The Effect Ethyl Alcohol has on Insect Colonization/Decomposition of Exposed Chickens Reveals Postmortem Interval Estimations May Be Influenced

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Abstract: Postmortem Interval Estimations are made using clues from the development of colonizing insects found on the individual. Insects are drawn to these bodies by chemical cues due to decomposition of the body. Many times in the forensics, the body has been burned, or in some way altered to make estimations difficult for investigators by altering the decomposition process, or effecting the colonization rate of insects to the body. To determine the effect of ethyl alcohol on insect colonization/decomposition, decomposing chickens that were previously soaked in 200 proof ethyl alcohol were compared to decomposing control chickens that were not soaked in alcohol. Insects in the orders Diptera and Coleoptera were collected and identified. Results from our experiment were also compared to other groups performing the same experiment in order to find further similarities or differences.

Keywords: Forensic entomology, alcohol, PMI, colonization

Forensic entomologists are commonly asked to assist with estimating a minimum post mortem interval which is the time period between when death occurred and when the body was discovered. Both insect colonization and decomposition can be used when determining PMI; however they can be affected by factors such as temperature, location of the body, or drugs. These factors can lead to an over or under estimation of minimum PMI. It is important to investigate factors that affect PMI calculations in order to obtain the best estimate.

Carvalho et al. (2001) discusses the importance of keeping in mind the factors that affect PMI estimations. Carvalho et al. (2001) hypothesized that diazepam would affect the development and growth of larvae, and also the time of emergence of adult flies. The authors tested their hypothesis by rearing Chrysomya *albiceps* (Weidemann) and *Chrysomya putoria* (Wiedemann), both from the family Calliphoridae, on liver tissue from male rabbits that were administered 50mg of diazepam via ear vein infusion. This dose given is twice the lethal dose. The authors found that for the first few hours exposed to diazepam, the larvae showed no significant differences as compared to the control group. However, it was found that the larvae feeding on the diazepam tissue from 18-54 hours developed more rapidly than the control sample. It was also found that pupariation times and the time of emergences took longer for the larvae that fed on the diazepam tissue compared to the control group. However, in this study we investigated the effects specifically of ethanol. Ethanol, like

diazepam, has a depressing the effect nervous system. Carvalho et al. (2001) reveals the importance of our current study of the effects of alcohol. Tabor et al. (2005) aimed to investigate "[w]hether ethanol or alcohol, in general, can alter the development and behavior of forensically important insects [since the effect] is unknown. A thorough search of the literature [even] found no published studies of the effect of antemortem ethanol ingestion on the development or successional patterns of carrion insects" (Tabor 2005). The authors also stated that the antemortem ingestion of alcohol may lead to an erroneous estimate of a post-mortem interval of a cadaver due to the effects alcohol may have on the succession patterns of insects that colonize a body. This study, performed in Blacksburg VA, investigated the effects that ethanol ingestion had on the postmortem insect successional patterns and the decomposition of domestic pigs. The study was conducted by collecting insect samples from the carcasses of ethanol treated and untreated pigs to then determine the effects that ethanol had on decomposition rates and insect development. The results showed that antemortem intake of ethanol did not seem to have an effect on the insect's successional patterns. There were also no clear changes in the decomposition rates of the carcasses between the ethanol-treated and untreated pigs.

We hypothesize that soaking a chicken in 200 ethyl alcohol for 24 hours will slow decomposition and delay arthropod colonization of the chicken. We expect to find the control chicken to be more decomposed than the test chicken and more colonized than the test chicken. Our null hypothesis is the ethyl alcohol will have no effect on decomposition nor alter arthropod colonization.

Materials and Methods

Whole frozen feathered chickens (including beaks and claws) were thawed on April 8, 2013. The treated chicken was submerged completely in a cooler (Igloo, Katy TX) in 200 proof ethyl alcohol (Sigma-Aldrich, St. Louis MO) for 24 hours. Control chickens were thawed completely (about 24 hrs at room temperature) on April 8, 2013 and left untreated. The chickens were then bagged (Gladware, Oakland CA) according to their treatment and transferred to the TAMU Rangeland Site on April 9, 2013 where they were placed in a brushy area and left to decompose at approximately 3:00pm. Figure 1 shows how our test chicken was placed.

