



HAL
open science

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

Malek Trimèche, Thouraya Boussoffara, Ifhem Chelbi, Saifedine Cherni, Sami Zhioua, Nourhen Msallem, Imen Labidi, Elyes Zhioua

► To cite this version:

Malek Trimèche, Thouraya Boussoffara, Ifhem Chelbi, Saifedine Cherni, Sami Zhioua, et al.. Effects of multiple feedings on sensitized rabbits on the fitness of *Phlebotomus papatasi* (Diptera: Psychodidae). *Acta Tropica*, 2022, 228, pp.106303. 10.1016/j.actatropica.2022.106303 . pasteur-03528309

HAL Id: pasteur-03528309

<https://riip.hal.science/pasteur-03528309>

Submitted on 22 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

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

7 Malek Trimèche ^{a,b}, Thouraya Boussoffara ^{a,b*}, Ifhem Chelbi ^{a,b}, Saifedine Cherni ^a, Sami
8 Zhioua ^c, Nourhen Msallem^a, Imen Labidi ^a, Elyes Zhioua ^{a,b*}

9 ^a Unit of Vector Ecology, Institut Pasteur de Tunis, Tunis, Tunisia

10 ^b Laboratory of Transmission, Control, and Immunobiology of Infections (LR11IPT02), Institut
11 Pasteur de Tunis, Tunis, Tunisia

12 ^c Laboratory of Bio-informatic, Mathematics, Statistic, Institut Pasteur de Tunis, Tunis, Tunisia

13 *Correspondence: Dr. Elyes Zhioua, elyes.zhioua@gmail.com Tel: +216 70 945 577; Fax:
14 +216 70 944 222, Unit of Vector Ecology, Pasteur Institute of Tunis, 13 Place Pasteur BP 74,
15 1002 Tunis, Tunisia; and Dr. Thouraya Boussoffara, boussoffara_thouraya@yahoo.fr,
16 Laboratory of Transmission, Control, and Immunobiology of Infections, Institut Pasteur de
17 Tunis, Tunis, Tunisia.

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 zooprophylactic 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).

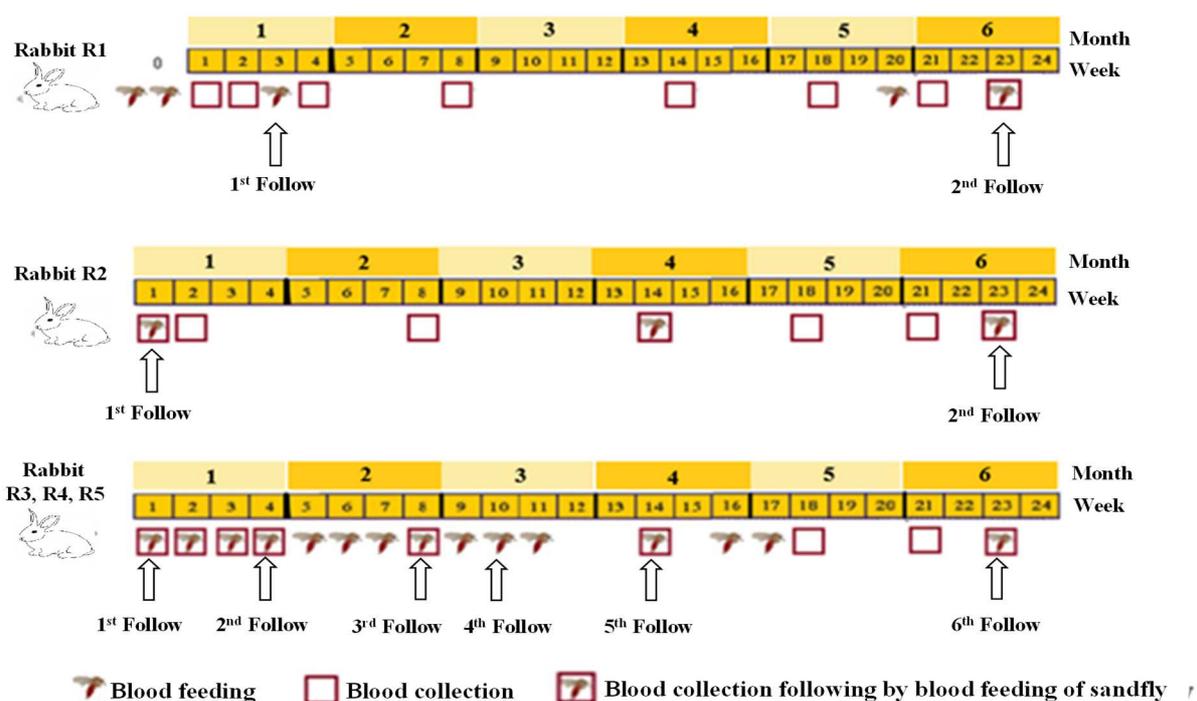
88 Among some of the most abundant molecules are anticlotting, antiplatelet, and vasodilatory
89 compounds that increase the haemorrhagic pool where sandflies feed (Valenzuela, 2004). In
90 addition, several salivary proteins are immunogenic and promote the development of specific
91 cellular and humoral 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*

