

1 **Annual bacterial epizooties impact albatross populations on a remote island**

2

3 **Electronic Supplementary Material**

4 **S1. Supplementary text and figures**

5 **1. Data collection**

6 **Additional methodological details**

7 All the Indian yellow-nosed albatrosses (*Thalassarche carteri*) included in the present study were
8 recruited as part of another study aiming at exploring the effect of an autogeneous vaccine to
9 induce a specific immune response and protection of nestlings against *Pasteurella multocida* (*Pm*)
10 on Amsterdam Island (37°49'S, 77°33'E; Bourret et al., 2018; Gamble et al. 2019b). Only birds that
11 had never been vaccinated against *Pm* were included in the present study. Most birds were part
12 of the control groups (NaCl-injected or unmanipulated, *i.e.*, observed without any handling) of the
13 *Pm* vaccination study (Bourret et al., 2018; Gamble et al., 2019b). Moreover, some birds were
14 parents of nestlings that were vaccinated against *Pm* (Bourret et al., 2018), but were not
15 vaccinated themselves, and were sampled before or soon after vaccination of their nestlings. It is
16 thus unlikely that vaccination of the nestlings could have had any impact on the probability of
17 infection of their parents by the time of sampling (which would not be the case later, *e.g.*, if
18 vaccination limits shedding or increase survival without limiting shedding). These parents of
19 vaccinated nestlings were included in the *Pm* PCR data exploration, but obviously not in the
20 nestling survival analyses.

21

22 **2. Survival analyses**

23 **Additional methodological details**

24 *Data collection and selection*

25 As albatross nestlings do not leave their nest until the first fledging attempt (late March in the
26 study population), nestlings could be individually identified without handling *via* plastic tags
27 hanged to their nests and engraved with an alphanumeric code. Yellow-nosed albatrosses usually
28 raise one nestling a year, so each nest contains maximum one nestling. Data included in the
29 nestling survival analysis were collected between the 13th of December (Time = 0 day in Table S1),
30 which fell after hatching for all the recruited nestlings but before the first sampling occasion, and
31 the 18th of March, before nestlings started fledging. Note that hatching is relatively synchronized
32 in yellow-nosed albatrosses (Pinaud et al. 2005). During each observation session, each nest that
33 previously contained a nestling was visually controlled and the presence or absence of a live
34 nestling was recorded. If a nest was observed empty, we considered that the nestling died
35 between the two observation sessions. To increase our sample size, up to 30 unmanipulated (*i.e.*,
36 visually monitored without any handling) nestlings were included in the analyses in 2014-2015,
37 2015-2016 and 2016-2017. We thus distinguish two groups of nestlings: the *Sampled* group
38 corresponds to nestlings that were handled for sampling, and the *Unmanipulated* group to
39 nestlings that have never been handled during the study.

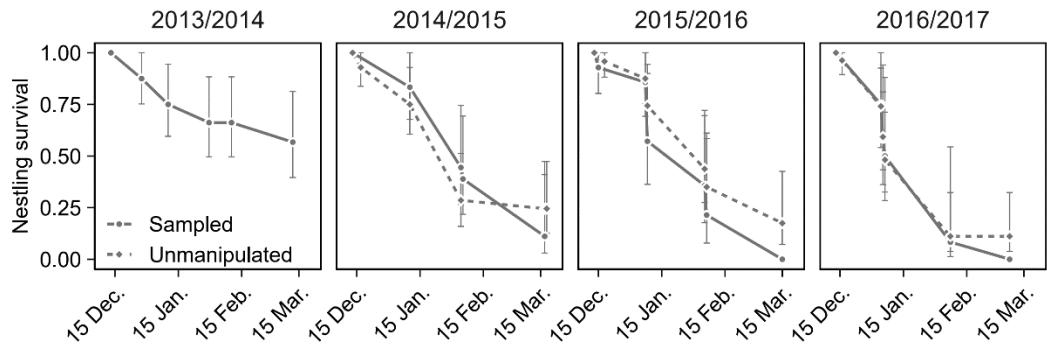
40 *Statistical analyses*

41 The potential effects of handling (sampled *versus* unmanipulated) and/or breeding season on
42 nestling survival were explored by fitting a Cox proportional hazards model (Andersen & Gill
43 1982) to the data using the “*survival*” package (Therneau and Lumley 2019). Such models are
44 classically used in biomedical studies to compare the risks of occurrence of a given event (here
45 death) among groups exposed to different factors (here handling or breeding season).

46 **Additional results**

47 None of the Cox models including an effect of handling (sampled *versus* unmanipulated) on
48 nestling survival (*i.e.*, *nestling survival ~ handling*; *nestling survival ~ handling + breeding season*;
49 and *nestling survival ~ handling : breeding season*) supported the hypothesis that handling could
50 impact nestling survival (Figure S1). All estimates for the effect of handling on nestling survival
51 were non-significantly different from zero (detailed results are available in the R notebook). As
52 we did not detect any effect of nestling handling on survival, sampled and unmanipulated
53 nestlings were grouped for all the following analyses.

54



55 **Figure S1.** Yellow-nosed albatross nestling survival during the four breeding seasons. The solid
56 line (*Sampled* group) corresponds to nestlings that were handled for sampling, and the dashed
57 line (*Unmanipulated* group) to nestlings that have never been handled during the study. Bars
58 represent the 95% confidence intervals; sample sizes are given in Table S1.

59 **Table S1.** Yellow-nosed albatross nestling survival during the four breeding seasons. The *Sampled*
 60 group corresponds to nestlings that were handled for sampling, and the *Unmanipulated* group to
 61 nestlings that have never been handled during the study. *Time* corresponds to time since the
 62 beginning of the monitoring study. # *Controlled nests* correspond to the number of nestlings at
 63 risk of death (*i.e.*, nest that contained a live nestling during the last observation session), and #
 64 *Dead nestlings* to the number of nestlings that had died among the nestlings that had been at risk
 65 of death since the last observation session.

