G6 Insect Succession Studies on Pig Carrion in Southwest Virginia and the Effects of Antemortem Ethanol Ingestion on Insect Succession and Development

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The goal of this presentation is to present the results of the effects of antemortem ethanol ingestion on insect development patterns on pig

carrion; and to present the results of insect succession studies performed on pig carcasses during different seasons over a two year period.

This presentation will impact the forensic community and/or humanity by presenting research which describes an interdisciplinary animal model, incorporating the expertise of forensic entomology and forensic toxicology, in characterizing factors that may or may not influence the succession and development of insect taxa on postmortem remains. As a collateral forthcoming project, the model will provide the means to investigate the feasibility of using biochemical markers of ethanol consumption (fatty acid ethyl esters) in discerning antemortem ingestion of ethanol from postmortem neo-formation during decomposition.

Learning Objective: one method of estimating the postmortem interval (PMI) uses results from studies on the faunal progression or succession patterns of carrion arthropods. The pattern of insect succession is specific to the location and environmental conditions in which a carcass occurs. Because taxa can vary greatly with locale, particularly at the species level, it is important to identify the forensically important insects that are specific to an area. To date, no such data have been published for the southwest Virginia region. It is possible that factors such as antemortem ingestion of ethanol or drugs can affect succession patterns and insect development rates, thereby rendering a PMI estimation based on insect evidence inaccurate.

Succession studies were performed over three seasons for two years using untreated pig carrion. Over 57 insect taxa were collected and identified. An occurrence matrix showing dominant species on a seasonal basis is presented.

In an additional study, two pigs (weighing 57 and 66 kg) were intravenously dosed with a mixture of 95% ethanol and saline using an intravenous catheter inserted into an ear vein and by oral gavage. Two untreated pigs of similar weight (53 and 49 kg) were used as controls. Antemortem blood samples were colleced from both groups 15 minutes following delivery of ethanol to the treated animals. Euthanasia immediately followed the collection of blood samples. Loin meat was removed from each carcass to be used as a rearing medium for field development studies of the black blow fly, *Phormia regina*. The carcasses were placed under cages in a partially wooded field within one hour of death. Insects were collected and an occurrence matrix was developed. Results of the succession studies indicate no differences in the insect taxa collected from ethanol-treated versus control pigs. Decomposition rates were similar for all animals.

All four carcasses were necropsied in the field two days postmortem. Blood, tissue and maggot homogenate specimens were analyzed for ethanol by headspace gas chromatography (HSGC) utilizing a HP 7694 HS Sampler configured to an AgilentGC-6890 Plus™ with a flame ionization detector (FID). The column was a Restek Rtx-BAC1™ and the internal standard utilized was n-propanol. Total run time was four minutes. The limit of quantitation (LOQ) is 0.01%.

For the development studies, *P. regina* egg clusters were collected from carcasses and placed in rearing cups containing pieces of loin meat from either treated or control pigs (n=6 each). The rearing cups were kept outdoors to monitor development under natural conditions. Temperature and relative humidity were recorded at the site using HOBO® data loggers. Following egg hatch, six maggots were removed from each rearing cup every eight hours until pupation. Size and larval stage were recorded for each sample interval. The time from pupation until adult emergence was also determined. Preliminary data indicate no difference in development on meat from ethanol-treated versus control animals. However, the concentration of ethanol in the loin meat of treated animals (0.07%) was only slightly higher than that of controls (ND). Additional *in vitro* studies using meat fortified with higher concentrations of ethanol are being conducted to determine if alcohol can affect maggot development.

An ethanol vitreous humor concentration of 0.14% was obtained for one animal (No. 2) and the loin ethanol concentrations (No. 1 and 2) of 0.07% suggest distribution within the pig model similar to that encountered in the vitreous and skeletal muscle of humans. The experimental paradigm seems to provide a reasonably comparable model to human postmortem tissues and fluids for elucidating the influence or effect of antemortem ingestion of ethanol on insect succession and development. The postmortem blood ethanol determinations in animals 3 and 4 are consistent with postmortem neo-formation encountered in decomposition. Maggot specimens analyzed as homogenates obtained from animals 1 and 2 had higher ethanol concentrations when compared to the control, untreated animals (No. 3 and No. 4). The low ethanol

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concentrations observed in the control animal maggot specimens are possibly the result of postmortem neo-formation during decomposition or being a metabolic by-product attributed to maggot and bacterial interaction. Additional data from a second experiment, derived from serial collection of maggot masses over a period of five days, from ethanoltreated and control animals is provided in the poster.

ETHANOL RESULTS-% (WEIGHT BY VOLUME)

Animal No.	Antemortem Blood	Postmortem Blood	Loin	Maggots
1	0.14	0.11	0.07	0.06
2	0.16	0.10	0.07	0.04
3	ND	0.02	ND	0.02
4	ND	0.01	ND	0.02

Animals 1&2 ethanol-treated Animals 3&4 non-treated controls ND indicates not detected

The research describes an interdisciplinary animal model, incorporating the expertise of forensic entomology and forensic toxicology, in characterizing factors that may or may not influence the succession and development of insect taxa on postmortem remains. As a collateral forthcoming project, the model will provide the means to investigate the feasibility of using biochemical markers of ethanol consumption (fatty acid ethyl esters) in discerning antemortem ingestion of ethanol from postmortem neo-formation during decomposition. (The Virginia Tech Animal Care Committee approved this study).

Forensic Entomology, Ethanol, Maggots

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