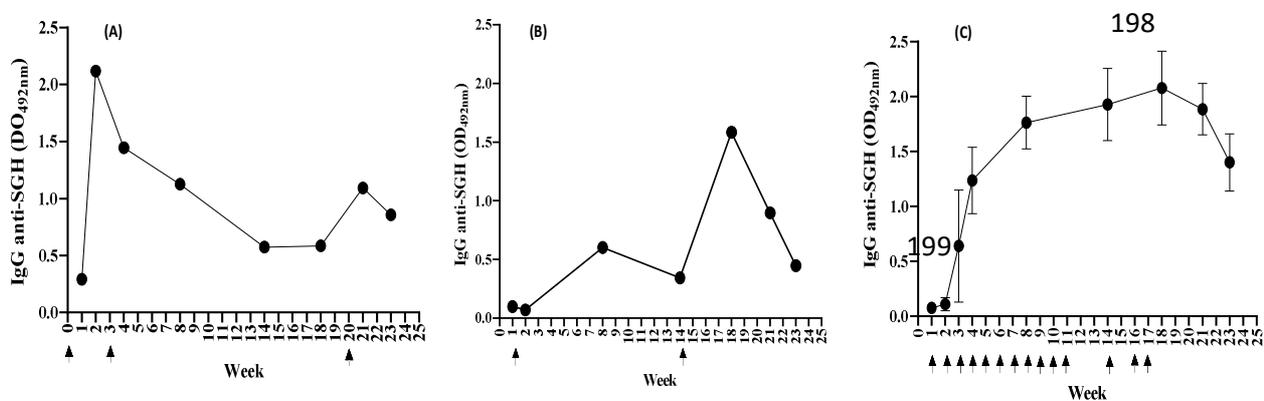
175 To investigate the kinetics and persistence of anti-*P. papatasi* saliva antibody response,
176 experimentally bitten rabbits were followed for 23 weeks. The different exposure patterns of
177 rabbits to the bites of *P. papatasi* females showed variation in IgG anti-SGH levels.

