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## Effects of multiple feedings on sensitized rabbits on the fitness of *Phlebotomus papatasi* (Diptera: Psychodidae)

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1 Acta Tropica

2 Research Article

3

4 **Title:** Effects of multiple feedings on sensitized rabbits on the fitness of *Phlebotomus*  
5 *papatasi* (Diptera: Psychodidae)

6

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18

19

20 **Abstract**

21 In the present study, we evaluated the effects of antibodies anti-sandfly saliva on the  
22 fecundity of *Phlebotomus papatasi*, vector of zoonotic cutaneous leishmaniasis in the Old  
23 World. Rabbits were repeatedly exposed to sandfly bites. Immune sera showed increased  
24 levels of anti-sandfly saliva antibody compared to the pre-exposition period. The analysis of  
25 biological parameters revealed no decline on the feeding success of females *P. papatasi* fed  
26 on rabbits repeatedly exposed to sandfly bites. Our results showed that anti-sandfly saliva  
27 antibodies of rabbits are not detrimental to the fitness of females *P. papatasi*. Thus, rabbits  
28 did not acquire resistance to sandflies following repeated exposures, and that contribute in  
29 maintaining a high density of *P. papatasi*. To control sandfly infestations and *Leishmania*  
30 transmission, more studies are needed for a better understanding of the mechanisms  
31 governing the resistance of hosts to bites of sandflies.

32

33 **Keywords:** *Phlebotomus papatasi*, Rabbits, antibodies anti-sandfly saliva, fitness.

## 34 1. Introduction

35 *Phlebotomus papatasi* is the main vector of *Leishmania major*, etiologic agent of  
36 zoonotic cutaneous leishmaniasis (ZCL) in the Old World (Ben Ismail et al., 1987; Derbali et  
37 al., 2012). ZCL a neglected tropical disease (NTD) is widespread in North Africa and the  
38 Middle East (MENA) affecting thousands of people annually and it is endemic in Central and  
39 Southern Tunisia (Ben Salah et al., 2007; Chelbi et al., 2009). ZCL is not fatal but the lesions  
40 produced may cause substantial disfigurement and severe distress to infected individuals  
41 with lifelong psychological and social consequences (Chahed et al., 2016). There is no  
42 effective ZCL vaccine available. Treatment is based on chemotherapy. Despite the existence  
43 of programs concerning vector and reservoir host control for the prevention against this  
44 neglected tropical disease in the MENA region, they are not at the necessary scale to control  
45 the disease effectively.

46 The fat sand rat, *Psammomys obesus*, and the desert's jird *Meriones shawi*, are the  
47 principal reservoir hosts of *L. major* in Tunisia (Rioux et al., 1986; Fichet-Calvet et al., 2003;  
48 Ghawar et al., 2011; Derbali et al., 2012). These rodents inhabit ecotones, which are the  
49 interface between natural ecosystems and human settlements (Chelbi et al., 2021).  
50 *Phlebotomus papatasi* is the most abundant sandfly species associated with *M. shawi*  
51 (Derbali et al., 2014), and *P. obesus* (Chelbi et al., 2021) at these ecotones resulting in the  
52 establishment of a direct link between the sylvatic and peri-urban cycles of ZCL (Müller et al.,  
53 2011; Chelbi et al., 2021; Barhoumi et al., 2021). In the peri-domiciliary areas, *P. papatasi*  
54 feed on a variety of other hosts available including humans, domestic animals such cows,  
55 dogs, rabbit, birds and rodents (*Mus musculus* and *Rattus norvegicus*) (Yaghoobi-Ershadi et  
56 al., 1995; Svobodova et al., 2003; Jaouadi et al., 2018). In a previous study, we showed that  
57 breeding rabbits in man-made underground holes located at the peri-domestic areas induced  
58 a deviation of the vector from entering bedrooms towards these holes and subsequently,  
59 exert a zooprophyllactic impact on the indoor abundance of *P. papatasi* (Chelbi et al., 2008).  
60 Therefore, rabbits bred in these man-made underground holes located at the peri-domestic

