## A-35: Estimation of time elapsed since death using entomological methods 2

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Fixing the age of the most advanced immature stages of fly species recovered from a corpse is the basis of estimation of the time elapsed since death. It is therefore essential to measure accurately the time taken for the eggs to pass through the various morphological changes before reaching the adult stage. Such a study is presented here together with application of the data to estimate the time of death in 6 human cases submitted to the Dept. of Forensic Medicine and Toxicology, Faculty of Medicine, Colombo.

Eggs obtained from wild *Chrysomya rufifacies* and *Ch.megacephala* on rabbit carcasses in the Colombo study were used for the age estimation of eggs and larvae of the species.

About 25 - 30 eggs with known time of oviposition were observed hourly under a stereoscopic microscope for visible morphological changes. Once morphological changes were pronounced, observations were made every 15 min until the hatching of first larvae. The time taken from oviposition to hatching of eggs was noted. The experiment was repeated 13 times in *Ch.rufifacies* and 15 times in *Ch.megacephala.*

A batch of 5 - 10 larvae were picked up and killed in hot water immediately after the hatching commenced. Length of the larvae were measured with a digital vernier caliper (accuracy 0.01 mm). Observations were continued at 6 h intervals until the first pupa was visible. Experiment was repeated 3 - 4 times.

Both eggs and larvae were reared at $30\pm3^{0}$C and $80\pm4$% relative humidity on human flesh.

Time elapsed since death of 6 human corpses with known time of death and 95% confidence intervals were estimated using the following formulae.

$$T = A + B\ (cd)$$

where, T = Time elapsed since death
A = Time of invasion of the insect species
B = Time elapsed since oviposition/Age of the most advanced insect form found on the corpse
cd = Climatic factor correction (wherever necessary)

95% confidence interval range = $(X \pm 2SD) + Y + T$

The median time to hatch eggs was 12 h in *Ch.rufifacies,* 10 h in *Ch.megacephala.* Segmentation and pumping movements of the eggs were seen, under the stereoscopic microscope about 2 h before hatching in both species. First ecdysis from first to second instar and the second ecdysis from second to third instar started between 12 - 18 h and 30 - 36 h respectively in the larvae of both species. The maximum body length recorded in

*Ch.rufifacies* was 14.26 mm while 14.85 mm in *Ch.megacephala*. The length of the larval period varied from 96 to 102 h in both species. In 4 cases where the known time of death was below 4 days the respective times were 39.30, 50.00, 75.00 and 88.00 h. The corresponding times based on entomological evidence were 34, 41, 73 and 84 h.

In 2 cases where the known time of death was above 4 days, the times were 156.00 and 172.00 h. The corresponding times based on entomological evidence were 122.00 and 139.0 h. All these values fell within the 95% confidence intervals.

The estimated time elapsed since death of all cases discussed, lies between the 95% confidence intervals. The estimated times based on entomological methods and the circumstantial evidence of these cases were comparable. The degree of comparison was closer in the cases where the time elapsed since death was less than 4 days.

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Repeated infections of malaria give rise to a partial degree of protection against the disease, as manifested by a reduction in both parasite densities in peripheral blood (anti-parasite immunity), and the intensity), and the intensity of clinical disease; this appears as a clinical tolerance to parasites i.e an anti-disease immunity. The objective of this study was to ascertain the degree to which clinical immunity is acquired by a community living in a malaria endemic region, as a result of repeated malaria infections, and whether this immunity is species specific, and age-acquired.

A population of 1942, resident in Kataragamja, in southern Sri Lanka where both *P.falciparum* and *P.vivax* are endemic, was chosen for this study. For a period of 18 moths commencing from January 1992, the malaria infections that occurred in this population were monitored with respect to age of patient, intensity of clinical disease and parasite density.

Malaria was monitored by (1) passive case detection at 2 diagonis and treatment centres in the area, and (2) an active case detection mechanism whereby 6 mass blood surveys of the entire population were conducted at approximately 3 monthly intervals. In every malaria patient thus detected the parasiteamia and the severity of the disease was measured using a questionnaire, in which 11 clinical symptoms were scored for their degree of severity according to the patients perception using a numerical scoring system. A clinical score was thus obtained for each patient, the higher one score the greater the intensity of disease. In a sample of patients, plasma levels of C-reactive Proteins (CRP) were assayed as an indicator of the degree of underlying pathology.

Records from 947 patients (547 due to *P.vivax* and the rest *P.falciparum* were analyzed for this study. The median clinical scores for primary infections of both *P.vivax* and *P.falciparum* infections were similar, the values being 13.0 and 13.3 respectively. When *P.vivax* infections were preceded by a single *P.vivax* infection the score was reduced to 11.0 and by multiple homologous infections, still further, to 9.2. When the parasite density of the patients was accounted for, the clinical score per parasite decreased 3-fold from a primary to multiple infections, suggesting the development of a clinical immunity.

When *P.vivax* infections were preceded by *P.falciparum* there was little effect on the clinical score of the *P.vivax* infection. Multiple infections with *P.falciparum* led, in contrast,to only a slight drop in the clinical score of a subsequent *P.falciparum* infection, from 13.3 to 11.0; When *P.falciparum* infections were preceded by *P.vivax* infections however, the clinical score decreased from 11.7 5 to 9.3 and the clinical score per parasite reduced two fold.

When the age of the whole population of patients was considered, both the clinical scores and the plasma C-reactive protein levels during an infection increased with increasing age.

These results indicate that over the short-term, repeated infections with *P.vivax* malaria confers a clinical immunity to subsequent infections of both *P.vivax* and *P.falciparum*. *P.falciparum* however, is less effective in inducing clinical tolerance; previous *P.falciparum* infections only conferred a mild degree of immunity to subsequent homologous infections and none to the heterologous species *P.vivax*.

Although clinical immunity is acquired and boosted in the short-term, it decreased with age of patients in this population. Clinical immunity thus appears not to be age-acquired. This loss of clinical tolerance in older age groups, in whom the incidence rate of malaria is low, is likely to be due to the lack of boosting by frequent infections, as a result of the acquisition of an anti-parasite immunity.