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

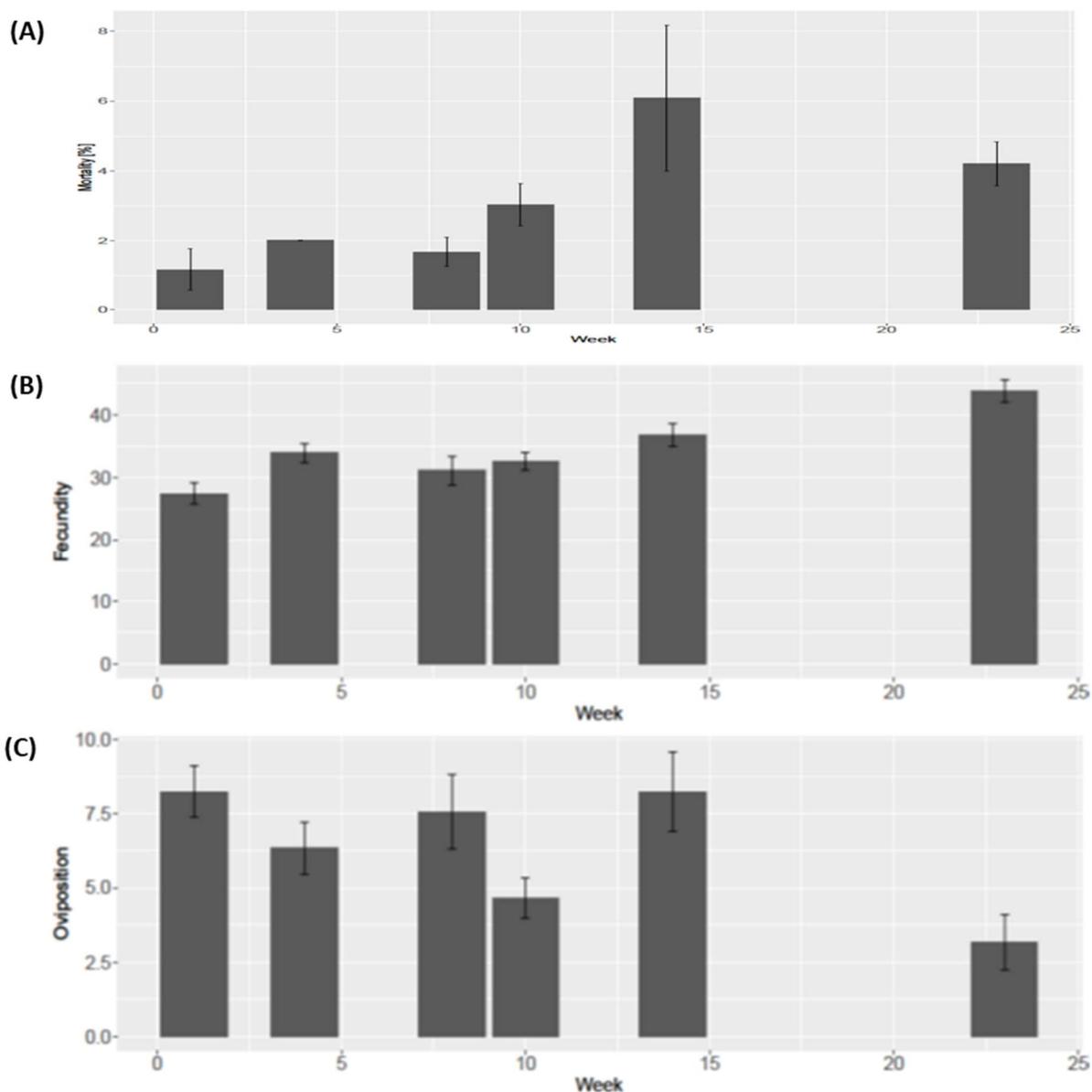
210 *3.2. Effect of immune sera on biological parameters of P. papatasi*

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**).

