Impact of anti-sandfly saliva antibodies on biological aspects of *Phlebotomus papatasi* (Diptera: Psychodidae), vector of cutaneous leishmaniasis

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**Abstract**

Sandflies are the main vectors of *Leishmania* parasites in tropical and subtropical areas. The immunization of vertebrate hosts with vector components through repeated bites may offer an alternative method for sandfly control. Aliquots of female *Phlebotomus papatasi* (Scopoli) (Diptera: Psychodidae) were weekly blood fed on 12 individual hamsters throughout 18 successive weeks. Significant biological and biochemical changes resulting from antibodies developed by immunized host sera against repeated biting were observed in sandfly females. Blood feeding and fertility rates of females significantly gradually declined to the end of the study period. No appreciable difference was observed in mortality rates among flies repeatedly fed on individual hamsters throughout weeks 9 and 18, compared to flies fed on naïve hamsters. Total salivary gland proteins of female sandflies were compared to proteins in sera of sensitized hamsters. SDS-page revealed bands common to both flies and hosts, indicating the development of anti-saliva antibodies in hamster sera. The importance of anti-sandfly saliva antibodies as a potential tool for vector control leading to the interruption of leishmaniasis is discussed.

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1. Introduction

Leishmaniasis, transmitted by phlebotomine sandflies, are found in 88 countries around the world, mainly in South and Central America, Africa, Asia, and southern Europe. However, over 90% of potentially fatal infections occur in just six countries: Brazil, Ethiopia, Sudan, South Sudan, India, and Bangladesh (Pigott et al., 2014). Annual incidence is nearly 0.2 ± 0.4 million and 0.7 ± 1.2 million cases for visceral and cutaneous leishmaniasis, respectively, ranking leishmaniasis to the ninth place of all human infectious diseases (Alvar et al., 2012). In Egypt, cutaneous leishmaniasis (CL) has been primarily identified in northern Sinai (Mansour et al., 1987) and was attributed to *Leishmania major* (Mansour et al., 1989). Visceral leishmaniasis (VL) caused by *L. infantum* has been found near Alexandria, in El-Agamy (Awadalla et al., 1987). Vectors for these parasites are well characterized across the Middle East and Africa: specifically, *Phlebotomus papatasi* and *P. sergenti* appears to be the potential vector for CL and *P. langeroni* for VL (Jacobson, 2003; Shehata et al., 2009). *Phlebotomus papatasi* constitutes more than 94% of the sandfly population in North Sinai (Hanafi et al., 2007). While probing for a blood meal, the infected sand flies salivate into the host’s skin. Sand fly saliva contains potent vasodilators, maxadilan and adenosine, described respectively in *Lutzomyia longipalpis* and *P. papatasi*, that prevent clotting at the biting site (Ribeiro et al., 1999). Additionally, as clearly demonstrated by several investigators, sand fly saliva contains immunomodulatory molecules that have been shown to enhance disease progression (Titus and Ribeiro, 1988; Rogers and Titus, 2003).
Frequent control measures against sandflies became largely ineffective (Consoli and Lourenço de Oliveira, 1994). In vectorborne diseases, the contribution of vector saliva to pathogen establishment in mammalian hosts is largely ignored. Vector saliva components delivered to a vertebrate host through biting, or injection of vector extracts, stimulate a wide spectrum of host immune responses with the production of anti-vector antibodies (Almeida and Billingsley, 1998; Morris et al., 2001). The anti-saliva antibody response correlates with the intensity of exposure to the host (Hostomska et al., 2008; Vlkova et al., 2011; Vlkova et al., 2012). This approach could be an interesting alternative for the control of hematophagous arthropods or of the diseases they transmit, by affecting their vital biological processes and hence potential vectorial capacity (Lal et al., 1994). In laboratory settings, immune responses to the sandfly salivary proteins have been consistently shown in mice, hamsters, dogs, and humans following repeated exposure to bites or by injection of salivary glands dissected from female P. argentipes, P. ariasi, P. papatasi, P. sergenti, L. longipalpis, and L. intermedia (Valenzuela et al., 2001; de Moura et al., 2007; Clements et al., 2010; Vlkova et al., 2012). Protection against CL and VL due to anti-saliva cellular immunity has been well established in rodents (Belkaid et al., 2000; Gomes et al., 2008).

