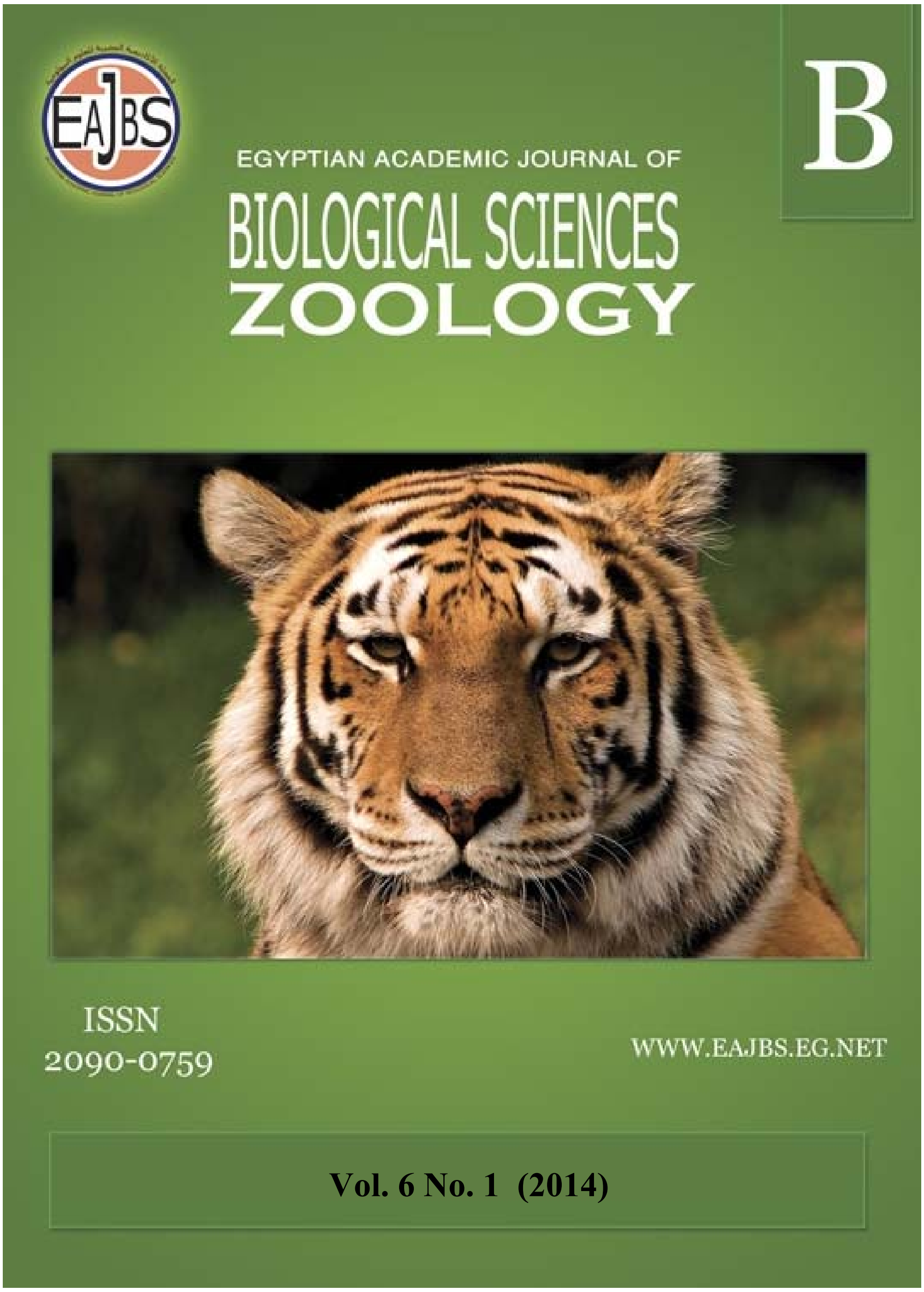

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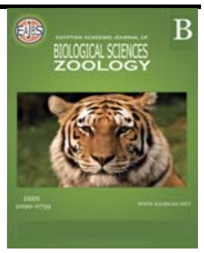

A study for the influence of mosquitoes saliva on immunity of a laboratory animal (pigeon) to control infectious diseases.