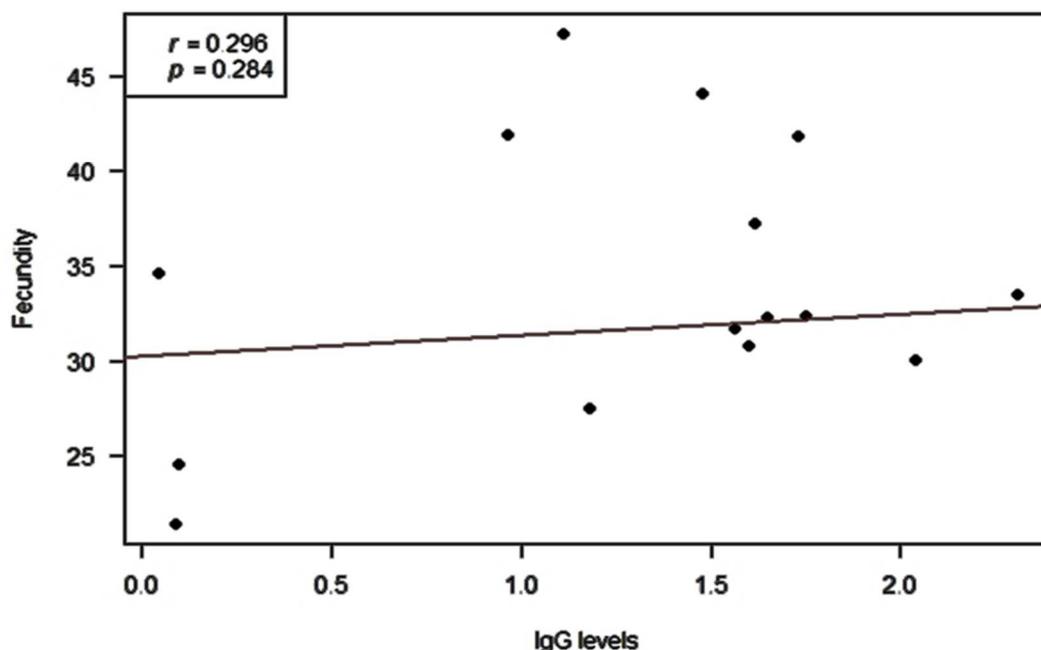


234

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.

336 **Financial disclosure statement**

337 This work was supported by the Institut Pasteur de Tunis and in part by a previous grant from
338 the US National Institutes of Health (grant number R21AI117107-01A1, EZ). The funders had
339 no role in study design, data collection, decision to publish or preparation of the manuscript.

340 **Conflict of Interest**

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

342 **References**

- 343 Andrade B.B. and Teixeira C.R., 2012. Biomarkers for exposure to sand flies bites as tools to
344 aid control of leishmaniasis. *Front Immunol.* 3 :121. Doi : 10.3389/fimmu.2012.00121
- 345 Anderson, J.M., Moore, I.N., Nagata, B.M., Ribeiro, J.M.C., Valenzuela, J.G., Sonenshine,
346 D.E., 2017. Ticks, *Ixodes scapularis*, feed repeatedly on white-footed mice despite
347 strong inflammatory response: an expanding paradigm for understanding tick-host
348 interactions. *Front. Immunol.* 8:1784. doi: 10.3389/fimmu.2017.01784.
- 349 Artigas-Jerónimo S., Villar M., Cabezas-Cruz A., Valdés J.J., Estrada-Peña A., Alberdi P., de
350 la Fuente J. 2018. Functional Evolution of Subolesin/Akirin. *Front. Physiol.* 9: 1612.
351 Doi: 10.3389/fphys.2018.01612
- 352 Barhoumi, W., Chelbi, I., Fares, W., Zhioua, S., Abbass, M., Derbali, M., Ramalho-Ortigao,
353 M., Elyes Zhioua, E., 2021. Risk assessment of the ecotones in the transmission of
354 zoonotic cutaneous leishmaniasis in Central Tunisia. *Int. J. Environ. Res. Public*
355 *Health* 2021, 18, 9274. doi: 10.3390/ijerph18179274.
- 356 Ben Ismail, R., Gramiccia, M., Gradoni, L., Helal, H., Ben Rachid, M.S. 1987. Isolation of
357 *Leishmania major* from *Phlebotomus papatasi* in Tunisia. *Trans. Roy. Soc. Trop.*
358 *Med. Hyg.* 81, 749.
- 359 Ben Salah, A., Kamarianakis, Y., Chlif, S., Ben Alya, N., Prastacos, P. 2007. Zoonotic
360 cutaneous leishmaniasis in central Tunisia: spatio-temporal dynamics. *Inter. J.*
361 *Epidemiol.* 36, 991-1000.
- 362 Brossard, M., Wikel, S.K. 2008. Tick immunobiology. In: Bowman AS, Nuttall PA, editors.
363 *Ticks: Biology, Disease and Control.* Cambridge: Cambridge University Press.
364 p. 186–204.
- 365 Chahed, M.K., Bellali, H., Ben Jemaa, S., Bellaj, T., 2016. Psychological and Psychosocial
366 consequences of zoonotic cutaneous leishmaniasis among women in Tunisia:

- 367 preliminary findings from an exploratory study. PLoS Negl. Trop. Dis. 10, e0005090. Doi:
368 10.1371/journal.pntd.0005090.
- 369 Chelbi, I. and Zhioua, E., 2007. Biology of *Phlebotomus papatasi* (Diptera: Psychodidae) in
370 the laboratory. J. Med. Entomol. 44, 597-600.
- 371 Chelbi, I., Derbali, M., AL-Ahmadi, Z., Zaafour, B., El Fahem, A., Zhioua, E., 2007.
372 Phenology of *Phlebotomus papatasi* (Diptera: Psychodidae) relative to the seasonal
373 prevalence of zoonotic cutaneous leishmaniasis in Central Tunisia. J. Med. Entomol.
374 44, 385-388.
- 375 Chelbi, I., Kaabi B., Derbali, M., Ben Hadj Ahmed, S., Dellagi, K., Zhioua, E., 2008.
376 Zooprophyllaxis: impact of breeding rabbits around houses on reducing the indoor
377 abundance of *Phlebotomus papatasi*. Vector-Borne Zoo. Dis. 8, 741-747.
- 378 Chelbi, I., Kaabi, B., Béjaoui, M., Derbali, M., Zhioua, E., 2009. Spatial correlation between
379 *Phlebotomus papatasi* Scopoli (Diptera: Psychodidae) and incidence of zoonotic
380 cutaneous leishmaniasis in Tunisia. J. Med. Entomol. 46, 400-402.
- 381 Chelbi, I. and Zhioua, E. 2019. Establishment, maintenance of *Phlebotomus sp* in the
382 laboratory, and infection with *Leishmania sp*. In *Leishmania: Methods and Protocols*,
383 Methods in Molecular Biology, 1971: 3 51-368. doi: 10.1007/978-1-4939-9210-2_19.
384 Joachim Clos (ed.), Springer Nature, New York, USA.
- 385 Chelbi, I., Mathlouthi, O., Zhioua, S., Fares, W., Boujaama, A., Cherni, S., Barhoumi, W.,
386 Dachraoui, K., Derbali, M., Abbass, M., Zhioua, E., 2021. The impact of the illegal
387 waste sites on the transmission of zoonotic cutaneous leishmaniasis in Central Tunisia.
388 Inter. J. Environ. Res. Public Health, 18, 66. doi: 10.3390/ijerph18010066.
- 389 Derbali, M., Chelbi, I., Ben Hadj Ahmed, S., Zhioua, E., 2012. *Leishmania major* Yakimoff et
390 Schokhor, 1914 (Kinetoplastida: Trypanosomatidae) in *Meriones shawi* Duvernoy,
391 1842 (Rodentia: Gerbillidae): persistence and transmission from natural reservoir to