The current study investigates the effects of anti-sandfly-saliva antibodies on the biological potentiality of laboratory-bred P. papatasi. In particular, we examined the temporal feeding ability, fertility and mortality of these flies when repeatedly exposed to its vertebrate host. Because of the potential use of sand-fly salivary proteins as anti-Leishmania vaccines and as markers of sand-fly exposure in a P. papatasi-prevalent area, it is important to have a more comprehensive repertoire of the salivary molecules present in this sand-fly species. Knowledge to be gained from our proposed studies would contribute to the development of novel strategies for preventing disease transmission in Egypt and other countries. Success in this domain may lead to the manufacturing of a biological vaccine and/or therapeutics for CL, contributing to a significant progress in public health.

2. Materials and methods

2.1. Sandfly colonization

A laboratory colony of P. papatasi was established based on field collections (May 2014) from El Agamy (31° 19' 8" N and 29° 91' 9" E), Egypt, and maintained at the Research and Training Centers on Vectors of Diseases (RTC) of Ain Shams University. Rearing was conducted at the RTC at controlled temperature, relative humidity, and light/dark photoperiod (Tesh and Modi, 1983). A sugar solution was offered as a carbohydrate source.

2.2. Exposure of vertebrate hosts to P. papatasi bites

Twelve laboratory male golden hamsters (100–120 gm) were obtained from the Nile Pharmaceutical Company and maintained at the RTC at controlled temperature, relative humidity, and light/darkness photoperiod. Hamsters were separated into four groups of three hamsters each. Prior to exposure to sandfly bites, hamsters were anaesthetized by intraperitoneal injection of sodium thiopental (0.4 ml/100 g body Wt.). Sandflies were deprived of food for 24 h prior to blood feeding. Starved females were weekly allowed to feed on individual hamsters for 90–100 min for 18 successive weeks. Immediately after blood feeding, fully engorged females were separated from partially fed and non-fed ones and blood feeding rate estimated by week throughout the study period. Fertility of blood fed females was estimated by counting eggs laid 3 days following feeds of weeks 1, 3, 6, 9, 12, 15 and 18. Mortality of bloodfed females was recorded every two days for weeks 1, 9 and 18. At the end of the study period, all hamsters were bled intracardially and sera separated by centrifugation for 5 min at 5000 rpm. Labelled sera were stored at –20 °C for potential detection of anti-saliva antibodies.

2.3. Salivary gland dissection

Three to five days-old female sandflies were separated into two groups. Females in one group were offered a sugar solution solely, while females in the second group were allowed to blood feed on hamsters. For each group, 20 sugar fed and 20 partially blood-fed or fully engorged females were retained and the remaining discarded. Salivary glands of females in either group were dissected out in phosphate buffered saline (PBS; 0.2 g KCl, 8 g NaCl, 0.2 g Na2PO4, 1.15 g KH2PO4 in 1 L deionised water, pH 7.2), as described by Haddow et al., (2002). To visualize salivary gland antigens resulting from blood feeding, dissected salivary glands from each group were sonicated in a clean Eppendorf tube containing 1 ml of ice-cold sterile 0.01 M PBS (pH 7.4) and stored at –70 °C for protein analysis.

2.4. Detection of antigens in fly salivary glands and of antibodies in hamster sera

After a first exposure to sandfly bites, three hamsters from one group were bled intrathoracically for protein analysis, constituting a control group for comparison with sera from hamsters repeatedly bitten. Three hamsters repeatedly bitten from a second group were bled at week 9 and from a third group at week 18. Salivary gland homogenates (SGHs) and sera were fractionated in a 12% SDS-PAGE polyacrylamide gel, in denaturing conditions. Protein samples from SGHs were prepared by mixing 20 ml with an equal volume of 2 × of treatment buffer (0.125 M Tris–Cl, pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.25% bromophenol blue). Samples of both SGHs, and sera were separated on the same gel. Molecular weights of samples were measured, using a mixture of 250 kDa, and 10 kDa as a protein marker (Cat# M3913, Sigma–Aldrich Corporation, St. Louis, MO 63103, USA). Each gel was rinsed with a Commassie stain and subsequently destained in solution I (50% methanol, 10% acetic acid), followed by destaining in solution II (5% methanol, 7% acetic acid) until protein bands were visible. Protein bands were analyzed by the Gel-Pro Analyzer software (Version 3.1, Windows 95/NT, Media Cybernetics 1993–1997, USA).

2.5. Statistical analysis

All data were analyzed and charted on a personal computer using Microsoft Office Excel 2010.

