency table with Yate's correction. The relationships between body condition, mean egg volume and intensity of infection were tested using the Pearson correlation coefficient. The associations of intensity with sex and intensity with location as well as the association of body condition with locality were tested using the Mann-Whitney U test, two-tailed (Zar 1999). The relationship between distance to the coast and prevalence was analyzed by means of the non-parametric correlation coefficient.

3. Results

Two blood parasite species were found infecting gulls: *Haemoproteus lari* (Yakunin 1972) (see Peirce 1981b), and *Babesia bennetti* (Merino 1998) in Yellow-legged Gulls. *B.bennetti* was only detected infecting one bird from Benidorm Island. Overall prevalence (number of individuals infected by *Haemoproteus lari* over total number of individuals sampled) was 100% for Benidorm Island gulls and 38.5% for Columbretes gulls. Prevalence for Benidorm males (infected males/ total number of gulls sampled) was 45.5% and 23.1% for Columbretes Islands males. Prevalence was 54.5% for Benidorm females (infected females/total number of gulls sampled) and 15.4% for Columbretes females. Data for both sexes were analyzed together as there were no significant differences between sexes in prevalence since all birds from Benidorm were infected (10 males and 12 females) as well as 3 males and 2 females from Columbretes Islands (χ^2_1=0.00, P = 1.00). Differences in intensity between sexes could not be tested for Columbretes gulls (intensities recorded were 1–2–2 infected cells/4000 red cells for the three infected males and 3–2 for the two infected females) due to the small sample size but there were no significant differences in intensity of infection between sexes for Benidorm gulls (U = 58.5; ns, Table 1). Hence, a 2 × 2 contingency table was used to test the independence

Table 1. Prevalence (individuals infected by *H. lari*/examined), intensity of infection (number of infected red cells in 40 fields) by *H. lari* and body condition (ratio of body mass to tarsus length) for Yellow-legged gulls from Benidorm and Columbretes Islands.

Sex	Prevalence	Intensity (SD)	Body Condition (SD)
BENIDORM ISLAND			
Males	45.5%	22.8 (32.40)	15.41 (1.97)
Females	54.5%	42.0 (79.86)	14.62 (0.89)
Both	100%	33.3 (62.34)	14.98 (1.49)
COLUMBRETES ISLANDS			
Males	23.1%	1.7 (0.58)	14.93 (1.97)
Females	15.4%	2.5 (0.70)	15.95 (4.39)
Both	38.5%	2.0 (0.71)	15.34 (2.66)

between status of infection (prevalence) and locality. The results of the contingency analysis show that gulls from Benidorm Islands had significantly higher prevalences than gulls from Columbretes Islands (Fisher's exact test, $\chi^2_1 = 14.2$, $P < 0.0001$). A similar result appeared for median intensities of infection, with Benidorm gulls showing much higher intensities than those from Columbretes ($U = 89.5$, $P < 0.05$).

Intensity of infection for birds from Benidorm was not correlated with body condition (males: $r_s = 0.12$; $P = 0.60$, females: $r_s = 0.06$; $P = 0.84$). Intensity was also not correlated with mean egg volume at Benidorm ($r_s = -0.10$, $P = 0.77$). The relationships between intensity and body condition and intensity and egg volume for Columbretes gulls could not be analyzed due to the small sample size. There were no differences in median body condition for gulls (infected + noninfected) from the two islands either using ratios ($U = 161$, $P = 0.83$, Table 1) or linear regression residuals ($U = 146$, $P = 0.51$) as a measure of body condition. Mean clutch size (±SD) was 2.50 ± 0.74 ($n = 22$) at Columbretes and 2.71 ± 0.54 ($n = 51$) at Benidorm ($U = 465$, $P = 0.162$). We found a strong inverse correlation between distance to the coast and prevalence after pooling our data from Columbretes and Benidorm with those from Chafarinas Islands (Ruiz *et al*. 1995, Ruiz *et al*. 1998) and Medes Islands (Bosch *et al*. 1997) ($r_s = -0.71$, $P < 0.001$). Data from Chafarinas and Medes Islands were collected during the breeding seasons (month of April) of 1994 and 1995. Data from the Ebro Delta were not included in the analysis because we restricted the comparison to sites with similar ecological features (Western Mediterranean islands).

