The effect of storage time on the antigenic stability of mosquito bloodmeals

Malkith Kaur[1], PKCLim[2] and Lokman Hakim S[1] [1] *Division of Parasitology, Institute For Medical Research, Kuala Lumpur; 2Division of Molecular Pathology, Institute For Medical Research, Kuala Lumpur*

Abstract

The enzyme-linked immunosorbent assay (ELISA) and the counter-current immunoelectrophoresis (CCIE) were used to determine the antigenic stability of bloodmeals of *Aedes togoi* and *Aedes aegypti* fed experimentally on 5 different hosts, namely man, monkey, cat, avian and rodent stored over a 5 month period. Results of the ELISA showed considerable variation in the mean optical density (OD) values of bloodmeals stored over the 5-month period. This wide variation could be due to the high sensitivity of the test and its ability to detect differences in the meal size and the rate of blood digestion by individual mosquitoes. However results obtained with the counter-current immunoelectrophoresis (CCIE) did not show much variation. This could be due to the fact that ELISA is more sensitive than the CCIE. It can be concluded that bloodmeals stored in the refrigerator for as long as 5 months do not show much loss in antigenicity.

Key words: bloodmeal; counter-current immunolectrophoresis; ELISA

Introduction

Bloodmeal identification remains an important tool in the investigation and study of arthropod-borne diseases. Investigation of insect vectors of diseases requires accurate information about their feeding habits and preferences. In recent years, the serological approach has been preferred to other methods for identification of the source of bloodmeals (Weitz, 1956) since such methods have been found to be accurate and consistent. Of the many available serological methods, the counter-current immunoelectrophoresis (CCIE) (Culliford, 1964) and the enzyme-linked immunosorbent assay (ELISA) (Burkot *et al.*, 1981) have been found to be almost similar in specificity and both are easy to perform. Thus these two methods have been used in this study to determine whether the blood meal samples deteriorate over time when stored in the refrigerator as no study has yet been done on this aspect of bloodmeal analysis.

Materials and Methods

Mosquito blood meal

Two species of laboratory reared mosquitoes, *Aedes togoi* (Liverpool strain), and *Aedes aegypti* (Selangor strain) were used in this study. At monthly intervals for a period of 6 months, 50 mosquitoes each of both species were fed on 5 hosts, namely man, monkey, rodent, avian and cat. Mosquitoes were killed 4 hrs after feeding and the stomach contents smeared onto a Whatman filter paper No. 1. The filters were then air dried and kept at 4°C in the refrigerator.

Preparation, standardization and absorption of antisera

Antisera were prepared in rabbits using the technique described by Weitz (1956). They were reacted with both homologous and heterologous sera in CCIE and absorped with cross-reacting heterologous sera to increase the specificity. The same antisera were further purified using the ammonium sulfate precipitation for use in the ELISA. Absorbed antisera were then rested using CCIE and ELISA.

Counter-current immunoelectrophoresis (CCIE)

CCIE was carried out according to the method described by Culliford (1964). Each sample was eluted in normal saline and the bloodmeal extract diluted at 1:150,1:300 and 1:450. The specimens were run on 1.3% agarose against the specific antisera for 100 minutes at 160 volts. After the run, the slides were soaked in normal saline overnight and examined for bands. A positive reaction is indicated by the formation of a white precipitate resulting from the insoluble product formed by antigen-antibody complex. The absence of a precipirate therefore indicates that no antigen antibody reaction has occurred.

Enzyme-linked immunosorbent assay (ELISA)

The ELISA was performed on all the specimen following the technique described by Burkot *et al.* (1981). The optimal dilution for the antigen and antisera obtained by chequerboard titration was 1:2500 while that for the conjugate (horseradish peroxidase goat anti rabbit IgG) was 1:40,000. The mean optical density (OD) reading using negative mosquito samples was 0.187 ± 0.039. The mean OD + 2 SD was taken arbitrarily as the positive cut-off value.

Results

Table 1 shows the mean OD ± SD values of the bloodmeals of the two mosquitoes species fed on the 5

different hosts. For *Ae. togoi*, statistical analysis, using analysis of variance, revealed no significant difference between the various mean OD values for human and avian bloodmeals over the 5 month period (F=1.06, P=0.381 and F=2.00, P=0.079 respectively). For monkey bloodmeal mean OD values at months 4 and 5 were significantly lower than that at month 0 (F=26.83, P=<0.001). Similarly, mean OD values of cat and rodent bloodmeals differed significantly (F=43.46, P=<0.001 and F=11.92, P=<0.001 respectively). For cat bloodmeal mean OD values for samples at months 2 and 4 were lower than that at months 0 while for rodent bloodmeal all the mean OD values from months 1 to 5 appeared to be higher than that at month 0. This may probably be due to the large variation in the size of bloodmeals taken in by the mosquitoes.