Breeding season	Handling	Time (day)	# Controlled nests	# Dead nestlings
2013/2014	Sampled	15	24	3
2013/2014	Sampled	28	21	3
2013/2014	Sampled	48	17	2
2013/2014	Sampled	59	15	0
2013/2014	Sampled	89	14	2
2014/2015	Sampled	28	18	3
2014/2015	Sampled	53	15	7
2014/2015	Sampled	54	8	1
2014/2015	Sampled	94	7	5
2014/2015	Unmanipulated	4	28	2
2014/2015	Unmanipulated	28	26	5
2014/2015	Unmanipulated	53	21	13
2014/2015	Unmanipulated	94	7	1
2014/2015	Unmanipulated	95	2	0
2015/2016	Sampled	2	14	1
2015/2016	Sampled	25	13	1
2015/2016	Sampled	26	12	4
2015/2016	Sampled	54	8	3
2015/2016	Sampled	55	5	2
2015/2016	Sampled	92	3	3
2015/2016	Unmanipulated	5	24	1
2015/2016	Unmanipulated	25	23	2
2015/2016	Unmanipulated	26	20	3
2015/2016	Unmanipulated	54	17	7
2015/2016	Unmanipulated	55	10	2
2015/2016	Unmanipulated	92	8	4
2016/2017	Sampled	22	12	3
2016/2017	Sampled	23	9	2
2016/2017	Sampled	24	7	1
2016/2017	Sampled	56	6	5
2016/2017	Sampled	85	1	1
2016/2017	Unmanipulated	3	27	1
2016/2017	Unmanipulated	22	26	6
2016/2017	Unmanipulated	23	20	4
2016/2017	Unmanipulated	24	16	3
2016/2017	Unmanipulated	56	13	10
2016/2017	Unmanipulated	85	3	0

67 **3. Temporal variations of *Pm* prevalence**

68 **Table S2.** Prevalence of *Pm* in yellow-nosed albatrosses of Amsterdam Island. *Pm* infection was
 69 determined by PCR on cloacal swab samples (see main text). Number of individuals testing
 70 positive are indicated between parentheses; 95% Clopper-Pearson confidence intervals between
 71 brackets. Note that February and March samples were grouped on Figure 1 a in order to increase
 72 sample size and late November samples were grouped with December samples.

Breeding season	Month	Stage	Prevalence
2013/2014	December	Adults	0.22 (15/67) [0.13; 0.34]
2013/2014	December	Nestlings	0.10 (2/20) [0.01; 0.32]
2013/2014	January	Nestlings	0.00 (0/16) [0.00; 0.21]
2013/2014	February	Nestlings	0.14 (2/14) [0.02; 0.43]
2013/2014	March	Nestlings	0.36 (4/11) [0.11; 0.69]
2014/2015	December	Adults	0.60 (70/117) [0.50; 0.69]
2014/2015	December	Nestlings	0.70 (14/20) [0.46; 0.88]
2014/2015	January	Nestlings	0.64 (9/14) [0.35; 0.87]
2014/2015	February	Nestlings	0.75 (6/8) [0.35; 0.97]
2014/2015	March	Nestlings	0.50 (1/2) [0.01; 0.99]
2015/2016	December	Adults	0.12 (11/91) [0.06; 0.21]
2015/2016	December	Nestlings	0.14 (2/14) [0.02; 0.43]
2015/2016	January	Nestlings	0.62 (5/8) [0.24; 0.91]
2015/2016	February	Nestlings	1.00 (3/3) [0.29; 1.00]
2016/2017	December	Adults	0.01 (1/73) [0.00; 0.07]
2016/2017	December	Nestlings	0.00 (0/9) [0.00; 0.34]
2016/2017	January	Nestlings	0.33 (2/6) [0.04; 0.78]
2016/2017	February	Nestlings	1.00 (1/1) [0.03; 1.00]
2016/2017	March	Nestlings	0.67 (2/3) [0.09; 0.99]

73

74 **Table S3.** Selection of the models explaining the temporal variations of *Pm* prevalence in yellow-
 75 nosed albatrosses of Amsterdam Island. Variation in prevalence was modelled using a logistic
 76 regression with PCR status (negative/positive) as the response variable. Individual identity was
 77 included as a random effect, in addition to the nest for adults in order to account for a potential
 78 pair effect. Breeding season was included in the model as a categorical variable and day as a
 79 continuous variable (corresponding to the interval between the actual sampling day and
 80 November 13th of the considered breeding season). Π : prevalence; Df: degrees of freedom; AIC:
 81 Akaike Information Criterion; “+” denotes an additive effect, “ \times ” denotes an interaction. The model
 82 with the lowest AIC was selected with a threshold ΔAIC of 2; when two models had a $\Delta\text{AIC} < 2$, the
 83 more parsimonious model was selected.

<i>Stage</i>	<i>Model</i>	<i>Df</i>	<i>AIC</i>
Adults	Π (.)	3	433.4959
	Π (breeding season)	6	334.9387
	Π (day)	4	413.3875
	Π (breeding season + day)	7	334.3225
	Π (breeding season \times day)	10	339.1930
Nestlings	Π (.)	2	235.8367
	Π (breeding season)	5	206.1508
	Π (day)	3	227.4529
	Π (breeding season + day)	6	187.9314
	Π (breeding season \times day)	9	185.1702

84
 85
 86 **Table S4.** Outputs from the selected generalized linear mixed model run on the *Pm* PCR data of
 87 adult yellow-nosed albatrosses of Amsterdam Island. Individual and nest identity were included
 88 as random effects in the model.