61 areas are exposed to multiple bites of *P. papatasi* during the season of sandfly activity  
62 (Chelbi et al., 2008). Specific antibodies have been characterized after bites of sandflies or  
63 injection of saliva in animal models under laboratory and field conditions as reviewed by  
64 Lestinova et al. (2017). While some authors attributed an impact of these antibodies on the  
65 fecundity and mortality of sandflies following repeated exposures (Gosh and Mukhopadhyay,  
66 1998; Milleron et al., 2004; Vilela et al., 2006), others did not report any effects (Kaburi et al.,  
67 2011; Martin-Martin et al., 2015). It is important to point out that most of studies concerning  
68 the impact of multiple exposures of animals on the fitness of the vector have been performed  
69 on ticks. Repeated bites of ticks result in the production of antibodies anti-saliva of the vector  
70 and this humoral immune response is responsible for the development of immunity in guinea  
71 pigs, affecting thus the physiology, the fecundity, and the vectorial competence of the vector  
72 (Wikel, 1981). Following repeated tick-feeding, several animal species including cattle,  
73 rabbits, mice, and guinea pigs developed an acquired tick resistance (ATR) which is  
74 manifested by reduced fecundity, reduced feeding success, prolonged duration of feeding,  
75 and increased mortality of ticks (Karasuyama et al., 2020). With some exception, the  
76 laboratory mouse does not become resistant and their immune responses to repeated tick  
77 bites do not lead to tick rejection (Mbow et al., 1994; Brossard and Wikel, 2008). Laboratory  
78 mice as well as the natural reservoir host, the white-footed mice (*Peromyscus leucopus*), are  
79 considered as a relatively “permissive” host when repeatedly bitten by *Ixodes scapularis*  
80 nymphs, supporting the “tick immune evasion” theory (Anderson et al., 2017).

81         During blood meal, sandflies salivate into the host’s skin. Saliva of sandflies saliva  
82 contains anti-hemostatic and immunomodulatory molecules that are inoculated into the host  
83 skin during blood-feeding. Beyond the functions associated with overcoming vertebrate  
84 homeostasis, sandfly saliva modulates the inflammatory response of the host and display  
85 many immuno-modulatory properties (Valenzuela, 2004). Sandfly saliva contains an array of  
86 bioactive molecules that allow the vector to successfully obtain a blood meal and transmit  
87 *Leishmania* promastigotes into a vertebrate host (Kamhawi, 2000; Lestinova et al., 2017).

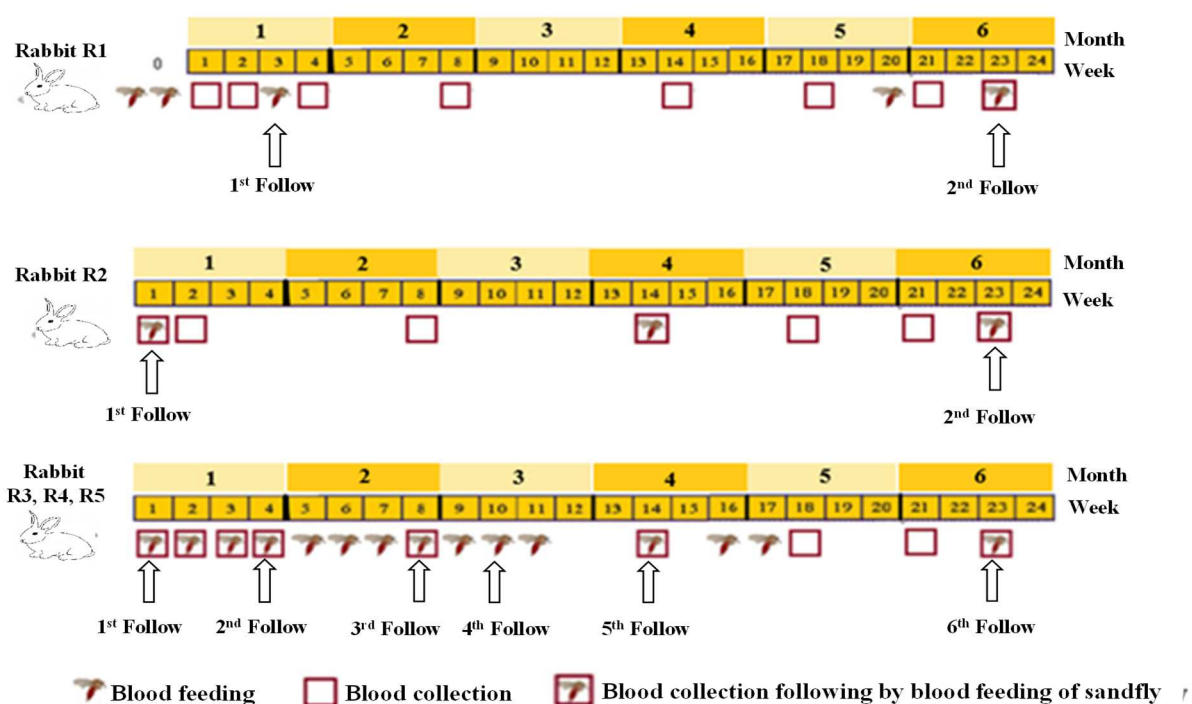
88 Among some of the most abundant molecules are anticlotting, antiplatelet, and vasodilatory  
89 compounds that increase the haemorrhagic pool where sandflies feed (Valenzuela, 2004). In  
90 addition, several salivary proteins are immunogenic and promote the development of specific  
91 cellular and humoral immune responses (Rohousova and Wolf, 2006). Hence, we aimed to  
92 investigate if anti-saliva antibodies induced by repeated *P. papatasi* bites affect the fitness of  
93 this important vector.

