SCIENTIFIC NOTE

Malignant Tumor Affects the Developmental Pattern of Feeding Larvae of *Chrysomya albiceps* (Wiedemann) and *Chrysomya putoria* (Wiedemann) (Diptera: Calliphoridae)

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Tumor Maligno Afeta o Padrão de Desenvolvimento de Larvas de *Chrysomya albiceps* (Wiedemann) e *Chrysomya putoria* (Wiedemann) (Diptera: Calliphoridae)

RESUMO - Larvas de *Chrysomya albiceps* (Wiedemann) e *Chrysomya putoria* (Wiedemann) foram criadas em temperatura ambiente em fígado de coelhos sadios e em fígado de coelho que apresentava tumor na região torácica. Ambas as espécies expostas ao fígado do coelho com patogenia desenvolveram-se significativamente mais rápido que as demais.

PALAVRAS-CHAVE: Ciência forense, entomologia forense, varejeira, desenvolvimento larval, linfoma

ABSTRACT - Larvae of *Chrysomya albiceps* (Wiedemann) and *Chrysomya putoria* (Wiedemann) were reared on liver tissues from a rabbit that had a malignant tumor in the thoracic cavity. Larval rearing of both blowfly species was conducted at ambient temperature. Larvae that fed on tissues from the rabbit with the tumor developed at significantly faster rates than those feeding on tissues from the control animal.

KEY WORDS: Forensic science, forensic entomology, blowfly, larval development, lymphoma

Insects are present in virtually all stages of decomposition of a body and are classified, based on their habits as: necrophages, predators, parasites, omnivores or incidental (Smith 1986). The role of the insects, primarily Diptera, in the decomposition process has been subject of numerous studies with a wide variety of different animal models, including swine, dogs, cats, mice, birds, humans and even elephants (Rodriguez & Bass 1983, Campobasso *et al.* 2001). Many species oviposit on a body and their larvae use the body as a food source during development (Carvalho *et al.* 2001a).

If we have data concerning insect life cycles, geographic distributions, successional patterns and affinities to different stages of decomposition, the insects can provide valuable information in estimating the time since death, place and manner of death (Goff 1993). The assumption to these estimates is basic for a given temperature, humidity, and level of precipitation, individuals of any species with an adequate food source will complete development from egg to adult in a predictable period of time (Goff 1993). However, recent studies using both flesh flies (Sarcophagidae) and blow flies (Calliphoridae) reared on tissues containing drugs of abuse and illicit substances have demonstrated departures from the expected patterns of development (Introna *et al.* 1990, Goff *et al*. 1992, Hedouin *et al*. 1999). The presence of these substances in tissues used as food source can either accelerate or retard rates of larval development and thus influence the estimate of the time since death (Introna *et al.* 2001, Carvalho *et al*. 2001b).

The present study was initially conceived as a drug-related study, but one of the rabbits used in the experiment was found to have a tumor and the objective of the study was changed in order to investigate the possible effects of this occurrence on the rate of development of fly larvae. Two fly species, *Chrysomya albiceps* (Wiedemann) and *Chrysomya putoria* (Wiedemann) were selected due to their involvement in the decomposition process and their medical and veterinary importance as myiasis-producing flies.

The animal model for this study was the domestic rabbit, *Oryctolagus cuniculus* L., weighing approximately 3.5 kg. Five

animals were used in the study. One rabbit had a lymphoma which affected the entire thoracic region. The other four rabbits were tumor-free and served as controls. In this manner, this is the first report of investigation involving forensic entomology in which the Diptera larvae had their development influenced by the presence of a tumor.

Rabbits were sacrificed mechanically, with no external lesions. Immediately following death, livers were excised, divided into two portions and each one received either 100 newly hatched larvae of *C. albiceps* or of *C. putoria*. The flies were obtained from laboratory colonies established from field-captured specimens. Every 6h, ten larvae were removed and weighed on an analytical scale until hour 54. Following this exposure, larvae were removed from the liver and placed in plastic containers with an artificial diet to complete development (Leal *et al.* 1982). The study was conducted at environmental conditions in the laboratory (approximately 27ºC).

A two way ANOVA was performed to analyse the results, using the Graphpad Instat® statistical program version 3.06 for windows 2003. The independent variables were the presence or the absence of the tumor and the developmental time, and the response variable was the larval weight. It was also performed the Dunnet multiple comparisons test.

Beginning 6h after exposure, the rates of development of larvae of both species feeding on the liver from the rabbit with the tumor were greater than those observed for the control larvae. Larvae 30h to 54h old, reared on the liver of the rabbit with the tumor were significantly heavier than those of the control groups, and no significant differences were observed among the control groups (Figs. 1, 2; Tables 1, 2). Both blowfly species, *C. albiceps* and *C. putoria* feeding on the liver from the rabbit with the tumor developed faster than the control ones. In addition, no significant differences were seen on the developmental rates between the species.