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ABSTRACT

The saliva of blood feeding arthropods has a variety of substances that are responsible for the success of blood meal. Repeated bites of mosquitoes stimulate immune responses to salivary antigen in the vertebrate host. In this study group of 4-pigeons were exposed to frequent bites of groups of *Culex pipiense* and *Anopheles pharoensis* (100 female mosquitos per pigeon, 6 times in 18 weeks) and the biting rate were estimated. The biting rate of mosquitoes on pigeons decreased through different attempts. Group of 12 different clean pigeons are used as control pigeons (6 pigeons for each mosquito's species) where one pigeon is exposed to the bites of 100 mosquitoes of one species 6 times in 18 weeks and the biting rate were calculated. The rate is relatively constant. The rate decreased in the case of the frequent bites may be due to the immunomodulative substances in mosquito saliva which stimulate immune responses in pigeons. Pigeons previously bitten frequently by *Culex pipiense* (*Cx.* sp.) and *Anopheles pharoensis (An.* sp.) mosquitoes are exposed once to bites of 100 female mosquitoes of the other mosquito species. The rate of feeding was calculated .This rate was increased. This increasing in the rate may be due to little cross- reactivity between the two mosquitoes species.

Further investigations are recommended to study the effect of mosquito saliva on human immune responses to evaluate the mosquito salivary proteins as vaccines for mosquitoes-borne diseases and also for decreasing or prevent the biting of mosquitoes.

Keywords:Mosquitoes saliva – Immunity - Pigeons

INTRODUCTION

Hematophagous insects are a major problem to vertebrate host as vectors of fetal diseases and also as nuisance pests. Mosquitoes transmit some of the world most serious vector-borne diseases such as, malaria, encephalitis, filariasis, yellow fever. (Esptin., 2001; Strobel and Lamury., 2001; Merlose., 2002).

In Egypt *Culicine* mosquitoes have been incriminated in transmission of rift valley fever virus (Gad *et al*., 1987) and the main vector of bancroftian filariasis

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(Southgate. 1979). While *Anopheline* mosquitoes were the main vectors of *Plasmodium vivax, P. falciparum and P. malaria.*

Vector saliva is an important factor in the transmission of disease agents by mosquitoes (Edwards *et al.,* 1998). The saliva of vectors possess an array of pharmacologically active compounds which helps them to locate blood vessels and to disrupt the hemostatic process of the host, mainly the blood clotting, platelet aggregation and vasoconstriction (Ribeiro., 1987; 1995).

Aim of work was to investigate the biting rates of certain mosquitoes species in experimental animals pre-exposed to frequent bites of mosquitoes. Have a preliminary observations on the developing of cross-reaction against the bites of certain mosquito species in experimental animals pre-exposed to separated bites by other species. Investigate the possibility that exposure to mosquito saliva may modulate immunity to mosquito bites in vertebrate host. Prevent spreading the infectious diseases by preventing bites of mosquitoes.

MATERIALS AND METHODS

Mosquitoes rearing

The laboratory strains of mosquitoes (*Culex Pipiens cx.* sp.) & *Anopheles Pharoensis* (*An.* sp.) used in this study were maintained in walk- in insectaries under controlled conditions of temperature (27 °C), relative humidity (70-80 RH) and a16 h photoperiod. Larval instars were maintained in enamel pans (30-35cm in diameter a 8-10 cm depth), half filled with tap water in case of *Cx*. sp. mosquitoes and in filtered water in case of *An. sp*. mosquitoes, and provided with fish food (Tetramin-Tetra Werk Gmb,W. Germany) to avoid scum formation in the rearing pans, larvae were poured daily into clean enamel pans. Developed pupae were transferred daily to plastic cups containing tap or filtered water that were introduced into 30cm wooden cages. Emerging adults were provided daily with 10% glucose solution and supplied with small water containers for egg laying. For oviposition, four days old adults were derived from food for 24h, then allowed to feed on a pigeon. Each egg raft was placed in a plastic cup containing tap water for hatching in case of *Cx*. sp. mosquitoes. In case of *An*. sp. mosquitoes the cub is supported by a filter paper for collecting their single layed eggs.

Calculation of feed and un feed mosquitoes

A- Group of 4 pigeons were exposed frequently to bites of groups of the two colonized mosquito species (100 female mosquito per a pigeon, 2 pigeons for each species). Where, the pigeons used not bitten before or during the experiment by any hematophagous insect.

B- Feeding of mosquitoes on pigeons was continued for 6 times over about 18 weeks and biting rates were calculated.

C- Group of 12 different clean pigeons are used as control pigeons (6 pigeons for each mosquito's species) where one pigeon is exposed to the bites of 100 mosquitoes of one species 6 times in 18 weeks and the biting rate were calculated.