For *Ae. aegypti*, analysis of variance showed that for all the 5 hosts, there were significant differences between the mean OD values (P=<0.001). For human bloodmeal, except for mean OD at month 5, all the orher mean values were lower than that at month 0 (F=10.69, P=0.001). For monkey bloodmeal, mean OD values from month 3 to 5 were lower than that at month 0 (F=50.94, P=<0.001). For cat bloodmeal mean OD values for all months except month 2 had higher mean OD values than month 0 (F=72.44, P=<0.001). For rodent bloodmeal, mean OD values for months 2 and 4 were lower than that for month 0 but mean OD values for months 1 and 5 appeared higher (F=28.21, P=<0.001). For avian bloodmeal, mean OD values from months 2 to 5 were higher than that of month 0 (F=22.76, P=<0.001).

CCIE was performed on bloodmeal samples of the two mosquitoes species fed on the 5 different hosts. Bands were obtained for all cat, human, rodent, and avian bloodmeals at all the three different dilutions tested. However in the case of the monkey bloodmeal a few specimens did not have any precipitin bands in the CCIE test. These included one bloodmeal sample from *Ae. aegypti* mosquito (month 0) which had no precipitin band at all for the three dilutions. In addition, bloodmeals at month 3 in three *Ae. aegypti* samples did not have any band at 1:450 dilution while another *Ae. aegypti* blood meal had no band at 1:300 dilution. For *Ae. togoi* at month 5, 3 mosquito samples had no precipitin band at 1:450 dilution.

Discussions

From this study, results obtained with the ELISA appear to have more variation than that of the CCIE test.

Table 1. Optical density (OD) readings (mean ± SD) of *Aedes togoi* and *Aedes aegypti* bloodmeals with enzyme-linked immunosorbent assay using homologous antigens

Month	*Aedes togoi*					*Aedes aegypti*				
	Man	Monkey	Cat	Avian	Rodent	Man	Monkey	Cat	Avian	Rodent
0	0.54 ± 0.12	0.55 ± 0.13	0.82 ± 0.20	1.33 ± 0.15	0.37 ± 0.14	0.46 ± 0.12	0.71 ± 0.17	0.64 ± 0.18	1.47 ± 0.09	0.47 ± 0.13
1	0.59 ± 0.10	0.66 ± 0.14	0.83 ± 0.19	1.33 ± 0.17	0.51 ± 0.14	0.48 ± 0.09	0.79 ± 0.19	0.81 ± 0.20	1.45 ± 0.10	0.54 ± 0.14
2	0.58 ± 0.13	0.50 ± 0.14	0.69 ± 0.15	1.34 ± 0.17	0.38 ± 0.18	0.42 ± 0.11	0.67 ± 0.14	0.56 ± 0.14	1.52 ± 0.07	0.37 ± 0.15
3	0.57 ± 0.11	0.59 ± 0.11	1.20 ± 0.16	1.39 ± 0.07	0.50 ± 0.16	0.44 ± 0.09	0.53 ± 0.17	1.02 ± 0.20	1.57 ± 0.05	0.39 ± 0.12
4	0.58 ± 0.16	0.37 ± 0.13	0.74 ± 0.20	1.33 ± 0.11	0.57 ± 0.30	0.43 ± 0.09	0.49 ± 0.13	0.92 ± 0.14	1.53 ± 0.08	0.42 ± 0.10
5	0.58 ± 0.13	0.49 ± 0.14	1.06 ± 0.19	1.37 ± 0.14	0.58 ± 0.15	0.58 ± 0.21	0.37 ± 0.11	1.10 ± 0.18	1.58 ± 0.05	0.63 ± 0.14

This may probably be due to the fact that the ELISA is more sensitive than the CCIE and is able to detect small differences in the antigenicity of bloodmeals. Other workers have demonstrated the high sensitivity of the ELISA. Edrissian & Hafizi (1982) showed that the ELISA is a specific and sensitive technique that could detect small amounts of human blood even 24 hours after ingestion by the mosquito vector. The CCIE on the other hand, showed very little differences in precipitin bands for bloodmeals stored over the 5-month period, except for a small number indicating the lower sensitivity of this test. There were considerable differences in the mean OD values for bloodmeals for both mosquito species over the 5-month storage period. In some instances, mean OD values for bloodmeals stored more than a month was higher than that at month 0. This wide variation observed is probably due to several factors such as the large variation in bloodmeal size of individual mosquitoes and their different rates of digestion. The feeding capacity of the insect vector is one of the most important limiting factors in this respect and the large variation is reflected in the ELISA readings.

The CCIE did not show much difference in the antigenicity of the bloodmeals stored over the 5-month period as most bloodmeals had precipitin bands. The few feeds that failed to react with anti-monkey antisera were probably due to the small meal size. The CCIE has been known to be less sensitive than the ELISA is; its sensitivity has been reported to be 1000 times less than the ELISA (Washino & Templis, 1983).

From the results of both the ELISA and the CCIE in this study, it has been shown that the bloodmeal stored in the refrigerator over a 5-month period did not appear to deteriorate and lose antigenicity significantly as results of both tests remain positive. This information is useful as the bloodmeal identification facility at the Institute for Medical Research, Kuala Lumpur provides its services to countries in Asia and South East Asia and often it takes some time for the samples to arrive in Malaysia. Of the two techniques used, the ELISA is much faster to perform than the CCIE. Further more, the ELISA can be automated rendering this test more useful in the field where large numbers of bloodmeals can be tested simultaneously for different host determination.

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