Haemoproteus vector (Culicoides) was trapped neither at Columbretes nor at Benidorm (island and garbage dump). However, we collected three female *Phlebotomus papatasi* and 1 female *Phlebotomus perniciosus* (Familiy Psychodidade) at the garbage dump of Benidorm, besides 5 *Culex pipiens* female mosquitoes. Phlebotoms are known to be the vectors of Leishmania (indicating a richer hematophagous diptera community at the dump than at both islands) and its role as possible vectors of Haemoproteus should be studied. We also trapped 7 female *Aedes mariae* (or *A. caspius*) at Benidorm Island. No haematophagous insect was trapped at Columbretes.

4. Discussion

Yellow-legged Gulls from Benidorm Island were more frequently parasitized and showed much higher parasite loads than gulls from Columbretes Islands. Our results suggest differential prevalence and intensity of parasitemia of *Haemoproteus lari* between the two islands. In addition, another blood parasite, *Babesia bennetti*, was present in gulls from Benidorm Island (Merino 1998). At least two hypotheses may explain these differences.

First, parasitism may be linked to intrinsic factors such as breeding effort or physical condition, as previously shown for other bird species (see for example Apanius *et al*. 1994, Merino *et al*. 2000, Oppliger *et al*. 1995, Oppliger & Christe 1996, Siikamäki *et al*. 1997), probably implying an energetically based trade-off between prevalence and increased breeding effort. Our data on Yellow-legged Gulls do not support that hypothesis since birds from Benidorm and Columbretes differed in prevalence of blood parasites despite mean clutch size and body condition was similar between islands.The fact that body condition or mean egg volume of infected females from Benidorm were not significantly correlated with intensity of parasitemia suggests that intensity of infection is not related to reproductive effort (but most likely to individual background of exposure to parasite vectors) although Bosch *et al*. (1997) found that more heavily infected females tended to lay smaller clutches and to be in leaner body condition at Medes Islands. Alternatively, the absence of a relationship between intensity and body condition also may be interpreted as Haemoproteus parasites having little effect on the health of gulls.

Secondly, prevalence may be determined by extrinsic factors like vector abundance. Recently, Sol *et al*. (2000) presented both observational and experimental evidence that vector abundance is the major influencing the spatial variation in prevalence of *Haemoproteus columbae* in pigeons. In fact, the general absence of haemoparasite infections in seabirds has been attributed to the lack of vectors in oceanic islands (Bennett *et al*. 1992). The abundance of vectors may be higher closer to the mainland (as our trapping results suggest) thus explaining the pattern found for Yellow-legged Gulls from Benidorm and Columbretes Islands and also the strong inverse relationship between distance to the coast and prevalence among the four islands analyzed. The proximity to the mainland may account for more frequently and more intensely infected gulls from Benidorm Island. Conversely, gulls from Columbretes may be less frequently and less intensely parasitized due to the long distance from the islands to the coast. In addition, gulls from Benidorm Island are known to frequently use the large garbage dump of the city of Benidorm as a feeding ground (own data), where vectors could find suitable places for reproduction, like wet sediments with high levels of organic matter and low salinity (Blackwell *et al*. 1994, Lardeux & Ottenwaelder 1997). The same factor (absence of suitable places for vector breeding) may also explain the low prevalence reported by Ruiz *et al*. (1995) at the Ebro Delta, together with the fact that Yellow-legged gulls from the Ebro Delta do not make common use of refuse dumps since they can find abundant food in secondary foraging habitats (rice fields, sandy beaches) during periods of fishing moratorium (Bosch *et al*. 1994, Oro *et al*. 1995). Similarly, the high prevalence of gulls from the Medes Islands might be explained by the common use of nearby refuse tips as feeding grounds (Bosch *et al*. 1994, 1997). Finally, Yellow-legged Gulls from the Chafarinas are also known to exploit waste food from refuse tips when purse-seine fisheries do not operate (González-Solís *et al*. 1997 a, b).