RESULTS

1-Comparison between biting rates of *Culex pipiens* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon

Table 1 shows the significant difference between biting rates of *Culex pipiens* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon where there is no significant difference between the biting rates of *Culex pipiens* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon at attempt number 1 and attempts number 2 while, starting from attempt number 3 to attempt number 6 there is significant difference between the biting rates of *Culex pipiens* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon.

Table 1: Comparison between biting rates of *Culex pipiens* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon

No. of attempts	Fed frequently on a pigeon			Fed on clean pigeons (control pigeons)			p-value
	No of exposed	No. fed	%	No of exposed	No. fed	%	
1	200	196	98%	100	97	97%	0.9 (NS)
2	200	194	97%	100	98	98%	0.9 (NS)
3	200	168	84%	100	96	96%	0.005*
4	200	120	60%	100	97	97%	<0.001*
5	200	120	60%	100	95	95%	<0.001*
6	200	88	44%	100	98	98%	<0.001*
Total	1200	886	73%	600	581	96.8%	<0.001*

*Statistically significant difference (Chi square test)
NS: not statistically significant difference (Chi square test)

Comparison between biting rates of *Anopheles pharoensis* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon.

Table 2 shows the significant difference between biting rates of *Anopheles pharoensis* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon where there is no significant difference between the biting rates of *Anopheles pharoensis* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon at attempt number 1 and attempts number 2 while, starting from attempt number 3 to attempt number 6 there is significant difference between the biting rates of *Anopheles pharoensis* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon.

Table 2: Comparison between the biting rates of *Anopheles pharoensis* fed on clean pigeons (control pigeons) and those fed frequently on a pigeon

No. of attempts	Fed frequently on a pigeon			Fed on clean pigeons (control pigeons)			p-value
	No of exposed	No. fed	%	No of exposed	No. fed	%	
1	200	194	97%	100	98	98%	0.9 (NS)
2	200	192	96%	100	96	96%	0.8 (NS)
3	200	170	85%	100	96	96%	0.008*
4	200	144	72%	100	97	97%	<0.001*
5	200	136	68%	100	95	95%	<0.001*
6	200	128	64%	100	97	97%	<0.001*
Total	1200	964	80%	600	579	96.5%	<0.001*

*Statistically significant difference (Chi square test)
NS: not statistically significant difference (Chi square test)

The biting rate of *Culex pipiens* on pigeon previously bitted by *Anopheles pharoensis*.

The pigeon which was exposed previously to the biting of *Culex pipiens* was also exposed to the biting of *Anopheles Pharoensis*, the rate of biting 97% as shown in (Table 3).

Table 3: The biting rate of *Culex pipiens* on pigeon previously bitten by *Anopheles pharoensis*

No. of attempts	No. exposed	No. fed	Percentage
1	200	114	97%

The biting rate of *Anopheles pharoensis* fed on pigeon previously bitten by *Culex pipiens.*

The pigeon which exposed previously to the biting of *Anopheles Pharoensis* was exposed to the biting of *Culex pipiens*, the rate of biting 95% as shown in (Table 4).

Table 4: The biting rate of *Anopheles pharoensis* fed on pigeon previously bitten by *Culex pipiens*

Experiment No.	No. exposed	No. fed	Percentage
1	200	110	95%

DISCUSSION

It is clear that saliva of arthropod vectors contains vaso-modulatory factors and immune-modulatory factors (Riberio1987; Gillespie *et al.,* 2000& Wahba *et al*., 2005).These effects would benefit the arthropod vector in its quest to locate blood and may help to keep blood flowing without incurring a host inflammatory response.

This study shows that, the rate of feeding of *Culex pipiens* and *Anopheles pharoensis* on a pigeon decreased when these rates are compared with biting rate of *Culex pipiens* and *Anopheles pharoensis* fed on different clean pigeons (control pigeons) through the different attempts. Where, the feeding rate decreased starting from the attempt number 3 when compared with the feeding rate the control pigeons, These results are corresponding with the data obtained by (Wahba *et al*., 2005).They found that there was decreased feeding rate on hamster because the sand fly saliva have immunogenic molecules, which innate immune responses in the skin. Repeated bites of female mosquitoes stimulate immune responses to salivary antigen in the pigeons the rate of feeding decreased because of the immunity which elicited in the pigeon after being bitten frequently by the female mosquitoes.

This result is corresponding with (Owhashi *et al.,* 2001; Nuttal *et al*., 2000) this result also agreed with (Kamhawi *et al.,* 2000) where they found that, repeated bites of blood sucking insects stimulate immune responses to salivary antigen in the vertebrate host.