- 392 sand fly vector *Phlebotomus papatasi* Scopoli, 1786 (Diptera: Psychodidae). Bull. Soc.
393 Pathol. Exot. 105, 399-402.
- 394 Derbali, M., Polyakova, L., Boujaâma, A., Cherni, S., Burruss, D., Barhoumi, W., Chelbi, I.,
395 Poché, R., Zhioua, E. 2014. Laboratory and field evaluation of rodent bait treated with
396 fipronil for feed through and systemic control of *Phlebotomus papatasi*. Acta Trop. 135:
397 27-32.
- 398 Fichet-Calvet, E., Jomâa, I., Ben Ismail, R., Asford, R.W., 2003. *Leishmania major* infection
399 in the fat sand rat *Psammomys obesus* in Tunisia: interaction of hosts and parasite
400 populations. Ann. Trop. Med. Parasitol. 97, 593-603.
- 401 de la Fuente J., Moreno-Cid J.A., Canales M., Villar M., de la Lastra J.M., Kocan K.M.,
402 Galindo R.C., Almazan C., Blouin E.F. 2011. Targeting arthropod subolesin/ akrinin for
403 the development of a universal vaccine for control of vector infestations and pathogen
404 transmission. Vet. Parasitol. 181, 17-22. Doi: 10.1016/j.vetpar.2011.04.018
- 405 Ghawar, W., Toumi, A., Snoussi, M.A., Chlif, S., Zâatour, A., Boukthir, A., Bel Hadj Hamida,
406 N., Chemkhi, J., Diouani, M.F., Ben Salah, A., 2011. *Leishmania major* infection among
407 *Psammomys obesus* and *Meriones Shawi*: reservoirs of zoonotic cutaneous
408 leishmaniasis in Sidi Bouzid (Central Tunisia). Vector-Borne Zoo. Dis. 11, 1561-1568.
- 409 Ghosh, K.N., Mukhopadhyay, J., 1998. The effect of anti-sandfly saliva antibodies on
410 *Phlebotomus argentipes* and *Leishmania donovani*. Inter. J. Parasitol. 28: 275-281.
- 411 Gomes, R., and Oliveira F., 2012. The immune response to sand fly salivary proteins and its
412 influence on *Leishmania* immunity. Front Immunol. 3 :110. Doi:
413 10.3389/fimmu.2012.00110.
- 414 Hostomska J., Rohousova I., Volfova V., Stanneck D., Mencke N., Volf P., 2008. Kinetics of
415 canine antibody response to saliva of the sand fly *Lutzomyia longipalpis*. Vector Borne
416 Zoonotic Dis. 8(4): 443-50. Doi: 10.1089/vbz.2007.0214.

- 417 Ingonga, P., Mbatia, P.A., Anjili, C.O., Mutani, A., Wishitemi, B., Odango, S., Robert, L.L.,
418 Githurie, J.I., 1996. The effect of immune sera from hamsters immunized with sandfly
419 gut and whole extract antigens on the fecundity and mortality of *Phlebotomus duboscqi*
420 (Diptera: Psychodidae). *Acta Trop.* 60: 269-279.
- 421 Jaouadi, K., Bettaieb, J., Bennour, A., Salem, S., Ghawar, W., Rjeibi, M.R., Khabouchi, N.,
422 Gonzalez, J.P., Diouani, M.F., Ben Salah, A., 2018. Blood meal analysis of phlebotomine
423 sandflies (Diptera: Psychodidae: Phlebotominae) for *Leishmania* spp. Identification and
424 vertebrate blood origin, Central Tunisia, 2015-2016. *Am. J. Trop. Med. Hyg.* 98: 146-149.
- 425 Kaburi, J.C., Ngumbi P.M., Anjili, C.O., 2011. Sandfly-saliva injected during repeated feeding
426 on a sensitized hamster causes fecundity and mortality to female *Phlebotomus duboscqi*
427 (Diptera: Psychodidae). *J. Vector-Borne Dis.* 48: 61-63.
- 428 Kamhawi, S. 2000. The biological and immunomodulatory properties of sand fly saliva and its
429 role in the establishment of *Leishmania* infections. *Microbes Infect.* 2: 1765-1773. doi:
430 10.1016/s1286-4579(00)01331-9.
- 431 Karasuyama, H., Miyake, K., Yoshikawa S. 2020. Immunobiology of acquired resistance to
432 ticks. *Front. Immunol.* 11:601504. doi: 10.3389/fimmu.2020.601504.
- 433 Lawyer, P., Killick-Kendrick M., Rowland, T., Rowton, E., Volf, P. 2017. Laboratory and mass
434 rearing of sandflies (Diptera : Psychodidae). *Parasite*, 24: 42. Doi:
435 10.1051/parasite/2017041
- 436 Lestinova, T., Rohousova, I., Sima, M., de Olivera C.I., Volf, P., 2017. Insights into the
437 sandfly saliva: blood-feeding and immune interactions between sandflies, hosts, and
438 *Leishmania*. *PLoS Negl. Trop. Dis.* 11(7): e0005600. Doi: 10.1371/journal.pntd.0005600.
- 439 Martín-Martín, I., Molina, R., Rohoušová, I., Drahota, J., Volf, P., Jiménez, M., 2014. High
440 levels of anti-*Phlebotomus perniciosus* saliva antibodies in different vertebrate hosts