## 94 **2. Materials and Methods**

### 95 *2.1. Experimental exposure*

96 A colony of *P. papatasi* originated from El Felta, Sidi Bouzid, Tunisia is maintained at the  
97 Institut Pasteur de Tunis according to the methods described by Chelbi and Zhioua (2007).  
98 F17 generation was used in this study. Seven weeks old female New Zealand white rabbits  
99 obtained from the animal facility at the Institut Pasteur de Tunis were used in this study. Five  
100 rabbits were used as a source of blood meal for *P. papatasi* according to three different  
101 exposure patterns (**Figure 1**). We assessed the levels of IgG anti-salivary gland  
102 homogenates (SGH) produced according to each exposure pattern in order to choose the  
103 exposition model allowing us to determine the correlation between the anti-SGH IgG levels  
104 and the fecundity of *P. papatasi*. The R1 rabbit was exposed twice in a row at week 0 to 500  
105 females *P. papatasi* at each exposure. Three other exposures to 100 females *P. papatasi*  
106 each, were carried out at weeks 3, 20 and 23. The sera were collected at weeks 1, 2, 4, 8,  
107 14, 18, 21, and 23 (**Figure 1**). The R2 rabbit was exposed only three times to 100 females *P.*  
108 *papatasi* per exposure at weeks 1, 14 and 23 and sera were collected at weeks 1, 2, 8, 14,  
109 18, 21, and 23 (**Figure 1**). Since rabbits are bred in ZCL endemic areas as groups in animal  
110 shelter and thus exposed to sandfly bites during the 6 months of activity (Chelbi et al., 2008),  
111 a third exposure scheme was carried out for the three remaining rabbits (R3, R4, and R5)  
112 constituting group G1. Each rabbit was exposed weekly to a varying number of *P. papatasi*  
113 between (100 and 300). This protocol was extended over 6 months corresponding to the  
114 period of activity of *P. papatasi* in ZCL endemic areas in order to mimic the natural exposition

115 (Chelbi et al., 2007). The fitness of the sandfly vector was assessed through evaluating some  
 116 biological parameters including blood-feeding success, fecundity and mortality rate of  
 117 females at 24 hrs post-blood meal. Follow-ups of these parameters were carried out at  
 118 weeks 1, 4, 8, 10, 14 and 23. Blood samples were always taken 24 hrs before exposition to  
 119 females *P. papatasi* and were carried out at weeks 1, 2, 3, 4, 8, 14, 18, 21 and 23. Each  
 120 rabbit was anaesthetized by intramuscular (IM) injection of a mixture of 1.5 ml of ketamine  
 121 (15 mg/Kg) (Merial, Lyon, France) and 0.02 ml/Kg of acepromazine maleate (Kela, N.V.,  
 122 Hoogstraten, Belgium) and was placed in Barraud cage measuring (40 x 40 x 40 cm). Unfed  
 123 sandflies were 5 to 7 days old and deprived of sugar for 24 hrs prior to use in this  
 124 experiment. A minimum of 100 females and 20 males uninfected laboratory-colonized *P.*  
 125 *papatasi* (F17) were then released into the cage. Males *P. papatasi* were present for mating  
 126 and to encourage females feeding (Chelbi and Zhioua, 2007; 2019). The sandflies were then  
 127 allowed to feed on the sedated rabbit for 60 min in the dark at 27°C. After this time the  
 128 number of engorged and unfed females *P. papatasi* were counted to determine the feeding  
 129 success. In addition, the numbers of dead engorged females were counted at 24 hrs post-  
 130 blood meal to determine mortality rate.



132 **Figure 1.** Exposure patterns of rabbits to the bites of *Phlebotomus papatasi*.

133 Engorged females were held individually in glass vials (5 cm high and 2.5 cm diameter)  
134 containing a small wet filter paper, with access to cotton wool soaked in a sucrose solution  
135 (30%). Vials were maintained at 27°C and 90% relative humidity in sealed polythene  
136 containers. Flies were examined daily for up to 10 days post-blood feeding. For each female  
137 sandfly, the number of eggs laid after death was recorded, and the fecundity (average  
138 number of laid eggs per female *P. papatasi*) was determined. In addition, the number of  
139 retained eggs (average number of retained eggs per female *P. papatasi*) in the ovaries was  
140 recorded.

#### 141 *2.2. Detection of anti-SGH antibodies*

142 Colonized sandflies were dissected at three to seven days after emergence. Salivary glands  
143 were removed under a stereo microscope in cold phosphate-buffered saline (PBS) (pH 7.4)  
144 and stored in a pool of 20 pairs per 20 µl PBS at - 80°C. Immediately before use, salivary  
145 glands were disrupted by three cycles of freezing-thawing.