<i>ADULTS</i>	<i>Estimate \pm standard error</i>	<i>Odds ratio [95% confidence interval]</i>
Breeding season 2013/2014 (intercept)	-1.85 \pm 0.28	-
Breeding season 2014/2015	2.24 \pm 0.34	9.43 [4.88; 18.22]
Breeding season 2015/2016	-0.14 \pm 0.43	0.87 [0.38; 2.01]
Breeding season 2016/2017	-2.43 \pm 1.04	0.09 [0.01; 0.68]

91 **Table S5.** Outputs from the selected generalized linear model run on the *Pm* PCR data of yellow-
 92 nosed albatross nestlings of Amsterdam Island. ":" denotes an interaction.

<i>NESTLINGS</i>	<i>Estimate ± standard error</i>	<i>Odds ratio [95% confidence interval]</i>
Breeding season 2013/2014 (intercept)	-4.53 ± 1.14	-
Breeding season 2014/2015	4.35 ± 1.27	78.00 [6.47; 939.99]
Breeding season 2015/2016	-0.56 ± 2.16	0.57 [0.01; 39.40]
Breeding season 2016/2017	0.06 ± 1.91	1.06 [0.03; 45.17]
Day	0.03 ± 0.01	1.03 [1.01; 1.06]
Breeding season 2014/2015 : Day	-0.02 ± 0.02	0.98 [0.95; 1.01]
Breeding season 2015/2016 : Day	0.07 ± 0.04	1.07 [0.99; 1.16]
Breeding season 2016/2017 : Day	0.02 ± 0.03	1.02 [0.97; 1.07]

93

94 **4. Longitudinal monitoring of *Pm*-PCR status**

95 **Interpretation and discussion.** This text refers to Figures 2 and 3 of the main text. Interestingly,
 96 transition from PCR-positive to -negative status was observed in 47/191 adults (191 referring to
 97 the number of individuals for which at least two samples were collected) and 9/50 nestlings. Note
 98 that adults were sampled at the inter-annual scale, while nestlings were sampled at the intra-
 99 annual scale. This observation suggests that a non-negligible proportion of individuals may clean
 100 the infection. In contrast, some individuals remain PCR-positive across series of consecutive
 101 sampling occasions. It should be noted that in 2015/2016 and 2016/2017, the epizootics
 102 (mortalities of nestlings) occurred later than in 2014/2015, thus a possible rise of prevalence in
 103 adults may have occurred without having been detected because adults were sampled before the
 104 actual outbreak. Note that adults are much less present at the colony in January and, thus more
 105 difficult to sample, but also less likely to be exposed. Samuel et al. (2005) have suggested the
 106 existence of chronic carriers in wild *Anatidae* populations based on antibody detection. The
 107 present study is the first to our knowledge to report molecular evidence supporting this
 108 hypothesis. If yellow-nosed albatrosses could indeed be asymptomatic carriers of *Pm*, it could
 109 have important consequences regarding spillover risks to the endemic Amsterdam albatross
 110 nesting a few kilometres away. The size of the Amsterdam albatross population (less than 100
 111 breeding pairs; BirdLife International, 2018) makes this species particularly vulnerable to
 112 potential disease outbreaks, urging the need to better understand the epidemiological dynamics
 113 of *Pm* in yellow-nosed albatrosses, the most abundant species of Amsterdam Island, but also in
 114 other species potentially involved in the maintenance and/or the dissemination of the bacterium
 115 on the island such as the brown skua (*Stercorarius antarcticus*; Boulinier et al., 2016; Gamble et
 116 al., 2019a) and the introduced house mouse (*Mus musculus*) and brown rat (*Rattus norvegicus*;

117 Curtis, 1983). For instance, yellow-nosed albatrosses could be the maintenance host of the
118 bacterium (Haydon et al., 2002) and brown skuas could play the role of epidemiological bridge
119 with the Amsterdam albatross population (Caron et al., 2015). The molecular tool developed in
120 the context of the present study will allow to efficiently investigate this hypothesis by screening
121 other potential hosts of *Pm* on the island. Such epidemiological data, in addition to ecological data
122 (e.g., inter-species contact rates) should help to reach the ultimate objective of describing the full
123 transmission dynamics of *Pm* within the study system.

124 **5. Histology**

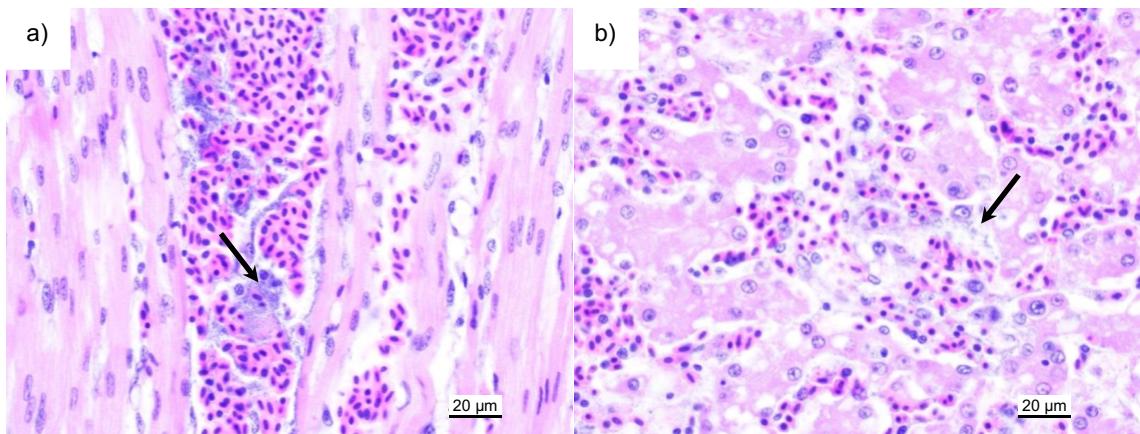
125 **Additional methodological details**