- 441 from the re-emerging leishmaniosis focus in Madrid, Spain. *Vet Parasitol.* 202 (3-4):207-
442 16. Doi: 10.1016/j.vetpar.2014.02.045
- 443 Martín-Martín, I., Molina, R., Jiménez, M., 2015. Kinetics of anti-*Phlebotomus perniciosus*
444 saliva antibodies in experimentally bitten mice and rabbits. *PLoS One*, 10(11):e0140722.
445 doi: 10.1371/ journal.pone.0140722.
- 446 Mbow, M.L., Christe, M., Rutti, B., Brossard, M. 1994. Absence of acquired resistance to
447 nymphal *Ixodes ricinus* ticks in BALB/c mice developing cutaneous reactions. *J*
448 *Parasitol.* 80:81–7. Doi:10.2307/3283349.
- 449 Milleron, R.S., Ribeiro J.C.M., Elnaime, D., Soong, L., Lanzaro, G.C., 2004. Negative effect
450 of antibodies against maxadilan on the fitness of the sand fly vector of American visceral
451 leishmaniasis. *Am. J. Trop. Med. Hyg.* 70: 278-285.
- 452 Moreno-Cid, J.A., Pérez de la Lastra, J.M., Villar, M., Jiménez, M., Pinal, R., Estrada-Pena,
453 A., Molina, R., Lucientes, J., Gortazar, C., de la Fuente, J., the SUB/AKR Vaccine study
454 group. 2013. Control of multiple arthropod vector infestations with subolesin/akirin
455 vaccines. *Vaccine*, 31: 1187-1196. Doi: 10.1016/j.vaccine.2012.12.073
- 456 Müller, G.C., Vasiliy, D., Kravchenko, V.D., Rybalov, L., Schlein, Y., 2011. Characteristics of
457 resting and breeding habitats of adult sandflies in the Judean Desert. *J. Vec. Ecol.* 36
458 (Suppl 1), S195-S205. Doi: 10.1111/j.1948-7134.2011.00131.x
- 459 Rioux, J.A., Petter, F., Zahaf, A., Lanotte, G., Houin, R., Jarry, D., Perires, J., Martini, A.,
460 Sarhani, S., 1986. Isolement de *Leishmania major* Yakimoff et Schokhor, 1914
461 [Kinetoplastida-Trypanosomidae] chez *Meriones shawi shawi* (Duvorney, 1842)
462 [Rodentia-Gerbillidae] en Tunisie. *Ann. Parasitol. Hum. Comp.* 61, 139-145.
- 463 Robles-Murguía, M., Bloedow, N., Murray, L., Ramalho-Ortigão, M., 2014. Effect of mouse
464 antisera targeting the *Phlebotomus papatasi* midgut chitinase PpChit1 on sandfly
465 physiology and fitness. *Mem Inst Oswaldo Cruz*, 109: 1064-1069.