3. Results

3.1. Temporal blood feeding of P. papatasi

A first group of three hamsters were each weekly exposed to 25 P. papatasi biting females for 18 successive weeks. As feeding repeats progressed, the number of female flies’ response to blood feeding significantly declined from 24.4 ± 0.24 to 1.55 ± 0.24 by the end of the 18th week (P = 0.001), indicating the impact of potentially developed anti-saliva antibodies in hamsters on the propensity of sandflies to blood feeding (Fig. 1).
3.2. Temporal fertility of females sandflies blood fed on repeatedly bitten hamsters

The fertility of aliquots of 20 bloodfed *P. papatasi* on each of three individual hamsters exposed to fly bites was estimated. A significant decrease in the mean number of eggs laid by females throughout exposure repeats was observed (*P* = 0.04). Females that have fed on a naïve hamster (week 1) laid 48.0 ± 2.3 eggs. Fertility was reduced the subsequent weeks, averaging 13.1 ± 3.0 eggs for sandflies that have ingested blood from hamsters repeatedly exposed to bites for 18 successive weeks.

3.3. Temporal mortality of female *P. papatasi*

Mortality of aliquots of 70 females each exposed to one of three hamsters was recorded on days 1, 3, 5, 7, and 9 post-blood feeding for week 1, week 9 and week 18 of biting hamsters. 24 h post-feeding, mortality rate did not vary significantly with repeated biting from week 1 to week 18 (Fig. 3). Mortality increased 3-, and 5-days post blood feeding (*P* < 0.005), to diminish once more at days 7, and 9 (*P* > 0.001). Nevertheless, temporal mortality was not significantly impacted following blood feeding on repeatedly bitten hamsters (*P* = 0.09).

3.4. Protein analysis of *P. papatasi* salivary glands

Total protein content of 20 pairs of salivary glands specific to blood fed females increased significantly compared to that of sugar fed females (*P* = 0.000). Protein electrophoresis exhibited 20 bands for blood fed females, compared to 17 bands for those fed on sugar (Fig. 4a). Protein bands ranged from 5.24 to 97.52 kDa for blood-fed females, compared to 9.12–77.5 kDa for sugar fed females. Such differences indicated potential protein induction proper to blood fed flies, in contrast to sugar-fed ones.

3.5. Protein analysis of sera of sensitized hamsters exposed to frequently fly bites

Total protein content of sera from hamsters bled at weeks 9 and 18 was compared with that collected from a control group (Week 1). Data revealed no significant difference in protein content in control group (35, 40, 38 mg/mL) compared to week 9 and week 18, respectively (Fig. 4b). Protein electrophoretic separation profile revealed that total protein was separated into 17 and 16 bands respectively for weeks 9 and 18, compared to 15 bands ranging in size from 96.6 to 34.0 kDa in the control group. Molecular weights of protein bands in the serum of immunized hamsters ran-
ged from 96 to 15.2 kDa (week 9) and from 96.6 to 12.2 kDa (week 18). Observed variation could refer to synthesis of antibody proteins at low molecular weight ranging from 28.9 to 12.0 kDa in experimental groups.

Total protein from salivary gland homogenates of blood fed females was compared to protein content of sera of sensitized hamsters. More than five protein bands were common to both flies and hamsters with molecular weight ranging between 12 and 70 KDa. Such common bands may tend to confirm the development of antibodies in hamster sera against female salivary gland antigen, as a result of multiple biting.

4. Discussion

The present study disclosed that hamsters that have been exposed repeatedly to *P. papatasi* bites develop anti-sandfly saliva antibodies, thus negatively impacting important biological parameters of sandflies: Blood feeding and fertility. Lowered affinity to blood feeding may be associated with a reaction to sensitized host skin. Similarly, the percentage of engorgement in females of *P. argenteipes* feeding on hamsters immunized by bites, gradually decreased (Ghosh and Mukhopadhyay, 1998). However, no differences were observed in relation to the success of first feeding among the groups fed on animals immunized with different extracts or bite and the controls (Vilela et al., 2006). Anti-saliva antibodies bind to the respective region of the antigen-presenting areas of the salivary gland. This causes the inhibition or even prevention of the normal flow of saliva, thereby disrupting the normal physiological process of the vector. Tripet et al., (2009) showed that egg production by *L. longipalpis* is not affected by feeding on immunized hosts, and studies on *P. duboscqi* and *P. perniciosus* also did not observe any differences in oviposition or mortality between experimental and control groups of sand flies (Tripet et al., 2009; Martin-Martin et al., 2015). Moreover, it is known that sand fly colonies thrive even on laboratory hosts that have been repeatedly exposed to sand flies.
(Volf et al., 2000). However, the marked decrease in fertility as observed in the case of *P. argentipes* (Kaburi et al., 2011). There was no significant difference between the numbers of eggs laid by females fed on immunized or naïve haemsters. Taken together, the effects of anti-saliva antibodies on sand fly physiology are not clear. The production of high levels of specific antibodies in hosts repeatedly exposed to *P. argentipes* did not lead to a deterioration of sand fly fitness, suggesting a minor effect of anti-saliva antibodies on sand fly feeding processes (Spitzova et al., 2020). A more promising approach to altering vector fecundity and mortality might be the immunization of hosts with body tissues, such as whole gut extracts or midgut chitinase (Robles-Murguia et al., 2014).