126 Dead birds opportunistically found on the colony were necropsied. Examinations of dead birds in
127 the study site are rare because of the high scavenging pressure imposed by brown skuas, brown
128 ratsskua and house mice (Gamble et al., 2019a; personal observations). Only apparently fresh and
129 intact carasses were considered. Considering the strong pressure applied on dead animals by
130 scavengers, we are confident that the delay between death and carcass detection did not exceed
131 24 hours. Necropsies were performed within a few hours after carcass detection. Tissue samples
132 from 21 yellow-nosed albatross nestlings and one adult were collected between December 2013
133 and January 2018. More specifically, heart (n = 21), liver (n = 20), kidney (n = 15), lung (n = 13)
134 and spleen (n = 5) samples were collected from those individuals, depending on the time available
135 to perform the necropsy. Tissue samples were collected and stored in 4% formaldehyde at room
136 temperature until further analysis. Samples were then routinely processed, sectionned at 4 µm
137 thickness and stained with haematoxylin-and-eosin for histological examination. When bacteria
138 were noticed on haematoxylin-and-eosin, additional Gram staining was performed for further
139 bacterial characterization.

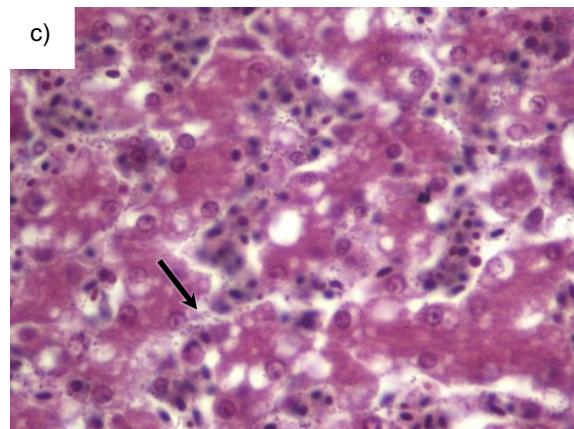
140 **Additional results**

141 Intravascular bacteria were detected in various tissues (notably liver, heart, spleen and/or lungs)
142 of nine necropsied yellow-nosed albatross nestlings sampled over the series of years (Figures S2-
143 4). These bacteria were often associated with hepatic and pulmonary congestion, haemorrhagic
144 myocarditis, and/or hepatic and splenic necrosis. Findings are consistent with bacterial sepsis as
145 cause of death of these individuals. The bacteria were identified as Gram-negative (Figure S2),
146 which would be in line with an infection by *Pm*.

147

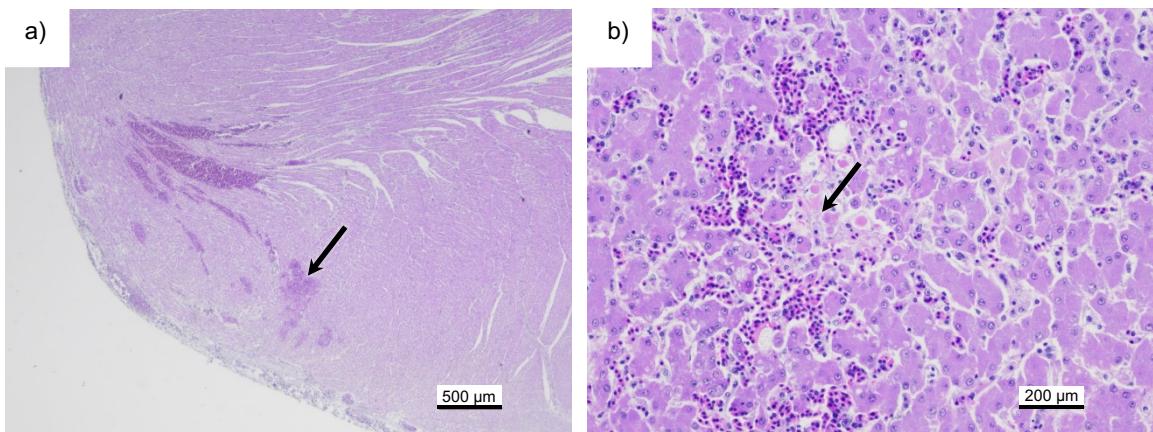


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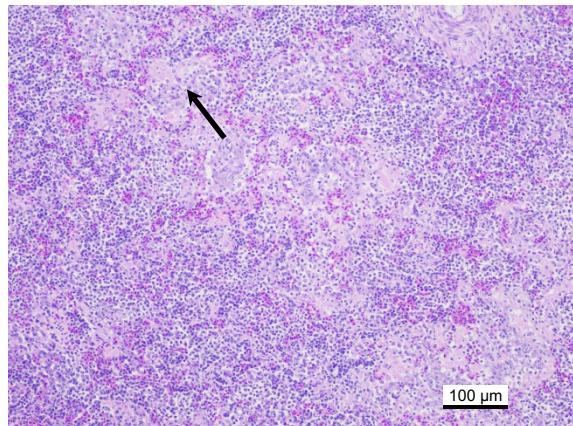
149 **Figure S2.** Photomicrography of a section of heart (a) and liver (b) from a yellow-nosed albatross
150 nestling (specimen AMS13-CAD12, found dead on the 12/01/2014) showing circulated bacteria
151 (black arrows). The bacteria were characterized as Gram negative (black arrows), here in the liver
152 (c). Haematoxylin-and-eosin staining (a and b) and Gram staining (c), magnification x 40.

153



154 **Figure S3.** Photomicrography of a section of heart (a) and liver (b) from a yellow-nosed albatross
155 nestling (specimen AMS13-CAD11, found dead on the 11/01/2014) showing bacteria associated
156 with haemorrhagic myocarditis and necrotic hepatitis respectively (black arrows). Haematoxylin-
157 and-eosin staining, magnification x 2 (a) and x 20 (b).

