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Biting activity of *Phlebotomus* sandflies fed on long term immunized hamster

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ABSTRACT

Phlebotomus papatasi is one of the common sand flies that found in Egypt. Three to five-days old 1080 non infected *P. papatasi* sandfly females were used in this study. They fed for 90-100 min. on blood of non immunized hamster for one time/week. Each hamster was re-exposed for an average of 18 exposures at one week interval after being immunized. immunized. Sand flies were observed to determine their biting activity as Fed or Half-fed. Then sera of control and immunized hamster was analyzed by using spectrophotometer. Three replicates of sand flies groups (each with 360 insects) were used.

The results indicated a remarkable change in biting activity of sand flies through the investigated period (18 weeks) where a powerful biting through the first seven weeks was observed, followed by a significant decrease in number of biting activity during the rest of weeks. This can be explained by acquiring hamster immunity due to formation of antibodies in his blood. Multiple exposures to sandfly saliva induce an immune response in hamster which can control leishmaniasis in this animal. This immune response was reflected in results of total protein estimation where the total protein increased after successive bits compared to control. Therefore, we could concluded that the antibodies which have been formed in hamster against saliva of female sandfly have a repellent effect as well as immunized effect.

Knowledge to be gained from the present study will contribute to recent trends in the future development of novel strategies for preventing disease transmission in Egypt.

Key words: Sandfly, Biting activity, Cutaneous Leishmaniasis, Hamster.

INTRODUCTION

Environmental changes give rise to numerous opportunities for unexpected or enhanced risks from vector-borne diseases. The best method to interrupt any vector-borne disease is to reduce human vector contact (Sharma and Singh, 2008). Vector control is the key intervention for the elimination of vector borne diseases. Currently, using Antigen Antibodies technique and immunological control are considered from the strong strategy recommended in vector control and widely used especially in mosquitoes and sand flies vectors (WHO, 2000).

Sandflies are flying, biting and blood-sucking insects that are found mainly in tropical and subtropical areas and occur in wide range of habitats; from hot dry deserts to dense tropical forests. The previous works in this field proved the ability of sandfly in acquiring and developing Leishmaniasis disease (Regis and Fabiano, 2012). Sandfly saliva consists of roughly thirty different salivary proteins, many with known roles linked to blood feeding facilitation. Apart from the anti-hemostatic capacity of saliva, several sandfly salivary proteins have been shown to be immunogenic. Immunization with a

single salivary protein or exposure to uninfected bites was shown to result in a protective immune response against leishmaniasis (Regis and Fabiano, 2012). Abdeladhim *et al*. (2014) gave a review with a comprehensive update of recent advances in the characterization of salivary molecules and their biological activities and offer insights pertaining to their protective effect against leishmaniasis and their potential as markers of vector exposure.

Multiple exposures to sandfly saliva induce an immune response that can control leishmaniasis in animal models (Belkaid *et al*., 1998). Cutaneous leishmaniasis (CL) is considered as an important health problem in many parts of Middle East especially in North Sinai; Egypt (WHO, 2000). So; the immunization of vertebrate hosts with vector components may be an alternative method for the control of disease transmitted by sandfly.

Phlebotomine sand flies are major biting insects of man and are the vectors of several viruses, the bacterium *Bartonella bacilliformis*, and, most importantly, the protozoan parasites that cause leishmaniasis. Worldwide, there are an estimated 2 million new cases of leishmaniasis annually, and 12 million people are currently believed to be infected with leishmaniasis (WHO, 2004). Throughout North Africa, the Middle East and Southwest Asia, *Phlebotomus papatasi* is the primary vector of *Leishmania major*, the causative agent of zoonotic cutaneous leishmaniasis (ZCL) (WHO, 2004). Although epidemiological and clinical findings are progressed but it we still need to evaluate the effect of the synthesized antibodies formed in hamsters during long-term sand flies feeding. Salivary glands of sand flies disease vectors are essential organ in blood- and sugar-feeding. They secret a salivary fluid that is rich in proteins with various functions including modulators of host physiological and immunological responses to insect bites in order to facilitate parasite invasion of the target host blood vessel and establishment of infection.

The present study was conducted to investigate the impact of biting activity of Phlebotomine sand flies fed on long term immunized hamster. The results will contribute to recent trends in the future development of novel strategies for preventing disease transmission in Egypt.

