Effects of 24-hour postmortem ethanol soaked chicken on insect succession

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Abstract: In the field of forensic entomology, experts are most frequently called upon to determine a minimum postmortem interval (PMI) for victims of death investigations. Many factors have a repellent effect on arthropod attraction to carrion, affecting the PMI, and this study examined the effects of soaking remains in ethanol for 24 hours before the body was made available for colonization. Eight whole chicken carcasses were used in this experiment. Four were used as controls and four were experimental carcasses soaked in ethanol for 24 hours. All chickens were taken into the field and remained for 7 days in wire cages. Arthropod specimens were collected and identified. Sarcophagidae larvae were the most abundant on both the control and test chickens. Five families of beetles were collected from the control and test chickens. Adult fly populations couldn't be distinguished between either the test or control chickens. No significant difference in insect succession between the control and experimental chicken was observed. However, there was a difference in gross decomposition between the control and experimental chickens. Further studies are needed to examine the behavior of adult flies with regard to colonization of ethanol-soaked chickens.

Keywords: Ethanol, soak, insect, succession, PMI, chicken, carcass, postmortem

In the field of forensic entomology, experts are most frequently called upon to determine a minimum postmortem interval (PMI) for victims of death investigations. The minimum PMI is defined as the time between insect infestations of a corpse and when the body is discovered. This can be determined using arthropod evidence obtained from the body itself, or the surrounding area. Due to the fact that some arthropods can detect, arrive at, and begin colonizing a corpse within minutes after death, a minimum PMI calculation through entomological evidence can be a very reliable aid in determining a range for approximate time of death in forensic investigations (George et al. 2009). Minimum PMI estimations are based on developmental data of specific species that experimentally determine how long each developmental stage takes at a given temperature (Tarone 2013b).

The developmental data used in minimum PMI estimations are typically for species of Diptera because, under normal conditions, they primarily colonize remains within minutes after death and/or exposure (George et al. 2009). However, there are many factors that have potential repellent effects on arthropod attraction to carrion, such as burning, burial, wrapping, the presence of a chemical substance, etc. (Charabidze et al. 2009, Tarone 2013a). These factors could delay arthropod succession and seriously inhibit entomologist's determination of an accurate minimum PMI (Charabidze et al. 2009). It is therefore important for forensic entomologists to understand which factors could potentially affect colonization, as well as the degree of that effect. This study examined the effects of soaking remains in ethanol for 24 hours before the body was made available for colonization. We hypothesized that soaking a chicken in alcohol would slow the rate of decomposition and delay arthropod colonization of the remains of an adult chicken. Our null hypothesis was that soaking a chicken in alcohol would have no significant effect on decomposition rate, nor would it alter the rate of arthropod colonization.

Materials and Methods

Eight whole adult chickens (Foster Farms, Livingston, CA), including claws, beaks, feathers, etc., were thawed on 8 April, 2013. Four were soaked in a cooler with 200-proof ethyl alcohol for 24 hours. The four control chickens were thawed on the same day and left untreated. The control chickens and treatment chickens were bagged separately and transported to Texas A&M University Rangeland Site on 9 April, 2013. They were deposited in a brushy area with a wire cage over them to discourage scavenging. After allowing the remains to be exposed in the field for 7 days, visual observations, data recordings, and specimen collection were performed on 16 April, 2013 for all four chicken carrion. Using a standard thermometer, the ambient and maggot mass temperatures were taken for both control and treatment groups. Arthropods present on control and treatment chickens were appropriately collected and stored for later identification. Adult flies were collected from above the carcasses using a sweep net and collected from around the chickens using sticky traps. Adult beetles were hand-collected with plastic spoons and sticky traps from under and around each chicken. Immediately following collection, adult beetles and flies were euthanized by in a glass kill-jar containing a towel soaked in ethanol. Adult specimens were later stored in glass vials and labeled according to the chicken from which they were collected. Maggots were hand-collected with plastic spoons from under the left wing of each chicken, hot-water killed, and subsequently stored separately from adult arthropods in glass vials containing ethanol. They were labeled accordingly. Upon return to the Texas A&M University's Entomology Department laboratory, all collected insects were identified through visual, microscopic analysis. Fly larvae were identified using the Seago Key and the Stojanovich et al. Key. Adult flies and beetles were identified using the Whitworth (2006) Key and the Arnett et al. (1980) Key, respectively.