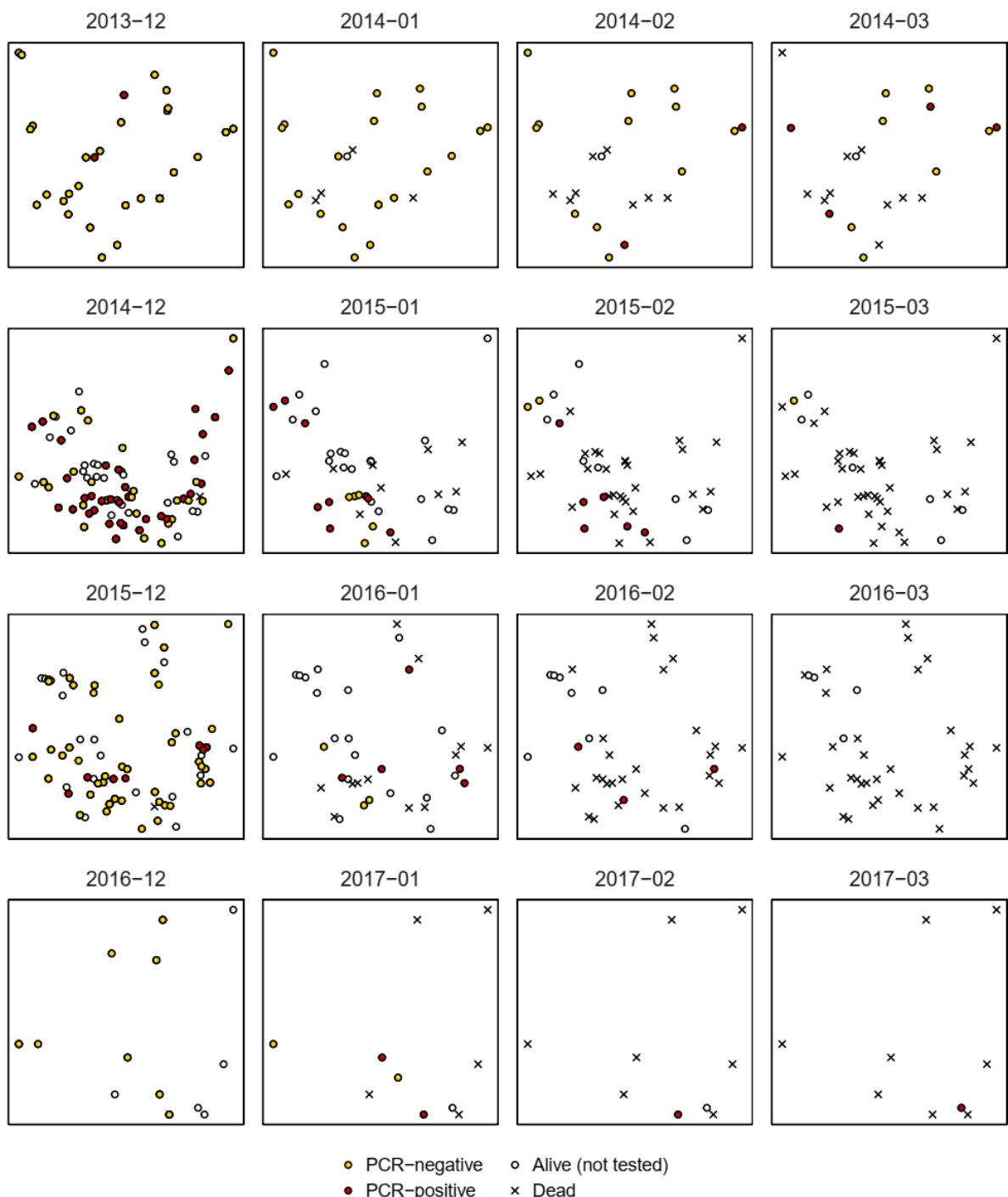
158



159 **Figure S4.** Photomicrography of a section of spleen from a yellow-nosed albatross nestling
160 (specimen AMS16-CAD5, found dead on the 16/12/2016) showing necrotizing splenitis with
161 intraleisional bacteria (black arrow). Haematoxylin-and-eosin staining, magnification x 10.
162