MATERIALS AND METHODS

1. Rearing of sandfly colonies:

A laboratory susceptible colony of *Phlebotomus papatasi* sand flies was used in this study. This colony was established from specimens collected from El-Agamy, Alexandria, Egypt, in May 2014 and were reared and maintained in an Insectary at Research and Training Centre on Vectors of Diseases (RTC) of Ain Shams University Cairo, Egypt. The Flies were reared by the procedure described by Modi and Tesh (1983) at 27°C ± 2°C, 80% ± 10% RH, and light: dark photo period 12:12 throughout their development.

Adults were offered 30% sucrose solution daily, and provided blood meal (necessary for egg production) on anesthetized Syrian golden hamster, *Mesocricetus auratus* (O: Rodentia, F: Muridae) once per week (Fig 1). After blood feeding, insects were aspirated using mechanical aspirator and placed in a plastic pot with a bottom made of plaster of Paris and a mesh cover. The bottom was humidified to provide a suitable surface to allow the females to lay their eggs.

After laying eggs, dead females were removed by fine forceps and eggs were allowed to hatch. Larvae were fed a mixture of aged rabbit feces and rabbit chow, and the moisture in the pots was monitored until adult emergence. Three to five- days old sandfly females were used in this study.

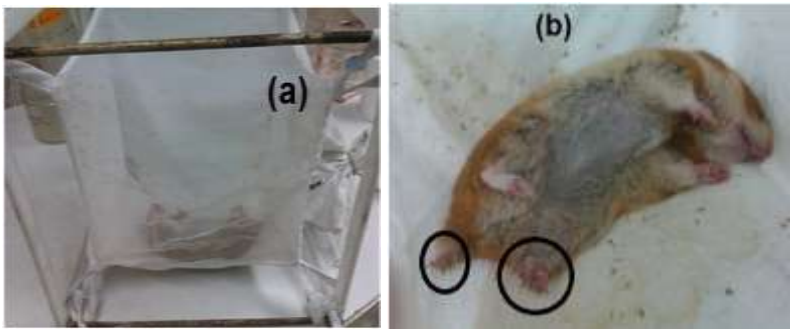

Fig. (1): (a) Adult sand flies in the mesh cage while feeding on anesthetized Syrian Golden hamster (b) closer view of adult females while feeding on hind foot and tail of the hamster.

2. Evaluation of biting activity of female *P. papatasi* fed on immunized hamsters:

A total of 1080 female sand flies and three hamsters were used under laboratory conditions in this study. All experiments had been conducted in accordance to the principles expressed in the declaration of Basel (Abbott, 2010). All hamsters were intra-peritoneal anesthetized by FDA-approved rapid-acting sodium thiopental at a dose of 0.4 ml/100 g body weight. Groups A, B, and C were used as replicates for the biting activity experiments.

A group of 20 non infected experimental female *P. papatasi* (3-5 days old) was allowed to feed on the immunized hamsters. Anesthetized hamsters were placed on the floor of the sandfly cage to allow sand flies feeding for 90-100 minutes in dark closed room (i.e. artificial nocturnal sandfly habit). After completion of the feeding period (90 min.) the animal was taken out and return to its rearing cage till next feeding trial. Each immunized hamster was re-exposed in this manner for an average of 18 exposures at one week interval. The were observed for the determination of their biting activity as one of two cases; Fed and Half-fed.

3. **Quantitative estimation of total protein of serum of hamster**:

Collected sera from experimental hamsters and control ones were subjected to quantitative analysis by spectrophotometer to measure the total protein of antibodies formed in the blood of repeated bitted hamsters. The total protein reagent was determined according to the method described by Bradford (1976). A total of 50µl of each sample of hamster' sera was pipetted into an Eppendorf® tube and adjusted final volume to 100µl by adding phosphate buffer (pH 6.6). Five ml of protein reagent (100 mg COBB dissolved in 50ml ethanol 95% then completed to 100 ml by 85% phosphoric acid) was added and allow the Eppendorf® to mix (inversion or vortexing). The total protein in each Eppendorf® was measured by spectrophotometer at 595 nm absorbance against blank prepared from 0.1 ml of phosphate buffer (pH 6.6) and 5 ml of protein reagent. The weight of protein was plotted against the corresponding absorbance resulting in standard curve used to determine total protein concentration in unknown samples.

RESULTS

The sand flies biting activity trials for each tested animal are graphically presented in Figures (2-4). It was obvious that there was a significant decline in biting activity of *P. papatasi* which fed on immunized hamster as a result of the sensitized antibodies in sera of host was

occurred after repeated biting for 18 weeks **(**Fig. 2). The general response of feeding activity of tested female sand flies against assayed animals was almost similarly at all replicates; which indicated the important role of synthesized antibodies in hamster against biting of sand flies; as well as against salivary gland homogenate of sand flies.

