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Sandfly-saliva injected during repeated feeding on a sensitized hamster causes fecundity and mortality to female *Phlebotomus duboscqi* (Diptera: Psychodidae)

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Various studies have shown that vertebrate hosts often acquire resistance after being parasitized by ticks[1]. This resistance is mediated in parts by the host's production of antibodies against tick antigens[2], and ticks fed on resistant animals display delayed development, decreased fecundity and increased mortality[3]. Studies conducted by Ramasamy *et al*[4] showed that *Aedes aegypti* Linn (Diptera: Culicidae) fed on rabbits immunized with mosquito antigens displayed a reduction in fecundity, and decreased viability of the progeny. Similarly, Alger and Cabrera[5] demonstrated increased mortality in *Anopheles stephensi* Liston (Diptera: Culicidae) fed on rabbits immunized mosquito gut antigens. Antibodies produced by hosts against tick saliva and those produced as a result of immunization with mosquito antigens are thought to be responsible for the increased mortality and reduced fecundity. The main antibody that is produced is IgG, particularly the IgG1 isotype that is capable of traversing the midgut of ticks and yet maintain its biological activity[1,4].

Phlebotomine sandflies which are vectors of the leishmaniases are known to inject saliva into the host when probing to locate blood capillaries[6]. Saliva triggers an immune response, which as also happens upon exposure to saliva of other arthropods, leads to the production of specific antibodies[7]. This response against salivary proteins has been used as an epidemiological marker of exposure to vectors as was shown in a recent study[8]. Using enzyme-linked immunosorbent assay, antibodies against saliva of *Lutzomyia longipalpis* Lutz & Neva, the vector for *Leishmania chagasi* Cunha & Chagas were detected in foxes, *Cerdocyon thous* Linn in Brazil which helped to establish the sylvatic cycle of the parasite[8].

Syrian hamsters (*Mesocricetus auratus* Whitehouse) have been shown to develop anti-sandfly saliva antibodies when they were used repeatedly to feed a colony of *Phlebotomos duboscqi* Neveu-Lemaire, the vector for *L. major* Yarkimoff & Schokhor in Kenya (Anjili, unpublished data). The amount of protein in a pair of salivary glands is estimated to be 1 µg[9]. In this protein estimate, there are a number of substances that are liable to interfere with vertebrate haemostatic and inflammatory responses[7,10]. One of the substances that has been recognized is a 44 kDa protein that is recognized by human, dog and fox antisera[8]. In the current study, we were interested in finding out whether these anti-sandfly-saliva antibodies have any effect on the fecundity and mortality of laboratory-bred *P. duboscqi* fed on hamsters that have been exposed to sandfly-saliva through continuous feeding on the same hamster.

An adult male hamster obtained from the Kenya Medical Research Institute (KEMRI) animal house was used to feed a colony of a Kenyan strain of female *P. duboscqi* twice a week for 4 months. This was done to generate anti-sandfly-saliva antibodies. A group of 30 experimental 3-day old female *P. duboscqi* was allowed to feed *ad libitum* on this hamster. Engorged sandflies were put into individual plastic vials with a plaster of Paris base for oviposition. A single male was added to each of the vials for continued mating which was then covered with fibre-screen top lids (12 holes per linear cm) through which they could feed. A control group of 30 female sandflies was similarly fed on a naïve control hamster that had never been exposed to sandfly bites. These were put in individual vials as explained above. The sandflies were then maintained at a temperature of 26°C and a relative humidity of 80% RH. All the engorged sandflies in two groups were given sugar syrup every morning and observed until they oviposited and died. Oviposition of sandflies started four days after blood feeding and completed egg-laying by the Day 10 after blood feeding. Emergence of the I instar larvae took place 15–16 days after blood feeding. During the observation period, time taken to oviposit, number of eggs laid per sandfly, egg morphology, number of days taken by eggs to hatch and time of death of adults were recorded.

The data were entered into a computer using MS Excel and thereafter imported into STATA 9.2, (STATACORP, TX, USA) for analysis. It was then analyzed using Chi-square. Comparisons of all the observa-

Table 1. Comparison of means (± standard error) of fecundity and mortalities between experimental and control sandfly groups

Sandfly group	Days taken for oviposition post-feeding	No. of eggs laid post-feeding	Days taken by eggs to hatch post-feeding	Life span of sandflies in days post-feeding
Experimental	17.9 ± 0.99	40.6 ± 4	9.7 ± 0.02	19.3 ± 1.20
Control	15.7 ± 0.07	43.2 ± 4.20	9.2 ± 0.02	19.6 ± 1.06

tions that were made between sandflies fed on the saliva-sensitized hamster and those fed on the control hamsters were not statistically significant as was shown using the, χ^2 analysis test. These were time taken to oviposit eggs (χ^2=0.184, p=0.669, df=1), number of eggs laid (χ^2=0.222, p=0.637, df=1), number of days taken by eggs to hatch (χ^2=0, p=1, df=1)) and mortality (χ^2=0.002, p=0.964, df=1). These comparisons were done using only the sandflies that were fed. In the group of sandflies that was given a saliva sensitized hamster, 13.3% did not feed, while in the control group, 10% also did not feed, representing a non-significant difference (χ^2=0.784, p=0.376, df=1) in the feeding success. Results regarding oviposition time post-feeding, number of eggs laid and life span of the sandflies are summarized in Table 1. The sandfly eggs are usually microscopic and these were examined under a microscope at 40× to observe any changes in shape, colour and structure. There was no change in egg morphology in both the groups of sandflies.