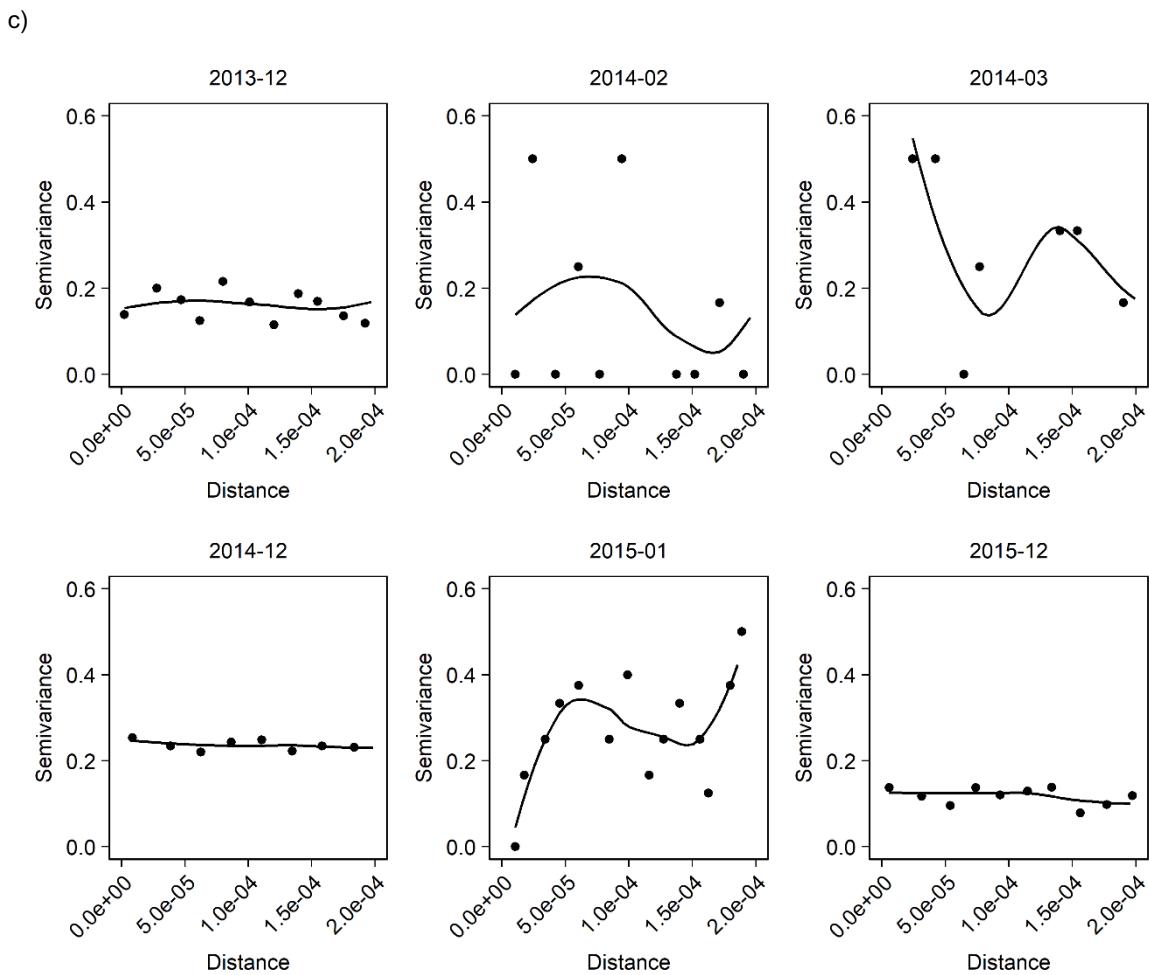
6. Spatial patterns of *Pm*-PCR status

a)





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170 See next page for figure legend (Figure S5).

171 **Figure S5.** (a) Spatial pattern of *Pm* detection by PCR in cloacal swab samples of Indian yellow-nosed albatrosses. Each dot represents a nest. Nests from which several individuals were tested (e.g., a parent and the nestling) within a month were considered PCR-positive if at least one of these samples tested positive. Late November samples were grouped with December samples. (b) The study colony (circled in blue) in Entrecasteaux Cliff, Amsterdam Island. Picture: Amandine Gamble/IPEV, 23 December 2016, taken from sea. (c) Variogram of the spatial distribution of *Pm* detection. Variograms were built using the in “*gstat*” R package (Pebesma et al., 2004). Dots correspond to empirical variograms and solid lines to local polynomial regressions. Variograms were built only for months during which more than 10 nests were sampled and both PCR-negative and -positive results were obtained.

181

182 **Interpretation and discussion.** We could not detect any strong spatial structuration of *Pm* detection in the study colony. More precisely, the variograms of March 2014 and January 2015 are the only ones supporting the hypothesis of spatial structuration of *Pm* circulation, but in different directions (different *Pm* status between nearby nests in March 2014 *versus* similar *Pm* status between nearby nests in January 2015). Several non-exclusive transmission routes have been suggested for *Pm* such as oral and fecal shedding (Samuel et al., 2003), environmental transmission (Bredy & Botzler, 1989) or *via* rodent bites (Curtis, 1983). Characterizing the spatial patterns of *Pm* circulation could help disentangling the relative contributions of these potential transmission routes. Seabird colonies are particularly relevant models to address such questions because of their high spatial structuration at small scales. For instance, a yellow-nosed albatross nestling will not leave its nest before its first fledging attempt, four months after hatching. The spatial pattern of infection among albatross nestlings is thus a resultant of the transmission processes, and not of the sampled hosts' movements. Few systems can allow discriminating transmission routes from sampled hosts' movements at such a fine spatial scale. However, because *Pm* exhibits an epizootic dynamics (rapidly increasing prevalence during the breeding seasons; Figure 1a), the spatial pattern of *Pm* infection is likely to change dramatically within a breeding season. A higher sampling frequency would allow to refine the mapping of *Pm* infection.

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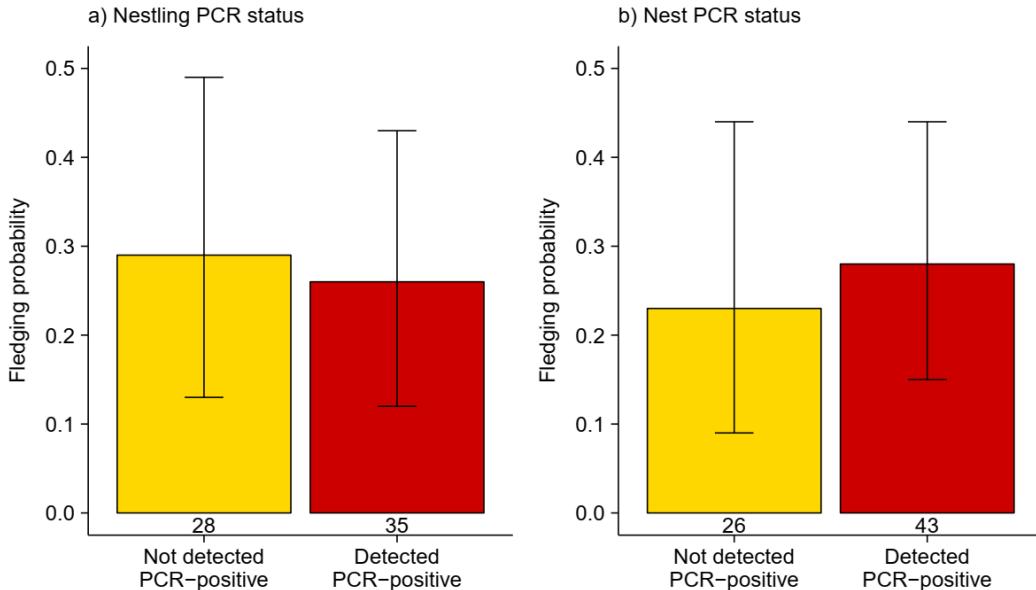
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201 **7. Exploration of the relationship between *Pm*-PCR status and fledging probability**