Most of the present results showed the high rate of biting activity ranged between 80 to 100% at the first ten weeks. Then, there was a significant decrease in the number of biting sand flies females till the 15th week from 80% to 32%, followed by a sharp decrease at 16th, 17th and the last 18th week of the experiment.

The first nine weeks showed a great sharing of completely fed sand flies compared with half fed ones. This result goes inversely by the time to reach the most of sharing of half fed sand flies to the end of the experimental time. This clearly shows that there is a great effect of formed antibodies in hamsters as a result of long-term sand flies feeding.

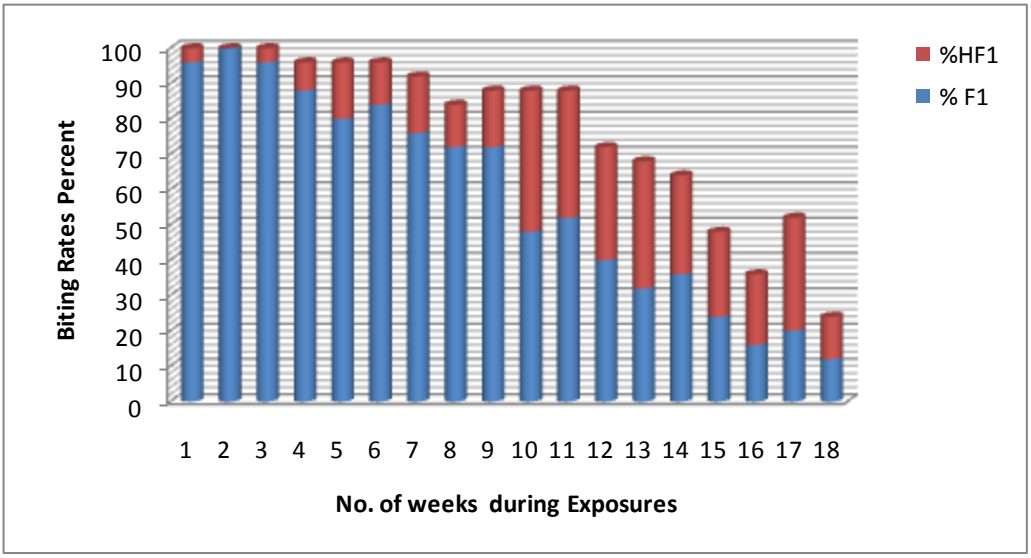

Fig.2: Biting activity of *Phlebotomus papatasi* (G.A) females during 18 weeks (exposure Intervals). HF1 : half fed, F1: completely fed.

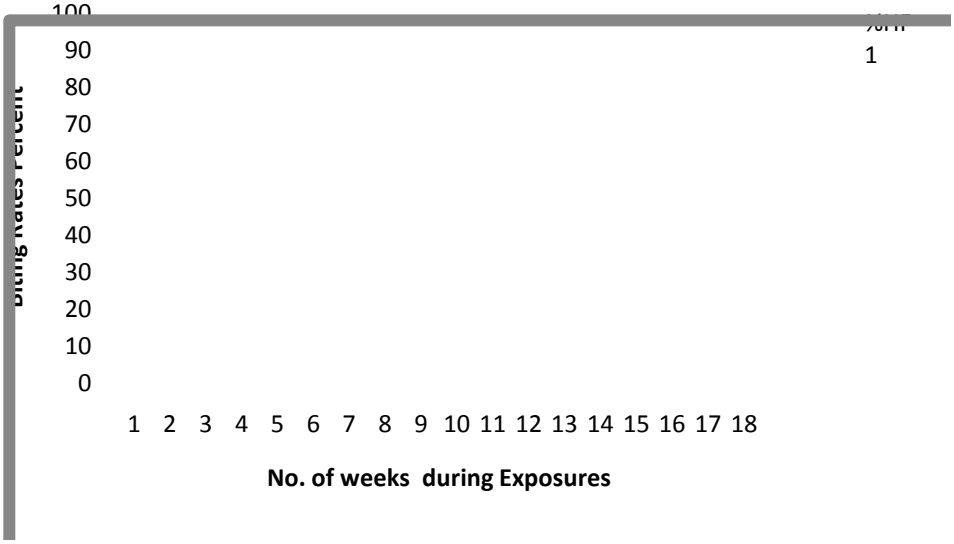

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Fig.3: Biting activity of *Phlebotomus papatasi* (G.B) females during 18 weeks (exposure Intervals). HF1 : half fed, F1: completely fed.