- 466 Rohoušová, I., and Volf, P., 2006. Sand fly saliva: effects on host immune response and
467 *Leishmania* transmission. *Folia Parasitol.* 53: 161–71.
- 468 Spitzova, T., Sumova, P., Volfova, V., Polanska, N., Poctova, L., Volf, P., 2020. Interactions
469 between host biogenic amines and sand fly salivary yellow-related proteins. *Parasites*
470 *Vect.* 13:237 doi: 10.1186/s13071-020-04105-2.
- 471 Sumova, P., Sima, M., Kalouskova, B., Polanska, N., Vanek, O., Oliveira, F.,
472 Valenzuela, J.G., Volf, P., 2019. Amine-binding properties of salivary yellow-related
473 proteins in phlebotomine sand flies. *Insect Biochem Mol Biol.* 115 :103245. Doi:
474 10.1016/j.ibmb.2019.103245.
- 475 Svobodova, M., Sadlova, J., Chang, K-P, Volf, P., 2003. Short report: distribution and feeding
476 preference of the sand flies *Phlebotomus sergenti* and *P. papatasi* in a cutaneous
477 leishmaniasis focus in Sanliurfa, Turkey. *Am. J. Trop. Med. Hyg.* 68: 6–9.
- 478 Tripet, F., Clegg, S., Elnaïem D.E., Ward, R., 2009. Cooperative blood-feeding and the
479 function and implications of feeding aggregations in the sand fly *Lutzomyia*
480 *longipalpis* (Diptera: Psychodidae). *PLoS Negl. Trop. Dis.* 3(8): e503.
481 doi:10.1371/journal.pntd.0000503
- 482 Valenzuela J.G., 2004. Blood-feeding arthropod salivary glands and saliva, in *Biology of*
483 *Disease Vectors*, W.C. Marquardt, Black IV, W. C., Freier, J. E., Hagedorn, H. H.,
484 Hemingway, J., Higgs, S., James, A. A., Kondratieff, B., Moore, C. G., Editors.
485 Elsevier Academic Press: Burlington. p.785.
- 486 Vilela, M.L., Souza, N.A., Olivieira, S.M., Costa-Pinto, D.C., Rangel, E.F., Traub-Cseko,
487 Y.M., 2006. Considerations on the effect of anti-sandfly antibodies on biological
488 parameters of *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae:
489 Phlebotominae). *Braz. J. Biol.* 66: 175-183.

- 490 Vlkova, M., Rohousova, I., Drahota, J., Stanneck, D., Kruedewagen, E.M., Mencke, N.,
491 Otranto, D., Volf, P., 2011. Canine antibody response to *Phlebotomus perniciosus* bites
492 negatively correlates with the risk of *Leishmania infantum* transmission. PLoS Negl
493 Trop Dis. 5(10): e1344. Doi : 10.1371/journal.pntd.0001344
- 494 Vlkova, M., Rohousova, I., Hostomska, J., Pohankova, L., Zidkova, L., Drahota, J.,
495 Valenzuela, J.G., Wolf, P., 2012. Kinetics of antibody response in BALB/c and C57BL/6
496 mice bitten by *Phlebotomus papatasi*. PLoS Negl. Trop. Dis. 6(7): e1719.
497 doi:10.1371/journal.pntd.0001719.
- 498 Wikel, S.K., 1981. The induction of host resistance to tick infestation with a salivary gland
499 antigen. Am. J. Trop. Med. Hyg. Doi: 10.4269/ajtmh.1981.30.284.
- 500 Xu, X., Oliveira, F., Chang, B.W., Collin, N., Gomes, R., Teixeira, C., Reynoso, D., My
501 Pham, V., Elnaiem, D.E. , Kamhawi, S., Ribeiro, J M.C., Valenzuela, J.G., Andersen,
502 J.F., 2011. Structure and function of a "yellow" protein from saliva of the sand fly
503 *Lutzomyia longipalpis* that confers protective immunity against *Leishmania major*
504 infection. J Biol Chem. 286(37):32383-93. Doi : 10.1074/jbc.M111.268904
- 505 Yaghoobi-Ershadi, M., Javadian, E., Kannani, A., 1995. Host preference pattern of
506 phlebotomine sandflies of Borkhar rural district, Isfahan province, Iran. Acta Trop. 60:
507 155–158.
- 508