The differences between experimental and control groups were not statistically significant in all the parameters compared, viz. days taken for oviposition, number of eggs laid, days taken by eggs to hatch and life span of sandflies. Nevertheless, the observed differences were caused by the fact that when a sandfly injects saliva into the blood it prevents clotting. This anticoagulant saliva has some effects on the capacity of the female sandfly to lay eggs as shown by the two groups (experimental and control).

The non-significant differences observed between the experimental and control groups of sandflies suggest that anti-saliva antibodies do not interfere with the physiological processes of *P. duboscqi.* Phlebotomine sandflies in captivity are known to feed on blood once during each gonotrophic cycle and in most cases die after oviposition. They therefore spend very little time on the host and may not take in a lot of anti-saliva antibody, unlike ticks that feed slowly and more than once on the same host and imbibe a large amount of blood that usually contain anti-saliva antibodies. Sandflies have small salivary glands that inoculate only small amounts of saliva into the host, even though the saliva is able to generate an antibody response. Ticks have larger salivary glands and inoculate large amounts of saliva into the host. They feed and spend a longer time feeding slowly on the same host and therefore imbibe large amounts of blood that usually contains more anti-saliva antibodies that can be detrimental to their physiological processes[3], like has been reported for *Ae. aegypti*[4].

Studies using *P. papatasii* Scopoli showed that sandflies imbibe 0.4–0.58 mg of blood by weight[11]. From our results, this amount of blood may not contain enough antibodies to interfere with feeding success, vitellogenesis and other physiological processes within the female sandflies. The short life span of sandflies could also hinder them from imbibing a large amount of anti-saliva antibodies over a much longer period of time. It is also possible that anti-sandfly saliva antibodies are not completely detrimental to the female sandfly unlike whole body supernatant-generated antibodies that are detrimental to *P. duboscqi* as was shown by Ingonga *et al*[12], and antibodies generated in a host following immunization with gut antigens of mosquitoes[5].

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REFERENCES

1. Need JT, Butcher JF, Zam SG, Wozniak EJ. Antibody responses of laboratory mice to sequential feeding by two species of argasid ticks (Acari: Argasidae). *J Med Entomol* 1991; *28*: 105–10.
2. Wikel SK, Allen, JR. Acquired resistance to ticks. I. Passive transfer of resistance. *Immunology* 1976; *30*: 311–6.
3. George JE, Osburn RL, Wikel SK. Acquisition and expression of resistance by *Bos indicus* and *Bos indicus* x *Bos Taurus* calves to *Ambyloma americanum* infestation. *J Parasitol* 1985; *71*: 174–82.
4. Ramasamy MS, Ramasamy R, Kay BH, Kidson C. Anti-mosquito antibodies decrease the reproductive capacity of *Aedes aegypti*. *Med Vet Entomol* 1988; *28*: 105–10.
5. Alger NW, Cabrera EJ. An increase in death rate of *Anopheles stephensi* fed on rabbits immunized with mosquito antigen. *J Econ Entomol* 1972 ; *65:* 165–8.
6. Ribeiro JMC, Rossignol PA, Spielman A. Blood-finding strategy of a capillary-feeding sand fly, *Lutzomyia longipalpis. Comp Biochem Physiol* 1986; *4:* 683–6.

7. Charlab R, Valenzuela JG, Rowton ED, Ribeiro JM. Toward an understanding of the biochemical and pharmacological complexity of the saliva of haematophagous sandfly *Lutzomyia longipalpis*. *Proc Natl Acad Sci USA* 1999; *96:* 15155–60.
8. Gomes RB, Medonca IL, Silva VC, Raus J, Cruz MSP. Antibodies against *Lutzomyia longipalpis* in the fox *Cerdocyon thous* and the sylvatic cycle of *Leishmania chagasi*. *Trans R Soc Trop Med Hyg* 2007; *101:* 127–33.
9. Theodos CM, Ribeiro JMC, Titus RG. Analysis of enhancing effect of sand fly saliva on *Leishmania* infection in mice. *Infect Immun* 1991; *59:* 1592–8.
10. Barral A, Honda E, Caldas A, Costa J, Vinhas V. Human immune response to sand fly salivary gland antigens: a useful epidemiological marker ? *Am J Trop Med Hyg* 2000; *62:* 740–5.
11. Theodor O. On relationship of *Phlebotomus papatasi* to the temperature and humidity of the environment. *Bull Entomol Res* 1936; *27:* 653–71.
12. Ingonga P, Mbati P, Anjili CO, Mutani A, Wishitemi B. The effect of immune sera from hamsters immunized with sandfly gut and whole body extracts on the mortality and fecundity of *Phlebotomus duboscqi* (Diptera: Psychodidae). *Acta Trop* 1995; *60:* 269–79.

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