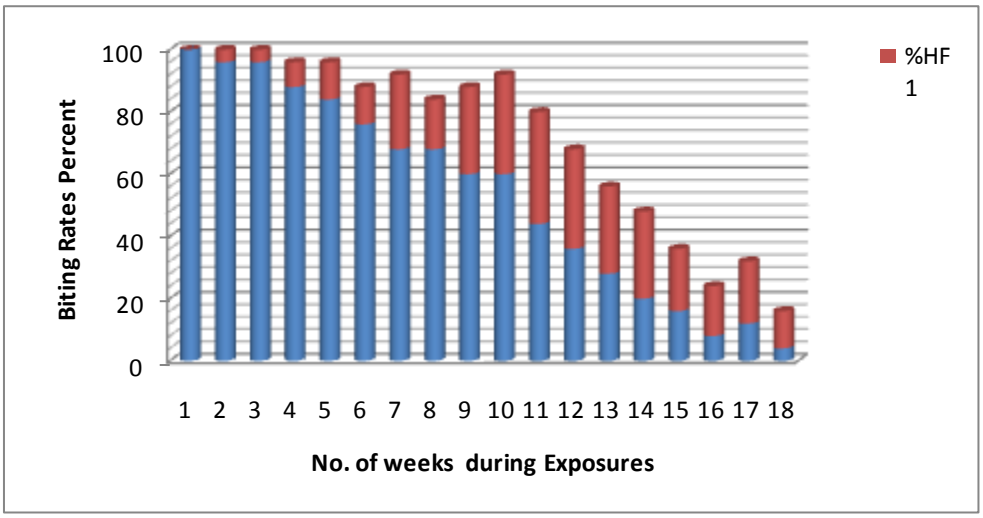

Fig.4: Biting activity of *Phlebotomus papatasi* (G.C) females during 18 weeks (exposure Intervals).

Spectrophotometer analysis of protein concentration of serum of control and bitted hamster:

Table (1) shows the results of serum samples from control and bitted hamster with san flies for 18 weeks. It was obvious that serum values was higher in bitted hamster (38 mg/ml) than in unexposed ones (35 mg/ml).

Table (1). Conc. of serum in blood samples of unexposed and bitted hamster by sand flies, *Phlebotomus papatasi*, for 18 weeks.

Type of hamster	Conc. of serum in blood sample
Unexposed hamster (Control sample)	35 mg/ml
Eighteen week biting exposed hamsters	38 mg/ml

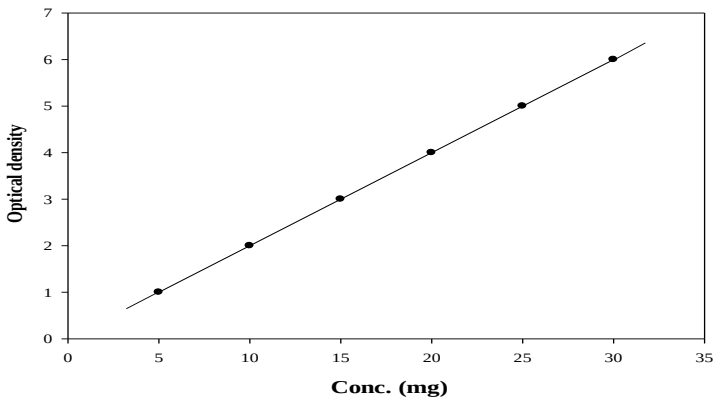

Fig.5 : Standard curve of total protein of serum of hamster exposed to bits of female sanflies for 18 weeks.

DISCUSSION

Immunization of the vertebrate host with arthropod vector's saliva components could disturb the biological aspects of these vectors; The most successful case for the use of this approach in arthropod control was for ticks by Trager (1939). He could protect cattle from the infection transmitted by ticks through inducing immunity by tick tissue injection.

Sandfly saliva has immune-modulatory properties, but it is important to point out that many of these components are proteins and therefore have the potential to be immunogenic. In fact it has been shown that multiple exposures to sandfly bites induce specific immunity that can be detected by the presence of antibodies and cellular immune responses. It is remarkable that all tested vector sandfly species from New and Old World were able to induce immune responses in several hosts. 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; Ahmed *et al*., 2010; Vlkova *et al.*, 2011).