146 Total IgG anti-*P. papatasi* saliva antibodies were measured using enzyme-linked  
147 immunosorbent assay (ELISA) according to Martin-Martin et al. (2014) with minor  
148 modifications. Briefly, microtiter plate wells (NUNC, Maxisorp, Roskilde, Denmark) were  
149 coated with *P. papatasi* SGH (0.5 glands per well). To block free binding sites, washed wells  
150 were incubated with 6% skim milk diluted in PBS with 0.1% Tween 20. Rabbit sera were  
151 diluted at 1:250 in 6% skim milk and incubated at 37°C for 3 hrs. Secondary antibodies (goat  
152 anti-rabbit IgG) conjugated with horseradish peroxidase (HRP) (Sigma-Aldrich, St. Louis,  
153 MO) were diluted at 1:2500 and incubated at 37°C for 1hr then visualized using  
154 orthophenyldiamine (OPD) in citrate buffer and hydrogen peroxide. Absorbance was  
155 measured using a microplate ELISA reader at 492 nm wavelength. Each serum was tested in  
156 duplicate. Quantification threshold was fixed at an OD equal to 0.1.

#### 157 *2.3. Statistical analysis*



158 The fluctuation of fecundity with overtime was tested by fitting generalized linear models  
159 (GLM). A generalized additive model for location scale and shape (GAMLSS) was performed  
160 to analyze the variation of laid/retained eggs, feeding success and mortality rate throughout  
161 weekly measurements. IgG anti- saliva levels in rabbits were analyzed using Theil-Sen single  
162 median model (MBLM). Person's correlation test was computed to analyze the relationship  
163 between IgG levels and fecundity, P-value < 0.05 was considered to indicate statistical  
164 significance. All tests were computed using R v. 3.6.0.

#### 165 *2.4. Ethical considerations*

166 The maintenance of animals and the experimental procedures used in this research program  
167 followed the Animal Care and Use Protocol which is approved by the Institutional Animal  
168 Care and Use Committee of the Institut Pasteur de Tunis, Tunisia (2016/02/I/UESV/V0). This  
169 work was performed under the Assurance of the US Office of Laboratory Animal Welfare  
170 [Assurance approval F-16-00170 (A5743-01)]. The Institut Pasteur de Tunis complies with  
171 the European Directive for the Protection of Vertebrate Animals used for experimental and  
172 other scientific purposes (2010/63/EU).

### 173 **3. Results**

#### 174 *3.1. Kinetics of anti-*P. papatasi* saliva antibody response in rabbit*

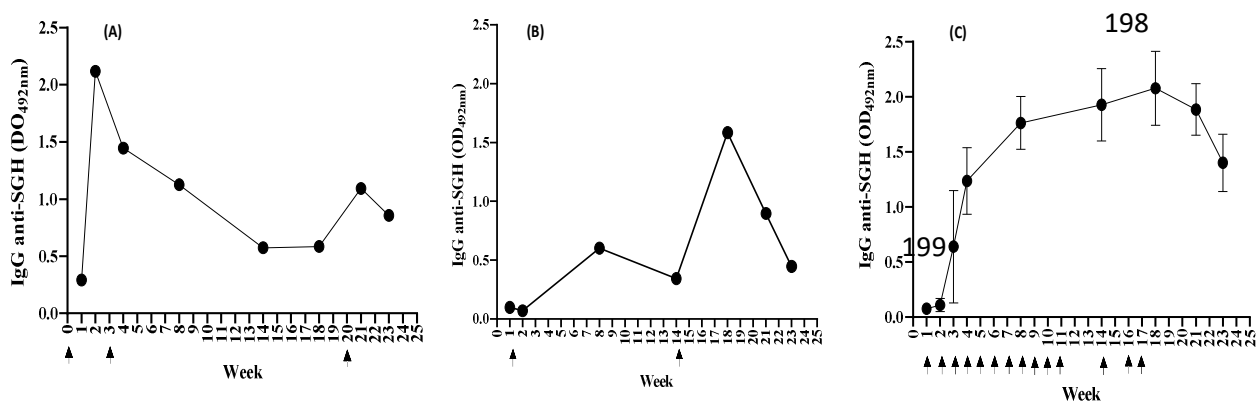
175 To investigate the kinetics and persistence of anti-*P. papatasi* saliva antibody response,  
176 experimentally bitten rabbits were followed for 23 weeks. The different exposure patterns of  
177 rabbits to the bites of *P. papatasi* females showed variation in IgG anti-SGH levels.

178 Exposure of the R1 rabbit to the bites of 500 females at three-day interval during the same  
179 week (Week 0) induced a significant increase in the anti-SGH antibodies level in the week 1  
180 to reach its maximum at week 2, followed by a decrease at the third week (**Figure 2A**).  
181 Despite a third exposure to 100 females *P. papatasi* at week 3, the IgG anti-SGH level in R1  
182 rabbit continued decreasing to week 20 (**Figure 2A**). With the last exposure to 100 females  
183 *P. papatasi* at week 21, a slight increase in IgG levels was noted (OD = 1). However, the IgG  
184 anti-SGH level decreased in the absence of another exposure to sandflies bites (**Figure 2A**).

