

## Hepatitis B Antigen in Laboratory Reared Mosquitoes

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**OBJECTIVE:** To determine the duration of carriage of hepatitis B antigen (HBsAg) by laboratory reared mosquitoes fed on a HBsAg carriers.

**BACKGROUND:** It has been suggested that the high prevalence of HBsAg carriers and anti-HBsAg in tropical areas may be due to the presence of biting insects capable of carrying and transmitting hepatitis B virus (HBV). Information accumulating over the past several years regarding the ability of insects to act as HBV vectors has been contradictory. The presence of HBsAg has been reported in eight (8) species of wild caught mosquitoes engorged with human blood; however, in unengorged mosquitoes no antigen was detected. (1) Conflicting data has been presented by other investigators which suggest that HBsAg does persist in certain mosquito species (*Aedes aegypti*) for up to six weeks following an antigen positive blood meal. (2) Further, one mosquito species (*Culex quinquefasciatus*) has been reported by a third investigator to develop antigen in the salivary glands approximately three weeks after feeding on HBsAg positive blood. (3) With an insectary capable of raising several genera of mosquitoes, with a number of HBsAg carriers available and with a sensitive radioimmune assay (RIA) to test for antigen, it was felt that the question of persistence and reappearance of HBsAg in laboratory reared mosquitoes could be approached.

**DESCRIPTION:** All mosquitoes used in this study were reared from eggs in the laboratory. After the adults emerged they were held for 48 hours and were deprived of fluids for 12 hours prior to use. Mosquitoes were fed on a known carrier of HBsAg/adr with a constant complement fixation titer of 1:512. Engorged mosquitoes were then removed and unfed mosquitoes discarded. A sample of 10 fed mosquitoes were quick-frozen and stored at  $-70^{\circ}\text{C}$ ; the remainder were placed in cages and allowed to feed on sugar water. Samples of 10 mosquitoes were withdrawn from the cage at varying times after feeding, quick-frozen and stored at  $-70^{\circ}\text{C}$ .

All mosquitoes were tested by RIA ("Ausria" kit, Abbott Laboratories) simultaneously for each mosquito species. Pools of 10 mosquitoes were triturated in 0.5 ml of 0.01 M Tris buffered saline pH 7.4 and centrifuged at 2000 rpm; 0.1 ml of the supernatant solution was placed in each of two Ausria tubes. Following this the test was run according to the directions provided with the Ausria kit. Included in each experiment was a pool of 10 unengorged mosquitoes of each species. Dilutions of serum of a HBsAg positive volunteer were run simultaneously by RIA to determine the concentration at which antigen could no longer be detected.

**PROGRESS:** At the time of this writing, *Aedes aegypti*, *Armigeres subalbatus*, *Anopheles minimus* and *Culex quinquefasciatus* have been tested in the above manner (Figure 1). All unengorged mosquito controls fell within 1 standard deviation of the mean of the negative controls run in the RIA. The first sample of the *Aedes* and *Armigeres* species were taken immediately after feeding. The initial sample of the *Anopheles* and the *Culex* species were taken 15 hours after feeding. Mosquito pools obtained within 24 hours of feeding show the presence of antigen in all four species. Antigen, as determined by the Ausria test, reached negative levels by 24-48 hours after feeding. In species which could be sampled as long as 15-20 days following feeding, antigen levels did not increase but remained within the limits of unfed mosquitoes. Unfortunately, because of the biting habits of *Culex quinquefasciatus*, engorged mosquitoes were obtained for