Sand flies do not feed for a long time on their host, they depend mostly on the salivary anti-hemostatic components to acquire a blood meal. However, a number of immuno-modulatory activities have been reported from the saliva of sand flies (Andrade *et al*., 2007; Prates *et al.*, 2012). The current work proved that hamsters are able to develop anti-sandfly saliva antibodies when they were used repeatedly to feed a colony of *Phlebotomos papatasi*. Similar observations have been revealed by Kaburi and Anjili (2011), when Syrian hamsters were used repeatedly to feed a colony of *Phlebotomos duboscqi*, the vector for *L. major* in Kenya. Through the present work, the amount of protein in sera of hamster was estimated to be 35 mg/ml in the case of control hamster and 38 mg/ml in exposed one.

In the current study a significant decline in biting activity of *P. papatasi* which fed on immunized hamster as a result of the sensitized antibodies in sera of host was occurred. The same effect was observed in Anophelus stephensi fed on mice immunized with various mosquito tissues (Almeida & Billingsley 1998). This kind of approach to control leishmaniasis may offer some advantages over currently employed measures, either in relation to insecticides, which are restrictive when used continuously, or in patient therapy normally involving high levels of toxicity. The injection into vertebrate hosts of haematophagous vector extracts, or saliva components delivered through bite, has been found to stimulate a wide range of immune responses with the production of anti-vector antibodies (Hatfield 1988; Ramasamy *et al.*, 1988; Srikrishnaraj *et al.*, 1993; Almeida & Billingsley 1998). This way of control might affect not only vital processes in the insects' biological development, but also interfere with their vectorial capacity (Sutherland & Ewen, 1974; Ramasamy & Ramasamy, 1990). This may be important from the standpoint of potential control of natural sandfly populations. This investigation could help us to define the salivary proteins that might be useful for vaccination against leishmaniasis.

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نشاط العض لذباب الرمل فليبوتومس التى تتغذى لمدة طويلة على دم الهامستر

اسلام عدلى[1] ، دعاء عبد الكريم[1] ، أبتهال الدمرداش[2] ، مجدى شحاته[1]

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المستخلص

فليبوتومس باباتاسي هي واحدة من ذباب الرمل الشائعة في مصر. و فى هذه الدراسة تم أستخدام 1080 عينة ذات عمر 3-5 أيام من العمر غير مصابة من اناث هذا النوع من ذباب الرمل . تغذت العينات لمدة 90-100 دقيقة على دم الهامستر غير المحصن بمعدل مرة واحدة / الأسبوع. أعيد تعريض كل هامستر لمتوسط 18 تعرضا في فترة فاصلة واحدة بعد التحصين. و تم ملاحظة ذباب الرمل المستخدم فى الدراسة لتحديد نشاطها العض لديها ومعرفة مدى امتلاء البطن (نصف تغذية أو تغذية كاملة) . كما تم تحليل مصل الدم و تقدير البروتين الكلى فى الاجسام المضادة المتكونة بعد العض فى دم الهامستر. واستخدمت ثلاث مكررات من مجموعات ذبابة الرمل (لكل منها 360 حشرة).

وأظهرت النتائج حدوث تغير ملحوظ في نشاط العض في ذباب الرمل خلال الفترة التي شملتها الدراسة (18 أسبوعا) حيث لوحظ وجود عض قوي خلال الأسابيع السبعة الأولى، يليه انخفاض كبير في عدد نشاط العض خلال بقية الأسابيع. ويمكن تفسير ذلك عن طريق تمون مناعة فى دم الهامستر بسبب تشكيل الأجسام المضادة في دمه. وقد أدى التعرض المتعدد للعاب ذبابة الرمل الى تحفيز استجابة مناعية في الهامستر مما يوضح امكانية السيطرة على داء الليشمانيا في هذا الحيوان. وقد انعكست هذه الاستجابة المناعية في نتائج تقدير البروتين الكلي حيث زاد البروتين الكلي بتكرار عملية العض المتعاقبة مقارنة مع كميتة فى مجموعة الهامستر المستخدمة للمقارنة (محموعة السيطرة). لذلك، يمكن أن نستنتج أن الأجسام المضادة التي تم تشكيلها في الهامستر ضد اللعاب من اناث ذباب الرمل لها تأثير طارد فضلا عن تأثير التحصين المناعى.

وستسهم المعرفة المكتسبة من نتائج هذه الدراسة في الاتجاهات الحديثة للتنمية المستقبلية لايجاد استراتيجيات جديدة لمنع انتقال الأمراض في مصر.