Anti-saliva antibodies did not interfere significantly with the longevity of *P. papatasi* as observed for non-variant temporal mortality, as previously reported in *P.argentipes* by Ghosh and Mukhopadhyay, (1998). Ingonga et al., (1996) observed that the mortality of *P. duboscqi* fed on hamsters immunized against various gut and carcass concentrations increased in the first three days of evaluation in relation to the control group. These observations indicate that the anti-sandfly saliva immune sera probably bind to the antigen-presenting sites of the sandfly salivary gland and, thus, cause sandflies death. It was reported that *P. papatasi* saliva consisted of 12 major protein bands with molecular weight ranging between 10 and 100 KDa (Valenzuela et al., 2001). Levels of anti-saliva IgG reflect the intensity of exposure to sand flies and thus can be used in epidemiological studies, e.g., to measure the effectiveness of vector-control campaigns (Lestinova et al., 2017). Sand-fly salivary proteins are potential targets to test human exposure to *P. papatasi* bites and to use them as epidemiological tools to assess the risk of contracting this neglected disease. Based on this fact, in our results, it could be concluded that the production of high levels of specific antibodies in hosts repeatedly exposed to *P. papatasi* did not lead to a deterioration of sand fly fitness, suggesting a minor effect of anti-saliva antibodies on sand fly feeding processes. Also, the synthesis of antibodies protein at low molecular weight ranging in the sera of hamsters exposed for biting for 9 and 18 weeks could be related to the previous explanation. In our study, many of these immunogenic proteins were poorly represented in a Coomassie-stained SDS-PAGE.

### 5. Conclusions

Vector borne diseases are still a neglected medical problem all over the world. Leishmaniasis is considered one of the most important diseases that transmitted by sandflies through most of the world especially in tropical and subtropical areas. The weakness of sand-fly salivary method of tropical disease control forced us to think out of the box where the immunization of vertebrate hosts with vector components through repeated bites may offer an alternative method for sandfly control. Our study suggested that the antibodies that formed due to the continuous bites of sandflies serve as a defense line to the expected infection of leishmaniasis. Biochemical and biological changes were observed in sandfly females as well as in hamsters due to successive sandfly biting. Also, a significant declined in blood feeding and fertility rates of females’ sandflies. The protein electrophoresis revealed bands common to both flies and hosts, representing the development of anti-saliva antibodies in hamster sera. The immunization of vertebrate hosts with vector components through repeated bites may offer an alternative method for sandfly control.

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### Declaration of Competing Interest

The authors declare that they have no known financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### References

Almeida, A., Billingsley, P.F., 1998. Induced immunity against the mosquito Anopheles stephensi Liston (Diptera: Culicidae): effects on mosquito survival and fecundity. Int. J. Parasitol. 28, 1171–1731.

Alvar, J., Vellez, I.D., Bern, C., Herrera, M., Desjeux, P., Cano, J., et al., 2012. Leishmaniasis Worldwide and Global Estimates of Its Incidence. PLoS ONE 7 (5). e35671.

Awadalla, H.N., Mansour, N.S., Mohareb, E.W., 1987. Further characterization of Leishmania isolates from children with visceral infection in Alexandria area, Egypt. Trans. R. Soc. Trop. Med. Hyg. 81 (6).

Belkaid, Y., Valenzuela, J.C., Kamhawi, S., Rowton, E., Sacks, D.L., Ribeiro, J.M., 2000. Delayed-type hypersensitivity to Phlebotomus papatasi sand fly bite: an adaptive response induced by the fly?. Proc. Natl. Acad. Sci. USA 97, 6704–6709.