Yellow-legged Gulls from Columbretes also visit garbage dumps on the mainland (probably during weekends and other periods during which the fishing trawler fleet is not active besides periods of dispersal) since some non-floating litter remains have been found scattered around the colony (authors, unpubl.). However, visits are considerably less frequent than in the case of gulls from Benidorm owing to the longer distance between Columbretes and the continental coast. Since intensity seem to be mostly influenced by individual background of exposure to parasites (see Allander & Bennett 1994), it is likely that the low intensities detected at Columbretes are explained by the fact that only older birds make use of resources from the mainland whereas all birds have an easy access to food resources at Benidorm.

Thus, prevalence of *Haemoproteus lari* in Yellow-legged gulls from the two colonies under study seems to depend on vector availability, which is influenced by distance to the coast. The fact that neither prevalence nor intensity was associated with sex of Benidorm gulls indicates a similar degree of exposure to vectors, probably due to a similar time spent by males and females on the mainland. Finally, we do not have any information on the presence and abundance of gull ticks (the supposed vectors of *Babesia*) at Columbretes. However, ticks are also common in other gulleries (Bosch & Figuerola 1999) and we have detected them infecting the brood patch of some adult gulls in Benidorm. A higher sample size might confirm the absence of this parasite in Columbretes, although the parasite may pass unnoticed, confounded with immature stages of infection by *H. lari*.

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Selostus: **Veriloisten esiintyminen kahdessa Välimeren länsiosan etelänharmaalokkipopulaatiossa**

Veriloisten esiintymisestä lokkipopulaatioissa on olemassa vain vähän tietoa. Kirjoittajat tutkivat Espanjassa paikkalintuna esiintyvän etelänharmaalokin infektoitumista *Haemoproteus lari* veriloisella Välimeren rannikolla. Taustamuuttujiksi kerättiin tietoja lokkien kunnosta ja pesimämenestyksestä. Aineisto kerättiin kahdelta saarelta, Columbretesin ja Benidormin saarilta. Columbretisin saari sijaitsee 57 kilometrin etäisyydellä mantereesta ja Benidormin saari sijaitsee 3 kilometrin etäisyydellä mantereesta. Columbretesin saarelta kerättiin pesimäaikana näytteet 13 lokista ja Benidormin saarelta 22 lokista. Kaikki yksilöt olivat aikuisia lintuja ja näytteiden sukupuolijakauma oli tasainen. Tuloksia verrattiin muilta alueilta kerättyihin aineistoihin. Kerätyistä näytteistä löytyi kaksi veriloislajia, *H. lari* ja *Babesia benetti*. Kaikki tutkitut Benidormin lokit olivat infektoituneita. Columbretesin lokeista oli infektoituneita 38.5 %. Sukupuolten välillä ei havaittu eroja infektoitumisasteessa. Tulosten mukaan lähempänä mannerta sijaitsevan Benidormin saaren lokkien infektoitumisaste ja infektion voimakkuus oli suurempi kuin Columbretesin saaren lokkien. Saarten välillä havaitut erot infektoitumisasteessa voivat selittyä lintujen kunnossa vallitsevilla eroilla tai saarten välisillä eroilla veriloisten väli-isäntien runsaudessa. Pesyekoossa ei kuitenkaan havaittu eroja Columbretesin ja Benidormin saarten välillä. Benidormin lokkien kunnon ja infektion intensiteetin tai munan koon välilä ei havaittu merkittäviä korrelaatioita. Havainnot viittaavat siihen, ettei veriloisinfektio vaikuttanut lokkien pesimämenestykseen. Kun aineistoon liitettiin aiemmin kerättyä tietoa Välimeren alueen etelänharmaalokkien infektoitumisasteesta Chafarinaksen ja Medesin saarilta, havaittiin infektoitumisasteen kasvan mitä lähemmäs mannerta tultiin. Lähellä mannerta pesivät lokit voivat infektoitua myös ruokaillessaan mantereella sijaitsevilla kaatopaikoilla. Kirjoittajat päättelevät, että veriloisille sopivien väli-isäntien esiintyminen oli tärkeä lokkien infektoitumisastetta selittävä tekijä. Veriloisille sopivia väli-isäntiä löytyy todennäköisesti enemmän lähellä mannerta sijaitsevilta saarilta sekä mantereelta kuin kauempana rannikkolta sijaitsevilta saarilta.

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