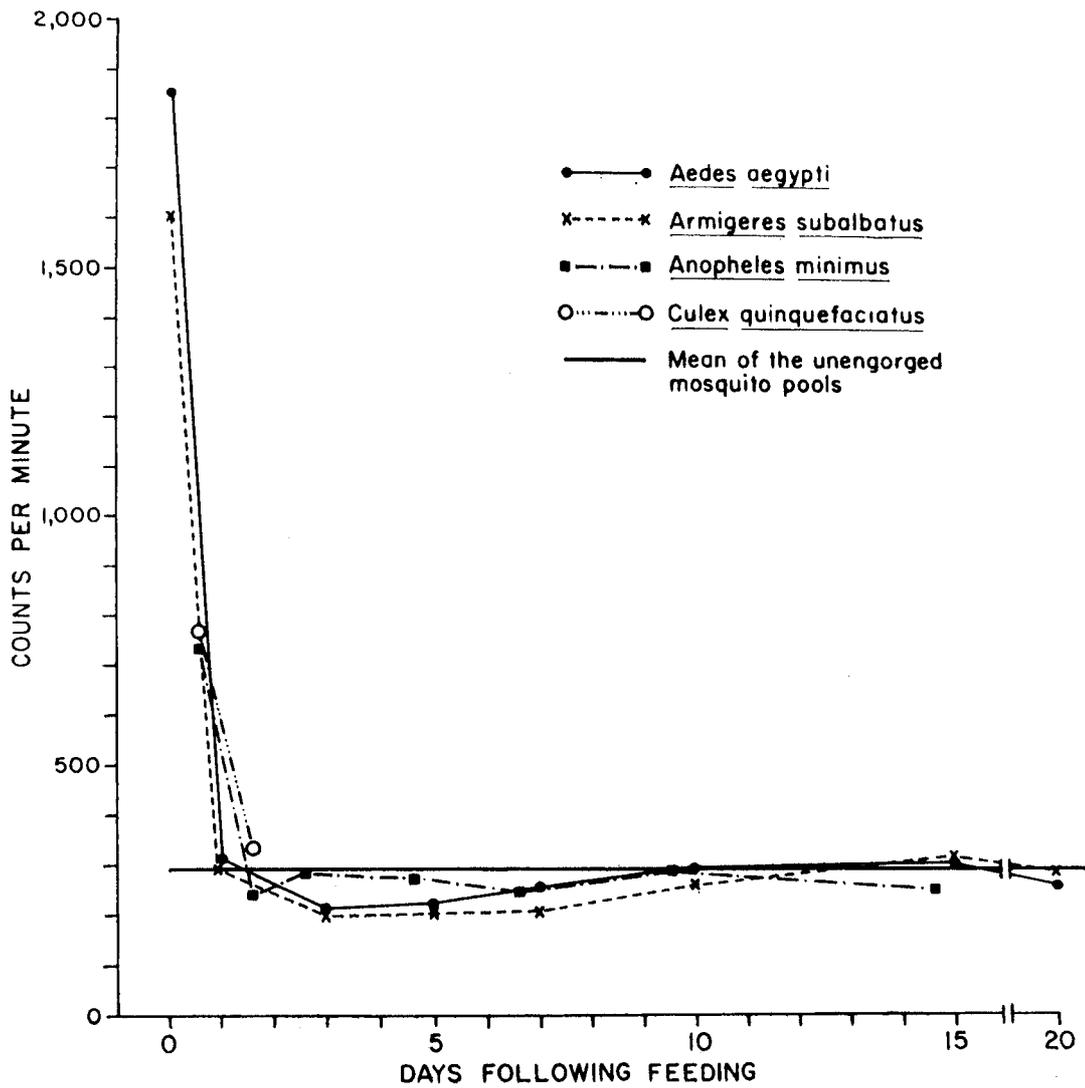


Figure 1. Radioimmune assay for HBsAg on pools of 10 mosquitoes collected following feeding on an HBsAg positive volunteer.

only two samples; however, in this species, too, a fall of antigen was noted within the first 36 hours. Negative levels were not reached and this species will be retested and followed for the full course of the experiment.

The serum dilution curve of the hepatitis B antigen positive volunteer demonstrated that antigen was no longer detectable at a dilution of 1:30,000. The range of counts per minute seen in mosquito pools would indicate that the pool of 10 mosquitoes contained an amount of antigen equivalent to a 1:1,000 dilution of serum.

**DISCUSSION:** The Ausria test (Abbott Laboratories) is probably only sensitive for the surface of hepatitis B virus (HBsAg). There is no evidence that we know of that it has any reactivity with core antigen of HBV. These data indicate that the disappearance of HBsAg from the mosquito pools parallels the digestion and elimination of the blood meal. In the three mosquito species followed for 15–20 days there was no reappearance of HBsAg; however, the presence of antigen in mosquitoes for 24–48 hours may allow them to serve as mechanical vectors if they refeed within this period of time.

It is unfortunate that there were insufficient engorged *Culex quinquefasciatus* to follow for the full course of 20 days. Studies on this species as well as *Aedes albopictus*, *Anopheles balabacensis*, and *Anopheles maculatus* remain to be completed. If, however, the antigen appears to dissipate in all species tested, the question of infection of the mosquito by HBV, with only the production of core antigen, remains a possibility and might provide an explanation of the reappearance of hepatitis B antigen in the salivary glands of *Culex quinquefasciatus* reported by others. Ways to investigate this question are being determined at present.

#### REFERENCES:

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