Clements, M.F., Gidwani, K., Kumar, R., Hostomska, J., Dinesh, D.S., Kumar, V., et al., 2010. Measurement of recent exposure to Phlebotomus argentipes, the vector of Indian visceral leishmaniasis, by using human antibody responses to sand fly saliva. Am. J. Trop. Med. Hyg. 82, 801–807.

Consoli, R.A.G.B., Lourenço de Oliveira, R., 1994. Controle quimico e biologico: Perspectivas. In: Principais mosquitos de importância sanitária no Brasil. Editora Fiocruz, pp. 155–159.

de Moura, T.R., Oliveira, F., Novais, F.O., Miranda, J.C., Clarenco, J., Follador, I., et al., 2007. Enhanced Leishmania braziliensis infection following pre-exposure to sandfly saliva. PLoS Negl. Trop. Dis. 1, e84.

Chosh, K.N., Mukhopadhyay, J., 1998. The effect of anti-sandfly saliva antibodies on Phlebotomus argentipes and Leishmania donovani. Int. J. Parasitol. 28, 275–281.

Gomes, R., Teixeira, C., Teixeira, M.J., Oliveira, F., Menezes, M.J., Silva, C., et al., 2008. Immunity to a salivary protein of a sand fly vector protects against the fatal outcome of visceral leishmaniasis in a hamster model. Proc. Natl. Acad. Sci. USA 105, 7845–7850.

Haddow, J.D., Poulis, B., Haines, L.R., Gooding, R.H., Aksoy, S., Pearson, T.W., 2002. Identification of major soluble salivary gland proteins in the sandfly (Diptera: Psychodidae). Acta Tropica 101 (2), 106–114.

Hostomska, J., Rohousova, I., Volfova, V., Stanneck, D., Mencke, N., Volf, P., 2008. Kinetics of canine antibody response to saliva of the sand fly Lucozmya longipalpis. Vector Borne Zoonotic Dis. 8, 443–450.

Ingonga, P., Mhutu, P.A., Angulo, C.O., Mutapi, A., Vishweshri, S.O., Robert, L.L., Githure, J.I., 1996. The effect of immune sera from hamsters immunized with sandfly gut and whole-body extract antigens on the fecundity and mortality of Phlebotomus duboscqi (Diptera: Psychodidae). Acta Tropica 60, 269–275.

Jordanov, R.L., 2003. Leishmania tropica (Kinetoplastida: Trypanosomatidae) – a perplexing parasite. Folia Parasitol. 50, 241–250.

Kaburi, J.C., Ngumbi, P.M., Christopher, O.A., 2011. Sandfly-saliva injected during repeated feeding on a sensitized hamster causes fecundity and mortality to female Phlebotomus duboscqi (Diptera: Psychodidae). J. Vector Borne Dis. 48, 61–63.

Kaburi, J.C., Ngumbi, P.M., Christopher, O.A., 2011. Sandfly saliva injected during repeated feeding on a sensitized hamster causes fecundity and mortality to female Phlebotomus duboscqi (Diptera: Psychodidae). J. Vector Borne Dis. 48, 61–63.

Lal, A.A., Schriever, M.E., Sacci, J.B., Goldman, I.F., Louis-Wileman, V., Collins, W.E., Azaz, A.F., 1994. Inhibition of malaria parasite development in mosquitoes by anti-mosquito-midgut antibodies. Infect. Immunity 62, 316–318.

Lestinova, T., Rohousova, I., Sima, M., de Oliveira, C.J., Volf, P., 2017. Insights into the sand fly saliva: Blood-feeding and immune interactions between sand flies, hosts, and Leishmania. PLoS Negl. Trop. Dis. 11(7): e0005600.35.

Mansour, N.S., Youssef, F.G., Mohareb, E.W., Dees, W.H., Karuru, E.R., 1987. Leishmania donovani visceral leishmaniasis in north Sinai. Trans. R. Soc. Trop. Med. Hyg. 81, 747–748.

Mansour, N.S., Youssef, F.G., Mohareb, E.W., Dees, W.H., 1989. Cutaneous leishmaniasis in the peace keeping force in East Sinai. J. Egypt. Soc. Parasitol. 19 (2), 725–732.