Results

On the day of collection adult flies were collected using a sweep net and, therefore, cannot be specifically associated with either

the control or the test chicken carcasses. During the time of the experiment, the average temperature ranged from 78°F to 79°F with approximately half an inch of rain during the exposure time. The most abundant species of adults collected were *Cochliomyia macellaria*, *Lucilia eximia*, *Musca domestica*, Muscidae (unidentified species), and *Phormia regina* (Table 1). Since adults could not be associated with either the test or control chicken, the larvae were the primary focus of the succession differences (Table 2). Sarcophagidae larvae were the most abundant on both the control and test chickens. *C.macellaria*, Lucilia *coeruleiviridis*, *Lucilia cuprina*, and *P. regina* larvae were all collected from the control chicken. Of these four species only *C. macellaria* and *P. regina* were found on the ethanol-soaked chickens. Maggots collected form under the left wing of the control were larger than ones collected from under the left wing of the test chicken. The number of larvae collected is not indicative of the number present on each carcass. This number reflects the number of larvae collected from each maggot mass, which varied with the collector. The only distinct difference is between the species present. Sarcophagidae larvae did not appear to have a preference for treated versus untreated chickens. *C. macellaria* and *P. regina* were found on both the control and test chickens, but were collected more commonly from the test chickens. *L. coeruleiviridis* and *L. cuprina* were only collected from the control chicken, suggesting an aversion to ethanol-soaked chickens.

Five families of beetles were collected from the control and test chickens: Dermestidae, Histeridae, Silphidae, Staphylinidae, and Trogidae (Table 3). There was no obvious pattern of beetle preference between the controlled and treated carcasses. While more obvious in some carcasses than others, there seemed to be a visible difference in decomposition rates between the control chicken (Fig. 1a), and the ethanol-soaked chicken carcasses (Fig. 1b). On the day of collection, adult flies were observed landing on the ethanol-soaked chickens at a much higher rate than on the control chickens. Overall, no significant difference in insect succession between the control and experimental chicken was observed.

Adult Species	No. of Specimens
Cochliomyia macellaria	40
Lucilia eximia	1
Musca domestica	2
Muscidae (unidentified)	2
Phormia regina	2

Table 1: Adult flies collected using sweep net from above both control and test carcasses.

Larvae Species	Control	Test
Cochliomyia macellaria	2	9
Lucilia coeruleiviridis	7	
Lucilia cuprina	2	
Phormia regina	1	7
Sarcophagidae larvae	12	11
Unidentified 1st Instar		1
Unidentified 2nd Instar	5	2

Table 2: No. larvae collected from control and test chickens.

Beetle Family	Control	Test	Unknown
Dermestidae		1	
Histeridae			1
Silphidae	5	1	
Staphylinidae	3	3	
Trogidae			1
Undentified	1		

Table 3: No. beetles collected from control and test chickens, as well as the surrounding area.

Figure 1: a) Control chicken on day seven (left) b) Test chicken on day seven (right)

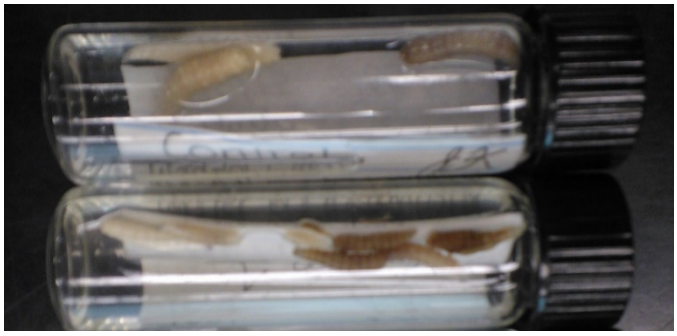

Figure 2: Top: Maggots collected from maggot mass under left wing of control chicken.
Bottom: Maggots collected from maggot mass under left wing of test chicken.

Discussion

The purpose of this experiment was to evaluate the effects of ethanol-soaked decomposing bodies on insect succession. Different drugs have been found to alter insect succession and development (Byrd and Castner 2010; Goff et al. 1989, 1991, 1993). It can be reasoned that ethanol-soaking would also alter insect succession. Tabor et al. (2005) evaluated antemortem ethanol ingestion on insect succession. Instead of having the test specimens consume alcohol in order to vary blood alcohol content as previous studies have, our experiment tested soaking an entire chicken carcass in ethanol for 24 hours. Despite being made available for colonization at the same time, the control chickens were in a later state of decomposition compared to the test chickens. This is in agreement with the results seen in the Tabor et al. (2005) study. There were also visible differences in insect colonization between the control and test chickens while Tabor et al. (2005) found no statistically significant difference between insect successions in pigs treated antemortem with ethanol compared to the untreated, control pig carcasses.

Maggots collected from the test chicken were visually determined to be smaller in size than those collected from the control chicken. However, larvae reared on ethanol-treated meat have a faster development rate (Tabor et al. 2005). Because the development rate was faster, this suggested delayed colonization of the ethanol-soaked chickens; however, there is no other evidence to support this. It is possible that rain could have affected ethanol concentration for the test- chicken; however, it is unlikely that this minimal amount of rain would have caused any effect. Control and experimental chickens were placed side by side and, therefore, exposed to the same weather conditions. Because the weather was consistent between the two, weather should not have been a source of any variation.

In conclusion, visible insect succession differences were not observed between the control and test chickens, although differences in time of colonization were apparent. Further studies are needed to examine the behavior of adult flies with regard to colonization of ethanol-soaked chickens. Future studies should also allow for more distance between the control and test chickens to ensure that there is no cross-colonization. More frequent monitoring of colonization and decomposition would also improve future studies.

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