Figure 1. Placement of Test Chicken

Control and test chickens were placed and covered with a wire cage to prevent scavenging; the control chicken was placed on the left and the test chicken on the right. This was replicated by three other groups placed at different locations on the TAMU Rangeland Site at the same time. Chickens were allowed to decay for one week in the environment.

After one week had elapsed, adult Diptera were collected using a sweep net and kill jar, and by using sticky traps. Adult Coleoptera were collected by hand and placed in a kill jar. Insects were in the kill jar until dead and

then placed in a vial. Larvae were collected by hand, blanched in hot water and preserved in 70% ethanol (Sigma-Aldrich, St. Louis MO). Adult flies were identified using the Whitworth key, fly larvae were identified using the Seago key, and the beetles were identified using the Arnett Jr. et al. key.

Results

The treated chicken still had skin on the body and was in the active decay state of

Figure 2. Test (left) and Control (right) Chickens

We found abnormally large maggots that were very lethargic on the test chicken whereas the maggots on the control chicken were actively moving around and of varying sizes. The maggot mass temperature on the test chicken was 100.58°F and the temperature on the control chicken was 98.24°F. The following insects were found on the test chicken: *Cochliomyia macellaria* (adult), Muscidae (adult), Sarcophagidae (larvae), *Lucilia coeruleiviridis* (larvae), *Lucilia cuprina* (larvae). The following insects were found on the control chicken: Staphylinidae (adult), Dermestidae (adult), Sarcophagidae (larvae), *Cochliomyia macellaria* (larvae). Sarcophagidae and *Cochliomyia macellaria* were common species found on both of the chickens. All of the species found along with their count and developmental stage can be seen in Table 1.

Some other groups showed similar results. Every group found large numbers of *Cochliomyia macellaria.* Groups 2 and 3 also collected large numbers of Sarcophagidae, while groups 1 and 4 found large numbers of *Phormia regina.* Group 3 and group 1 both showed slower decomposition in the test chicken, while the chickens in group 2 and 4 both were in the same stage of decomposition. Every group also reported obvious maggot masses except for group 1, whose treated chicken did not contain large maggot masses. One difference with group 3 as compared to the other groups was that the maggot mass temperatures were cooler than all of the other groups. For group 2 and 4 the control chickens contained hotter maggot masses than the treated chicken, however our treated chicken’s maggot mass was hotter than the control chicken maggot mass.

Table 1. Approximate number of roaches per 2.54 square centimeters.

Species Found on Test	Count	Stage of Development
Cochliomyia macellaria	28	Adult
Muscidae	2	Adult
Sacrophagidae	10	3rd Instar Larvae
Lucilia coeruleiviridis	2	3rd Instar Larvae
Lucilia cuprina	1	3rd Instar Larvae
Species Found on Control	**Count**	**Stage of Development**
Staphylinidae	1	Adult
Dermestidae	1	Adult
1 Unidentified Beetle	1	Adult
Sacrophagidae	3	3rd Instar Larvae
Cochliomyia macellaria	9	3rd Instar Larvae

Discussion

The hypothesis that soaking chicken in 200 proof ethyl alcohol for 24 hours will slow decomposition and delay arthropod colonization of the chicken was supported for only one out of four groups. It was determined that the lack of beetle activity on the treated chicken constituted a delay in arthropod colonization. However, group's 1, 2, and 4 reported finding beetles on their test chicken. Group's 2 and 4 reported no difference in the decomposition rate between the test and control chickens. Group 2's results most closely matched what Tabor et al. (2005) found in their study since no major differences were found. Group 1 did report that their test chicken was not as far along in the decomposition process as their control chicken, but a significant delay in insect colonization was not found.

This study, along with the other studies cited, show the importance of understanding factors that affect PMI estimations. Investigators should keep in mind these factors in order to determine a PMI estimation with the least amount of error. Further research is still needed on the specific effects of alcohol on decomposition rates and insect succession to further reduce inaccuracy in PMI estimations.

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