105, 7845–7850.
Martin-Martin, I., Molina, R., Jiménez, M., 2015. Kinetics of anti-Phlebotomus perniciosus saliva antibodies in experimentally bitten mice and rabbits. PLoS ONE 10, e0140722.

Morris, R.V., Shoemaker, C.B., David, J.R., Lanzaro, G.C., Titus, R.G., 2001. Sandfly maxadilan exacerbates infection with Leishmania major and vaccinating against it protects against L. major infection. J. Immunol. 167, 5226–5230.

Pigott, D.M., Bhatt, S., Golding, N., Duda, K.A., Battle, K.E., Brady, O.J., et al., 2014. Global distribution maps of the leishmaniasis e02851 eLife 3. https://doi.org/10.7554/eLife.02851.

Ribeiro, J.M., Katz, O., Pannell, L.K., Waitumbi, J., Warburg, A., 1999. Salivary glands of the sand fly Phlebotomus papatasi contain pharmacologically active amounts of adenosine and 5’-AMP. J. Exp. Biol. 202, 1551–1559.

Robles-Murguia, M., Bloedow, N., Murray, L., Ramalho-Ortigao, M., 2014. Effect of mouse antisera targeting the Phlebotomus papatasi midgut chitinase PpChit1 on sandfly physiology and fitness. Mem. Inst. Oswaldo Cruz. 109, 1064–1069.

Rogers, K.A., Titus, R.G., 2003. Immunomodulatory effects of Maxadilan and Phlebotomus papatasi sand fly salivary gland lysates on human primary in vitro immune responses. Parasite Immunol. 25, 127–134.

Shehata, M.G., Samy, A.M., Doha, S.A., Fahmy, A.R., Kaldas, R.M., Furman, B.D., Villinsk, J.T., 2009. First Report of Leishmania tropica from a Classical Focus of L. major in North-Sinai, Egypt. Am. J. Trop. Med. Hygiene 81 (2), 213–218.

Spitzova, T., Sumova, P., Volfova, V., Polanska, N., Povtora, L., Volf, P., 2020. Interactions between host biogenic amines and sand fly salivary yellow-related proteins. Parasites Vectors 13, 237.

Tesh, R.B., Modi, G.B., 1983. Development of a Continuous Cell Line from the Sand Fly Lutzomyia longipalpis (Diptera: Psychodidae), and its Susceptibility to Infection with Arboviruses. J. Med. Entomol. 20 (2), 199–202.

Titus, R.C., Ribeiro, J.M., 1988. Salivary gland lysates from the sand fly Lutzomyia longipalpis enhance Leishmania infectivity. Science 239, 1306–1308.

Tripet, F., Clegg, S., Elnaem, D.E., Ward, R.D., 2009. Cooperative blood-feeding and the function and implications of feeding aggregations in the sand fly, Lutzomyia longipalpis (Diptera: Psychodidae). PLoS Negl. Trop. Dis. 3, e503.

Valenzuela, J.G., Belkaid, Y., Garfield, M.K., Mendez, S., Kamhawi, S., Rowton, E.D., Sacks, D.L., Ribeiro, J.M., 2001. Toward a defined anti-Leishmania vaccine targeting vector antigens: characterization of a protective salivary vaccine. J. Exp. Med. 194, 331–342.

Vilela, M.L., Souza, N.A., Oliveira, S.M.P., Costa-Pinto, D., Cabello, P.H., Rangel, E.F., Traub-Cseko, Y.M., 2006. Considerations on the effect of anti-sandfly antibodies on biological parameters of Lutzomyia longipalpis (Lutz & Neiva, 1912) (Diptera: Psychodidae: Phlebotominae). Braz. J. Biol. 66 (1A), 175–183.

Vlkova, M., Rohousova, I., Drahota, J., Stanneck, D., Krueckwagen, E.M., Mencke, N., et al., 2011. Canine antibody response to Phlebotomus perniciosus bites negatively correlates with the risk of Leishmania infantum transmission. PLoS Negl. Trop. Dis. 5, e1344.

Vlkova, M., Rohousova, I., Hostomska, J., Pohankova, L., Zidkova, L., Drahota, J., et al., 2012. Kinetics of antibody response in BALB/c and C57BL/6 mice bitten by Phlebotomus papatasi. PLoS Neg. Trop. Dis. 6, e1719.

Volf, P., Tesarova, P., Nohynkova, E.N., 2000. Salivary proteins and glycoproteins in phlebotomine sandflies of various species, sex and age. Med. Vet. Entomol. 14, 251–256.