202 A potential association between *Pm*-PCR status and fledging probability at the individual or nest
203 level was investigated. To do so, we classified as PCR-positive all the nestlings from which *Pm* DNA
204 was detected in at least one sample (individual level), or that belong to a nest from which *Pm* DNA
205 was detected in at least one sample (i.e., including its parents; nest level).

206 We first analysed the pooled data (i.e., no distinction between the breeding seasons). We could
207 not detect any significant difference of the fledging probability between nestlings not detected as
208 PCR-positive (proportion of fledged nestlings [95% confidence interval]: 0.29 [0.13; 0.49]) and
209 nestlings detected PCR-positive (0.26 [0.12; 0.43]). The same result was obtained at the nest level
210 (0.23 [0.09; 0.44] and 0.28 [0.15; 0.44] respectively).

211 Considering that *Pm* prevalence varies among breeding seasons (see main text, Figure 1), we ran
212 additional analyses accounting for a potential interactive effect of breeding season and *Pm*-PCR
213 status on fledging probability. We fitted logistic regressions, with fledging success used as the
214 response variable, and *Pm*-PCR status (negative or positive), breeding season (categorical) and
215 their interaction as potential explicative variables and compared the models based on their Akaike
216 Information Criterion (AIC; Burhman & Anderson, 2002). At the individual level, the two models
217 with the lowest AIC either did not include PCR status (*fledging ~ breeding season*, AIC = 67.123),
218 or included PCR status with a non-significant effect on fledging probability (*fledging ~ breeding*
219 *season + PCR status*, AIC = 67.828, estimate \pm standard error of *PCR status* = 0.84 ± 0.75). All other
220 models had AIC > 70. Similarly at the nest level, the three models with lowest AIC either did not
221 include PCR status (*fledging ~ breeding season*, AIC = 72.77), or included PCR status with a non-
222 significant effect on fledging probability (*fledging ~ breeding season + PCR status*, AIC = 71.758,
223 estimate \pm standard error of *PCR status* = 1.23 ± 0.73 ; *fledging ~ breeding season × PCR status*, AIC
224 = 70.92, estimate \pm standard error of *PCR status* = 1.54 ± 0.92). All other models had AIC > 80.