These results may indicate that the tumor in the rabbit released a substance or substances which increased the rate of development in the larvae independently of the species, and that the effect was similar in both species, resulting in a weight increase in the maggots. As seen in Table 1, mean

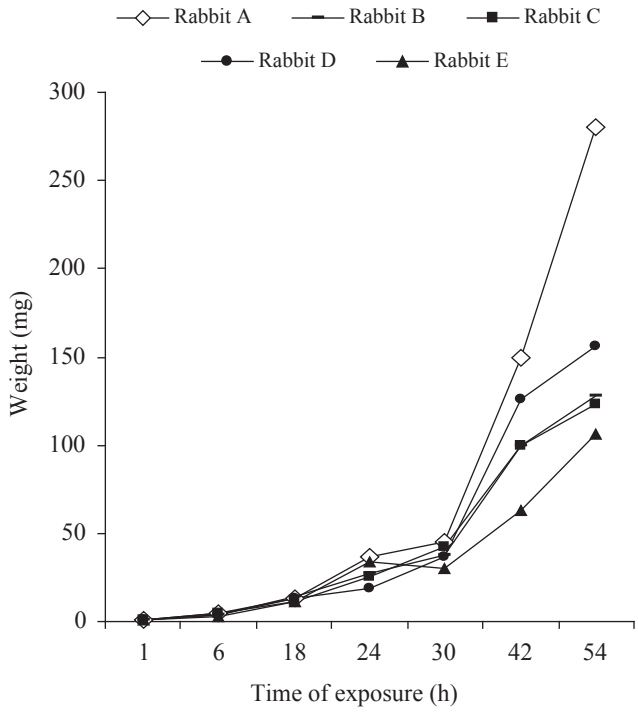

Fig. 2. Weight (mean of ten larvae) per hour of exposure of *C. putoria* larvae fed on the liver of the rabbit. A – exposed to tumor; B, C, D, E – exposed to normal tissue.

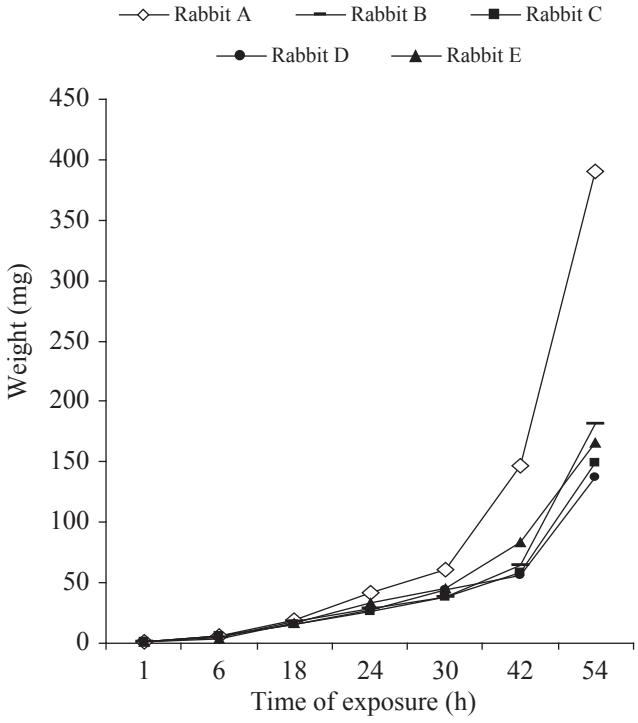

Fig. 1. Weight (mean of ten larvae) per hour of exposure of *C. albiceps* larvae fed on the liver of the rabbit. A – exposed to tumor; B, C, D, E – exposed to normal tissue.

Table 1. Weight (mg) of *C. albiceps* and *C. putoria* larvae exposed to the liver (tumor and tumor-free) of the rabbit during 54h ($\bar{x}$ ± SD) (n = 10).

Rabbit	*C. albiceps*	*C. putoria*
A[1]	390 ± 61.03	280 ± 44.49
B	182 ± 48.62	128 ± 26.60
C	149 ± 19.80	123.5 ± 26.13
D	137 ± 33.54	156 ± 13.20
E	166 ± 47.42	106 ± 13.81

[1]A = experimental rabbit (tumor)
B. C. D and E (tumor-free)

Table 2. Comparison of the weight (mean) of larvae of both fly species exposed to the liver of the rabbit containing a tumor and tumor-free.

Comparison	Weight of larvae ($\bar{x}$)	Mean difference	q[1]	P value
A[2]	335			
A/B	155	180	4,060	P < 0.05
A/C	136	199	4,489	P < 0.05
A/D	146.5	188.5	4,252	P < 0.05
A/E	136	199	4,489	P < 0.05

[1]q = if the value of q is greater than 3,480 then the p value is less than 0.05 and considered significant; [2]A = experiment rabbit (tumor); B. C. D and E (tumor-free)

weight of *C. albiceps* maggots feeding on the tissues from the rabbit containing the tumor were approximately double of those of the control groups. A similar, although not as great, increase was observed for *C. putoria* maggots (Table 1). The variation among means was significantly greater than expected by chance and the p value was considered significant (Table 2) to larval weight.

Although the experiment clearly demonstrated a difference in weights related to tumor feeding, the actual mechanism causing the difference was not determined. The difference might be related to a substance or substances associated to the presence of the tumor, which stimulated a faster feeding by the individual maggots or, alternatively, to a substance that was released by the tissues, that caused a change in the metabolic rate of the maggots, since there were different patterns of distribution that may be attributed to differences in the physiology of the flies (Campobasso *et al*. 2004). Feeding substrate does appear to matter when considering the rate of blowfly larval development. Therefore, it is important to know the origin and what substances the sample contains, such as drugs or others contaminants (Kaneshrajah & Turner 2004).

Even though the actual mechanism for the effect reported here remains unknown, it does serve to point out the need for cooperative efforts among different specialists in the death investigation team. While the entomologist collects and identifies the insects from a corpse, the estimates may be biased if the information concerning the state of the body from the pathologist is not available (Campobasso & Introna 2001). It is clear that although the necrophagous insects can both be utilized as physical evidence in a criminal investigation and contribute to the determination of the time, cause, way and place of the death, it is necessary to be alert to factors or simple details that can affect the results.

The knowledge of the effects of pathological processes present in an organism associated with the effects of different drugs in the development rates of immature necrophagous insects must be stimulated, because of their importance in the estimate of time of death. In this case, the faster development rates of the maggots would have resulted in a significantly longer estimated period of insect activity on the body and thus a longer minimum estimated postmortem interval (PMI).

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