185 Rabbit R2 was exposed to 100 females *P. papatasi* at week 1. The anti-SGH immune  
 186 response was delayed and only peaked at week 8 (OD = 0.6) and then declined (**Figure 2B**).  
 187 A second exposure to 100 females *P. papatasi* carried out at week 14 induced a significant  
 188 increase in the IgG anti-*P. papatasi* saliva level to reach a peak, 4 weeks after the last  
 189 exposure (OD = 1.584), followed by a decrease in the absence of another exposure to  
 190 sandfly bites (**Figure 2B**).

191 The weekly exposure of the G1 rabbits (R3, R4, R5) to the bites of varying number between  
 192 100 to 300 females *P. papatasi* per exposure caused a progressive increase of the IgG anti-  
 193 *P. papatasi* saliva level, reaching a peak at week 18. When the exposure to sandfly bites  
 194 ceased, the IgG anti-*P. papatasi* saliva level decreased (**Figure 2C**). Overall, IgG anti-*P.*  
 195 *papatasi* saliva levels have increased significantly with repeated exposure to sandfly bites  
 196 (MBLM: *v*-value = 71326, *df* = 28, *p*-value < 0.001).

197



200

201

202 **Figure 2.** Humoral responses against salivary gland homogenates. Total IgG anti-SGH  
 203 levels were measured within sera from rabbit R1 (**A**), rabbit R2 (**B**) and group G1 including  
 204 the rabbits R3, R4, and R5 (**C**). ELISAs were used to measure the rabbit total anti-SGH IgG  
 205 levels. Results were expressed as OD measured at 492 nm. For rabbit R1 and R2 each point  
 206 represents the mean of the duplicate values for the same rabbit serum. For the group G1,  
 207 each point represents the mean of IgG levels measured in sera from the three rabbits.  
 208 Vertical bars represent standard errors. The arrows indicate the blood feeding time.

209

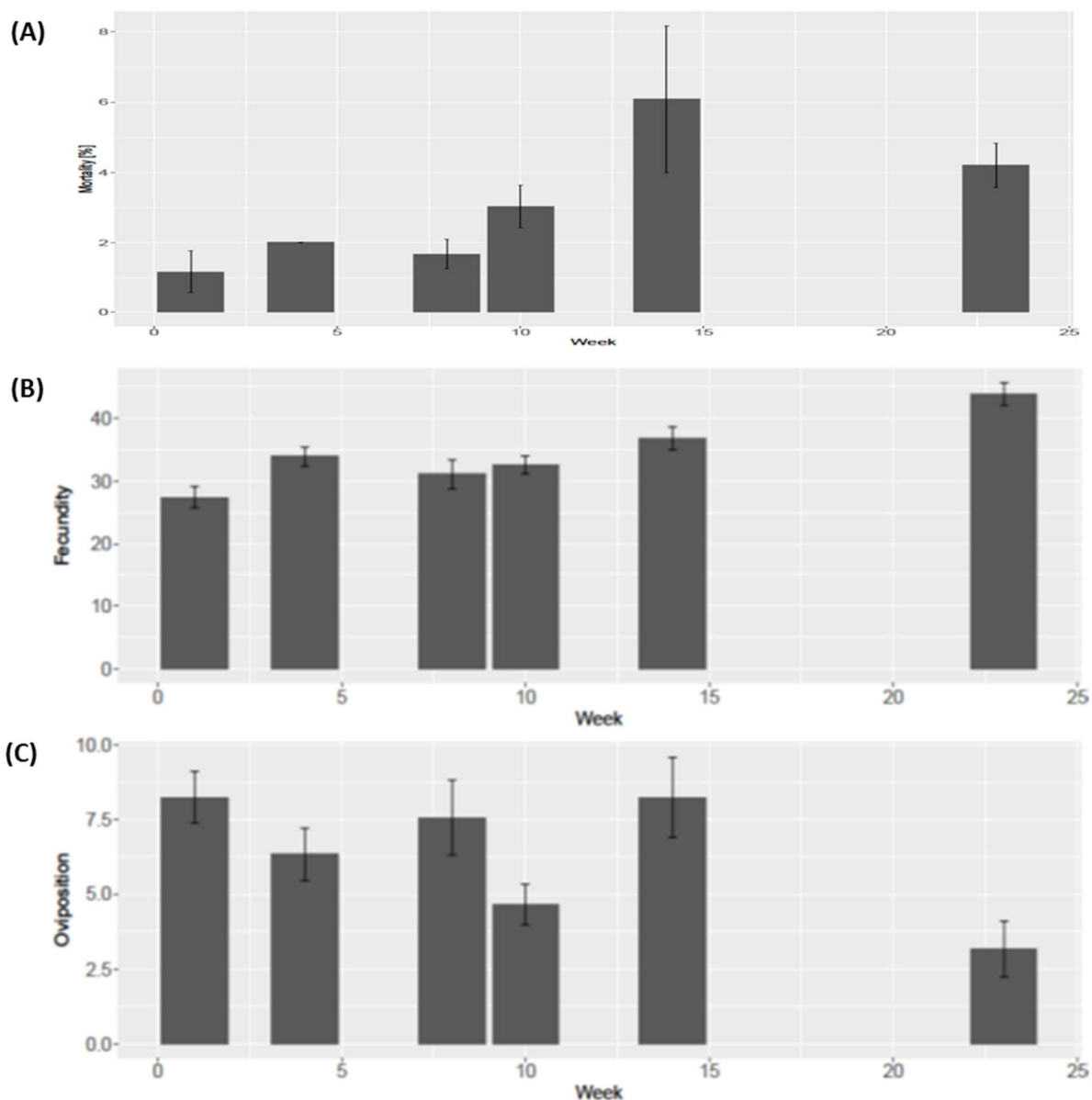
210 *3.2. Effect of immune sera on biological parameters of P. papatasi*