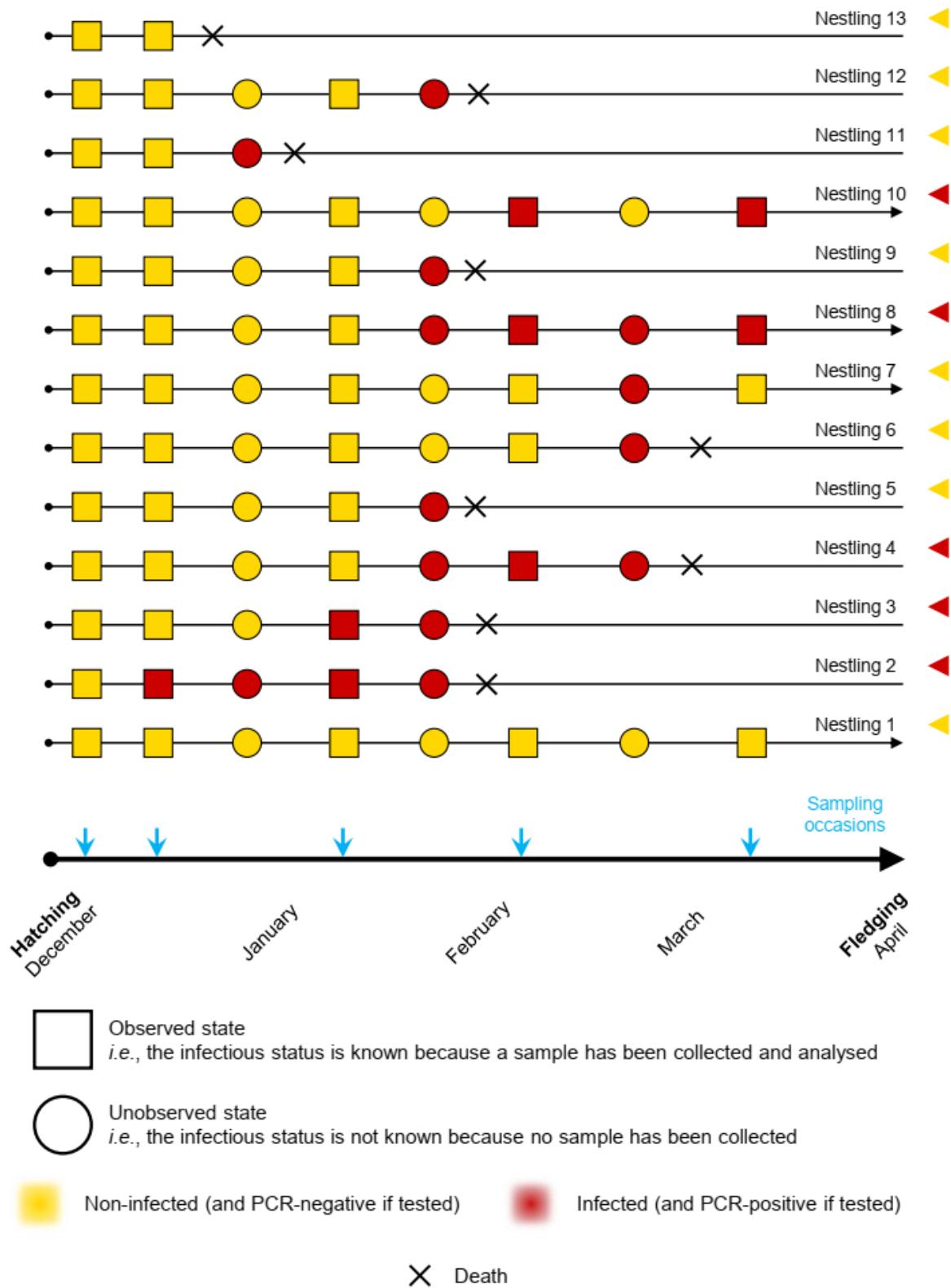
225

226 **Figure S6.** Relationship between fledging probability and *Pm*-PCR status at the individual nestling
 227 (a) or whole nest (*i.e.*, including the parents' samples; b) levels. The proportion of fledged nestlings
 228 are reported with their 95% Clopper-Pearson confidence intervals; sample sizes are indicated
 229 below the bars.

230

231 **Interpretation and discussion.** Despite previous reports of a negative effect of *Pm* on albatross
 232 nestling survival on Amsterdam island (Weimerskirch, 2004; Bourret et al., 2018; Jaeger et al.,
 233 2018), we could not detect a correlation between *Pm*-PCR status and fledging probability at the
 234 individual or nest level in the context of this study. This seemingly contradictory observation is
 235 likely a consequence of a discrepancy between the temporal scales of the sampling (once a month)
 236 and that of the epidemiological process, avian cholera being considered as an acute disease with
 237 a high lethality rate in most cases (Wobeser, 1997). For instance, if a nestling is detected as PCR-
 238 negative during the February sampling occasion and dead at the following sampling, we cannot
 239 exclude the possibility that this nestling became infected between the two occasions. Estimating
 240 the lethality rate of *Pm* infection in the study population would require more frequent sampling
 241 occasions at the epizootic peak. The data collected as part of this study provide useful elements to
 242 optimize future field sampling designs.

243



244

◀ Has never been detected PCR-positive ◀ Has been detected PCR-positive at least once

245

See next page for figure legend (Figure S7).

246 **Figure S7.** Schematic illustration of the importance of the timing of sampling for the inference of
247 epidemiological processes. In the presented fictive situation, 11 nestlings got infected (dark red
248 discs and squares; nestlings 2-12), among which 8 died following infection (nestlings 2-6, 9, 11
249 and 12), but only 5 were detected as PCR-positive (dark red triangles). Hence, more than half of
250 the infected nestlings were “missed”. More precisely, in this fictive biological situation, 8/12
251 (67%) infected nestlings died *versus* 1/2 (50%) non-infected nestlings died; but the fictive study
252 presented in this schema (following the sampling design illustrated with the blue arrows) would
253 report that 3/5 (60%) PCR-positive nestlings died *versus* 6/8 (75%) PCR-negative nestling died
254 leading to the false conclusion that infected nestlings have a lower mortality rate than non-
255 infected nestlings. For illustrative purpose, we have chosen small sample sizes but the
256 phenomenon would be the same with higher sample sizes as long as the timing of sampling
257 relative to the epidemiological process is the same, potentially leading to statistical significant
258 differences between infected and non-infected nestlings that do not represent the actual
259 biological situation (which gives the opposite result). The same phenomenon (*i.e.*, discrepancy
260 between the timing of the biological process and of sampling) will also interfere with the
261 possibility to test other hypotheses such as the existence of a spatial structuration of the
262 epidemiological dynamics (Figure S5).

263 This can occur if nestlings have time to get infected and die between two sampling occasions (e.g.,
264 in the case of acute diseases causing rapid deaths). They might thus never get detected as infected
265 even if they actually got infected (e.g., nestlings 5, 9, 11 and 12). In the case of the present study,
266 such issue could have arisen if the time delay between two sampling occasions (one month as of
267 January) is longer than the delay between *Pm* infection and induced death, which is likely to be
268 the case considering the pathogenicity of avian cholera (Wobeser, 2007; note however that the
269 infection period is not known). In addition, (1) non-infected nestlings can die from other causes
270 (e.g., predation by skuas or rats, poor parental care...; e.g., nestling 13) and (2) infected nestlings
271 can survive until fledging (as shown for the first time in the present study; e.g., nestlings 7, 8 and
272 10). These two situations contribute to the blurring of the correlation signal between infection
273 status and fledging probability. This highlight the benefits of experimental approaches that allow
274 to indirectly control infection probability (e.g., Bourret et al., 2018 in the same study system using
275 vaccination) and properly test causal links between two variables, which is not the objective of
276 the present observational study.

277 Note that for the sake of simplicity, we did not consider state misclassification (*i.e.*, we considered
278 sensitivity and specificity of test equal to 1), which is not likely to be the case, leading to additional
279 blurring of the correlation signal between infection status and fledging probability. Designs
280 accounting for uncertainty at all the stages of the observation process are called for (sampling and
281 laboratory analyses; DiRenzo et al., 2018). The basic data we report in the present study will thus

282 help to design future field studies in a iterative process (Restif et al., 2012), notably because such
283 basic knowledge is essential to propose optimized repeated sampling designs that will help
284 precisely describing key epidemiological parameters, such as the probability to clear the infection,
285 while accounting for the test detection probability (MacKenzie & Royle, 2005).
286 Overall, Figure S6, in the light of the explanation provided in this Figure S7, illustrates the
287 importance of the timing of sampling in disease ecology studies and the necessity to describe
288 epidemiological dynamics as a first step to design appropriate sampling designs to answer specific
289 questions (see Yoccoz et al., 2001 for an analogy with biodiversity monitoring).

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