Pigeons previously bitten by *Culex pipiense* and *Anopheles pharoensis* mosquitoes are exposed once to bites of the other mosquito species. The rate increased in comparing with the final attempts feeding rates it was relatively similar to the rate of feeding of control pigeons and the first attempt. This rate increased due to little cross- reactivity between the two mosquitoes species. This study showed that there is no significant difference in cross-reactivity against the bites of certain mosquito species in experimental animals pre-exposed to separated bites by other species.

These results are corresponding with the data obtained (Matsuoka *et al;* 1997) they reported that antibody responses to mosquito salivary proteins are genus-specific, indicating little cross-reactivity between salivary proteins from different arthropods (i.e. *Anopheles sp.*, *Culex sp.* or *Aedes sp.*) or that both species-shared and species-specific allergens exist and with (Brummer-Korvenkontio *et al*., 1997) they showed that anopheles saliva antigen reacted with immune serum to *An. stephensi*, but not with immune serum to *Aedes communis* or *A. aegypti,* indicating species-specific antibody production. While, these results disagree with (Peng *et al*., 2006; Rizzo *et al*., 2011), they had demonstrated the presence of cross-reactive antibody responses against salivary proteins from different hematophagous arthropod species.

The present study findings also not agree with the observation of (Volf *et al*., 2000; Rispail and Leger., 1998) where they observed distinct cross-reactivity only between saliva proteins of two taxonomically closely related mosquito species.

In conclusion, the present study showed that, the saliva of mosquitoes exert immunomodulatory effect on vertebrate host. These saliva as protein fractions act as antigen, which can enhance the production of antibodies in the host. This study indicated that the primary effect of mosquito saliva could affect the feeding rate.

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ARABIC SUMMARY

دراسة تاثير لعاب البعوض علي مناعة احدى حيونات التجارب (الحمامه) للسيطره على الامراض المعديه

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استهدفت الدراسه الحاليه عمل رصد لمعدلات تغذيه نوعين من عشائر البعوض علي حيوانات التجارب من خلال تعريضها للتغذيه المتكرره وعمل ملاحظه مبدئيه للتفاعل المتصالب اذا استحدث داخل حيوانات التجارب ضد نوع من البعوض حيث ان الحيوان قد تعرض من قبل لعضات متكرره من نوع اخر من البعوض ولمنع انتشار الامراض المعديه التي تنتقل بواسطة البعوض. تم تربية البعوض لعمل مستعمرات منه تحت ظروف محدده من درجات الحراره والرطوبه ومدة الاضاءه.

كما تم تغذية الطور الناضج من البعوض علي محلول الجلوكوز 10% ثم حرمانه من الطعام لمدة24 ساعه ثم تغذيته على حمامه وتوضع له اوعيه صغيره ممتلئه بالماء ليبيض فيها.

تم تعريض مجموعه من 4 حمامات ليتغذى عليهم البعوض تغذيه متكرره من نوعى الكيولكس بيبينز والانوفيليس الفرعونى بواقع 100 بعوضه لكل حمامه وذلك لست مرات في 18 اسبوع وتم تعيين معدل التغذيه في كل مره .فوجد انه قد انخفض معدل تغذية البعوض على الحمام خلال مرات التغذيه المختلفه.

.ومجموعه من 12 حمامه مختلفه تم استخدامهم كتجربه قياسيه بحيث تعرضت ست حمامات للعض من كل نوع من البعوض بواقع حمامه لكل 100 بعوضه من كل نوع وذلك لست مرات في 18 اسبوع وتم تعيين معدل التغذيه في كل مره.فلم ينخفض معدل التغذيه خلال المرات المختلفه.

قد يرجع الانخفاض في معدل التغذيه المتكرره الى العامل الموجوده في لعاب البعوض والتي ادت الى اثارة المناعه ضد هذا اللعاب لدى الحيوانات.

تكوين اللعاب في الحيوانات المتغذيه علي الدماء هو مركبات بروتينيه تعمل كاجسام مولده ينتج عنها اجسام مضاده لهذه المركبات البروتينه مما يفسر انخفاض معدل التغذيه.

الحمام الذي قد تغذى من قبل عليه بعوض الكيولكس والانوفيليس تم تعريضهم للنوع الاخر من البعوض وقياس معدل التغذيه في كل منهم .

فقد ازداد معدل التغذيه في كل منهم مره اخرى مما يدل على انخفاض التفاعل التصالبي بين النوعين.