211 To assess the effect of the development of host anti-saliva antibodies on the sandfly fitness  
212 we evaluated some biological parameters including blood-feeding success, fecundity,  
213 number of retained eggs, and mortality rate of females 24 hrs post-blood meal.

214 Following exposure of the R1 rabbit to the bites of 500 females at three-day interval during  
215 the same week (Week 0), the monitoring of the engorgement at week 3 and week 23 showed  
216 a feeding success of 100 % (N = 100) and 97.2 % (N = 74), respectively. Mortality rates  
217 observed at week 3 and week 23 were 6 % and 0 %, respectively. Fecundity observed at  
218 week 3 and week 23 were 52.4 (N = 53) and 49.1 (N = 45), respectively.

219 Following exposure of the R2 rabbit to the bites of 100 females *P. papatasi* at week 1, the  
220 monitoring of the engorgement at week 1 and week 23 showed a feeding success of 100 %  
221 (N = 100) for both sampling times. Mortality rates observed at week 23 were 2 % (N = 100).  
222 Fecundity observed at week 1, week 14 and week 23 were 37.6 (N = 40), 26.4 (N= 24), and  
223 43.5 (N = 49), respectively. At week 23, no difference was found in mortality and fecundity of  
224 sandfly groups fed on rabbit 1 and rabbit 2.

225 Following repeated exposure of the G1 rabbits to the bites of varying number (100 to 300) of  
226 *P. papatasi* females per exposure during a 6 month period, mortality rate recorded 24 hrs  
227 after blood meal increased after weekly exposures of rabbits to sandflies (GAMLSS:  $t =$   
228  $3.472$ ,  $df = 14$ ,  $p = 0.00374$ ) (**Figure 3A**). However feeding success of sandflies showed no  
229 significant variation with time (GAMLSS:  $t = 1.722$ ,  $df = 14$ ,  $p = 0.107$ ). In all intervals, the  
230 feeding success was more than 95%. On the contrary, fecundity increased significantly with  
231 frequency of exposure to sandfly bites (GLM:  $t$ -value =  $6.275$ ,  $df = 847$ ,  $p$ -value < 0.001  
232 (**Figure 3B**). Similarly, the number of retained eggs in the ovaries varied significantly with  
233 time (GAMLSS:  $t$ -value =  $8.606$ ,  $df = 846$ ,  $p$ -value < 0.001 (**Figure 3C**).

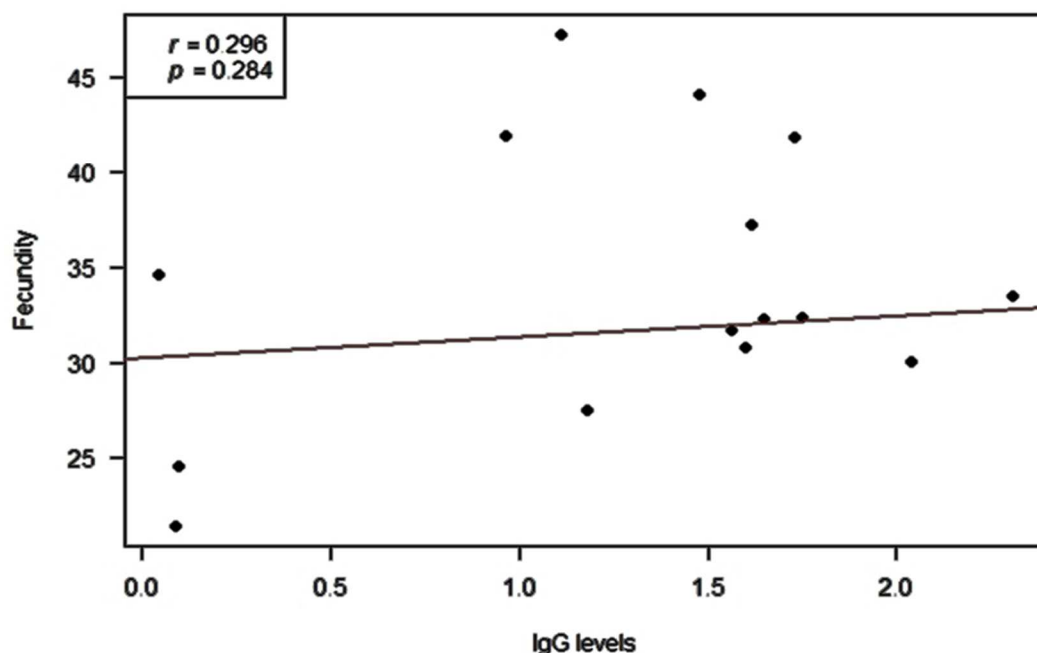


234

235 **Figure 3: Monitoring of the biological parameters of sandflies over time.** Histograms  
 236 correspond to mortality rates (A) fecundity (B) and oviposition (C) of *Phlebotomus papatasi*  
 237 fed on rabbits R3, R4 and R5 repeatedly exposed to sandflies' bites. Bars represent the  
 238 mean and the errors are the standard error of the mean.

239 The increase in fecundity is not correlated with the increase in IgG levels of rabbits  
 240 (Pearson's correlation:  $t = 1.1186$ ,  $df = 13$ ,  $p = 0.2836$ ) (Figure 4). In addition, results  
 241 suggest no significant effect of IgG levels of rabbits on the fecundity (GLM:  $t = 1.102$ ,  $df = 13$ ,  
 242  $p = 0.29$ ).

243



244

245 **Figure 4:** Correlation between anti-SGH IgG levels found in rabbits versus sandfly fecundity  
 246 (number of laid eggs). Black dots are the measurements. The line represents the intercept  
 247 and the slope of the model.  $r$ : Pearson correlation coefficient.

248

#### 249 4. Discussion

250 During blood-feeding, sandfly saliva is injected into the host triggering immune  
 251 response. Specific anti-saliva antibodies have been characterized after sandfly bites or  
 252 injection of saliva in humans and several animal models (Gomes and Oliveira, 2012;  
 253 Andrade and Teixeira, 2012) and multiple uses were attributed to this antibody response. In  
 254 addition to being a marker of exposure in several hosts and a marker of risk for *Leishmania*  
 255 transmission, anti-saliva antibody response was considered as an indicator of putative  
 256 reservoirs (Lestinova et al., 2017). To date, only a few studies have been performed  
 257 concerning the kinetics of anti-sandfly saliva antibodies in leporids (Vilela et al., 2006; Martin-  
 258 Martin et al., 2015). Taking into account that rabbits are highly attractive and represent a  
 259 major source of blood for *P. papatasi* in ZCL endemic areas (Chelbi et al., 2008; Jaouadi et  
 260 al., 2018); we evaluated the anti- saliva immune response within rabbits exposed to repeated  
 261 sandfly bites. Our results showed that exposure of rabbits to sandfly bites elicited production

262 of anti-saliva antibodies at variable levels depending on the bite's frequency. Indeed, a  
263 significant decrease of the anti-saliva IgG levels was noted in absence of blood feeding  
264 which increases again after a new exposition. Moreover, repeated exposure to *P. papatasi*  
265 bites are needed to maintain high levels of anti-saliva IgG. These results differ from those  
266 described by Martin-Martin showing that the exposure of rabbits to *P. perniciosus* bites once  
267 a week induces high levels of anti-saliva IgG, which persisted for seven months (Martin-  
268 Martin et al., 2015). The observed difference might be due to the sandfly's species used but  
269 also to the immunization schedule. In our study, rabbits were exposed to 100 *P. papatasi*  
270 females while in the aforementioned study rabbits were exposed to 500 *P. perniciosus*  
271 females. Furthermore, on dogs experimentally bitten by *P. perniciosus* the levels of anti-  
272 saliva antibodies positively correlated with the number of blood-fed sandflies (Vlkova et al.,  
273 2011). In other studies, the IgG anti-SGH remained detectable for six month after the last  
274 exposure of dogs or mice to *Lu. longipalpis* (Hostomska et al., 2008) and *P. papatasi* (Vlkova  
275 et al., 2012) respectively. Herein we report for the first time the kinetics and persistence of  
276 anti-*P. papatasi* saliva antibodies in rabbits exposed to sandfly bites under laboratory  
277 conditions. Thus, detecting sandfly saliva specific humoral responses in rabbits, as a major  
278 blood source for *P. papatasi*, may contribute to a better understanding of the potential role of  
279 leporids in the eco-epidemiology of leishmaniasis.

280         Nonetheless, it seems that developing anti-sandfly saliva antibodies in rabbit exposed  
281 to sandfly is not detrimental to the fitness of females *P. papatasi*. While no significant  
282 difference was observed with feeding success, fecundity increased significantly following  
283 repeated exposure to bites of sandflies. However, the observed increase in fecundity was not  
284 correlated to anti-saliva antibodies levels. Despite low mortality rates noticed overtime  
285 following repeated exposure to bites of sandflies, fecundity of survivors was not affected. It is  
286 important to note that a low mortality rate of females 24 hrs post-feeding mainly due to stress  
287 factors is expected during the colonization of sandflies (Chelbi and Zhioua, 2007; 2009;  
288 Lawyer et al., 2017). Our results are similar to those reported by Vilela et al., (2006) showing

289 a low mortality rate of *L. longipalpis* females 24 hrs post-feeding on rabbits repeatedly  
290 exposed to bites. Thus, the reported low mortality rates cannot be attributed to the effect to  
291 multiple exposures of rabbits to bites of *P. papatasi* females. Actually, the effects of anti-  
292 saliva antibodies on biological parameters of sandflies remain controversial. Kaburi et al.  
293 (2011) reported that repeated exposure of the Syrian hamster to the bites of *P. dubosqi*,  
294 vector of *L. major* in Sub-Saharan Africa did not interfere with the physiological process of  
295 this sandfly species. However, repeated exposure of hamsters immunized with sandfly gut or  
296 whole body extract (without gut) have a significant impact on the survivor, fecundity, and egg  
297 hatching of *P. dubosqi* (Ingonga et al., 1996). Hamsters exposed to multiple feedings of *P.*  
298 *argentipes* acquired immunity affecting the feeding success as well as the fecundity of this  
299 important vector (Gosh and Mukhopadhyay, 1998). However, the marked decrease in the  
300 fecundity observed in *P. argentipes* repeatedly fed on hamster was not observed in *P.*  
301 *papatasi* (Gosh and Mukhopadhyay, 1998). To the contrary, there were no differences in  
302 oviposition or mortality between *P. argentipes* females fed on immunized or naive hamsters  
303 (Spitzova et al., 2020). Milleron et al. (2004) showed that mice sensitized by bites of *L.*  
304 *longipalpis* or by injection with the maxadilan protein, produced antibodies that reduced  
305 vasodilation and negatively affected egg production by 39.5%. Females *L. longipalpis* fed on  
306 rabbits exposed to repeated bites or immunized with sandfly gut antigens showed high death  
307 rate and lower fecundity (Vilela et al., 2006). *Lutzomyia longipalpis* fed on repeated exposed  
308 hamsters tended to produce less egg per unit of time spent feeding compared to those fed  
309 on naive hosts (Tripet et al., 2009). An important role is attributed to salivary yellow-related  
310 proteins, which once inoculated into the host's skin these proteins will bind biogenic amines  
311 such as serotonin and histamine (Sumova et al., 2019; Xu et al., 2011). It was advanced that  
312 the serotonin binding by salivary yellow-related proteins may affect negatively the feeding  
313 success of sandflies belonging to the genus of *Phlebotomus* feeding on mammalian hosts  
314 (Spitzova et al., 2020). However, the feeding success of *Sergentomyia* sp. adapted to  
315 reptiles is not affected (Spitzova et al., 2020).

316 It will be of major epidemiological importance to study the impact of rabbit immunized  
317 with body tissues such as whole gut extract (Vilela et al., 2006), mid-gut chitinase (Robles-  
318 Murguia et al., 2014), or subolesin/akirin vaccines (Moreno-Cid et al., 2013) on the fitness of  
319 this vector and thus on the ZCL transmission. Vaccination with subolesin/ akirin is a  
320 promising approach since those proteins are transcriptional factors well conserved across  
321 vector species (Artigas-Jerónimo et al., 2018; de la Fuente et al., 2011). Subolesin/ akirin are  
322 candidate antigens for the control of many arthropod vector infestations such Ticks,  
323 mosquitoes and sandflies (Moreno-Cid et al., 2012).

## 324 **5. Conclusion**

325 Even though sandflies modulate and dampen host immune responses to ensure  
326 successful feeding, we cannot ascertain that the effects of anti-saliva antibodies upon  
327 repeated infestations may result in decreased feeding success on the host, delayed time of  
328 engorgement, and decreased fecundity. It seems that anti-sandfly saliva antibodies of rabbits  
329 are not detrimental to the fitness of female *P. papatasi* and subsequently, rabbits do not  
330 acquire resistance to blood-feeding after repeated exposures to sandflies. Our results are  
331 corroborated by the fact that despite domestic animals in the peri-domestic areas in ZCL  
332 endemic foci are significantly exposed to repeated bites of *P. papatasi* during the 6 months  
333 period of activity, they still contribute in maintaining high vector densities as source of blood  
334 meals. Further studies are needed to unravel the role of anti-sandfly saliva antibodies on the  
335 fitness of this important vector.

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## 340 **Conflict of Interest**

341 The authors declare that they have no conflicts of interest.



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