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Case Report

The first case of forensic entomology applied to a roadkill dog carcass in the Colombian Andes

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ABSTRACT

An uncommon case of forensic entomology applied to a roadkill animal is detailed; the minimum period of insect activity (PIA*min*: 72 h) and the postmortem interval (PMI: ≤48 h) were calculated. Inferences and possible events pre- and postmortem are related and proposed, including the possible explanation of the occurrence of colonizer carrion flies on dry remains. The accelerating effect of vertebrate scavengers on the decomposition stages of the carcass was measured (7.4 time faster). This study is the first forensic entomology case applied to a roadkill animal in the neotropics.

1. Introduction

Forensic entomology is broadly defined as applying entomological evidence to legal proceedings [1]. However, the meaning is more commonly associated these days with using insects in legal investigations of deliberate criminal acts, most frequently homicides [2,3]. Animal remains, including human corpses, provide numerous resources to insects; about 400 species have been reported seasonally associated with pig carcasses [4]. These ecological associations (of insects with carcasses) allow for an estimation of the time at which a cadaver was colonized by insects [5]. Blow flies (Diptera, Calliphoridae) are the primary insect group of corpse colonizers in open natural environments and are usually associated with the first decomposition stages [6,7]. This colonizing tendency and the later insect species succession makes them ideal as forensic indicators [5], and their occurrence may serve as a biological clock; this biological data allows the calculation of an approximate time of death [3,8,9]. This period had been commonly interpreted as the postmortem interval (PMI); however, several authors claim that time-of-death inferences must be reconsidered in terms of the period of insect activity (PIA), and eventually, the use of both estimators will provide better interpretations for improved event reconstructions [10]. Scavenging is one of the critical biotic factors affecting the insects succession on carrion. Vertebrate scavengers increase the decomposition rate and may eliminate decompositional stages, affecting considerably the amount of available tissue to colonize and reduce the insect diversity (richness and abundance) over the successional stages [11]. In tropical environments, the effect of vertebrate scavengers on the remains decomposition rates is still unknown; these lacks influence accurate PMI and PIA estimations. Based on calculations above commented, we briefly discuss a preliminary PMI and a minimum period of insect activity (PIA*min*) from an entomological forensic case involving the dry remains of a roadkill dog. The uncommon occurrence of blow flies during the skeletal stage of decomposition is discussed, as is the eventual use of forensic entomology techniques applied to roadkill fauna.

2. Case presentation

On November 27, 2020, at 11:00 am, during a routine car inspection of roadkill wildlife in the mid-range of the Colombian Andes in the province of Antioquia, La Pintada municipality, a large dog carcass in the dry remains decomposition stage was located on the right side of the westbound road (La Pintada-Bolombolo) at 5°45′19.22″N; 75°38′4.06″W (610 m), a location surrounded by a matrix of tropical dry forest and pastures. A wake of four black vultures (*Coragyps atratus*) facilitated spotting of the corpse's location from a considerable distance. During the approach, the scavenger birds were frightened away, and the remains were then inspected for entomological samples. The carcass seemed to be clean of arthropods since no soft tissue was evident (Fig. 1a). Nevertheless, an exhaustive search inside the skull allowed the manual collection of maggots with entomological forceps.

3. Materials and methods

Larvae were placed in a rearing container consisting of a glass container with a vermiculite layer (2 cm); larvae and a small piece of flesh

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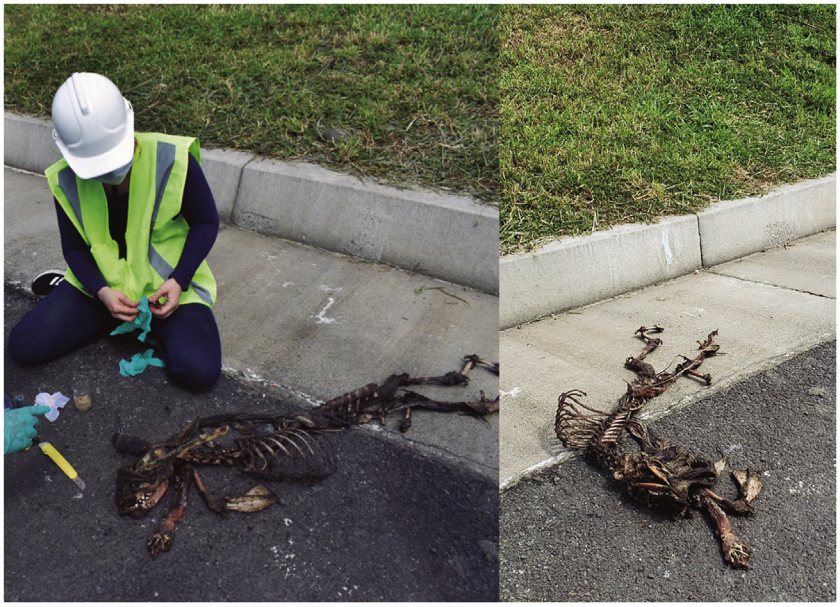

Fig. 1. Dog carcass in the skeletal stage, a) before the exhaustive entomological inspection; b) after first vertebra broken and foramen exposition.

(obtained from the corpse) were wrapped in a sheet of aluminum foil. The container was covered with a fine muslin to ensure breathing and taken to the laboratory in Medellín. Adults reared were identified using the keys of Amat et al. [12] and deposited in the entomological collection of Tecnológico de Antioquia [CETdeA]. To estimate the minimum period of insect activity (PIA) and a preliminary postmortem interval (PMI) based on the entomological samples, the accumulated degrees days (ADD) calculations reviewed in Amendt et al. [13] were applied using the developmental life cycle data (ADD based on reared specimens at 29 °C considering a minimum threshold of 10 °C) provided by Alvarez Garcia et al. [14] and daily climatological data during the study in La Pintada and Medellin obtained from http:www.weather.com. Finally, to calculate the acceleration effect of vertebrate scavengers, the time of a dry carcass elapsed without scavengers (37days) was divided by the sum of the PIA*min* (3 days) + PMI (2 days) estimated for the case.

4. Results and discussion

Thirteen live larvae of the secondary screwworm *Cochliomyia macellaria* (Fabricius, 1775) in the second instar of development were extracted from the liquefied brain inside the dog's skull. The larvae's pupation occurred synchronically on December 1 with some hours of difference among specimens, while the adults' emergence occurred on the December 7. Calculations of the accumulated degrees day (ADD) of the larvae collected allowed the inference of a minimum range of insect activity (PIA*min*) from November 24 or 25 and a postmortem interval (PMI) from November 25 backward (Fig. 2).

Entomological succession studies on pigs' carcasses carried out in the same Andean range recorded larvae of *C. macellaria* from days 2–6 after death and adults from bloated to dry decomposition stage [15,16]. At first sight, inspection of the dry carcass led to the consideration of a minimum preliminary PMI of October 22 since in standard conditions and in the absence of vertebrate scavengers, a pig corpse takes an average of 37 days to become dry remains in a similar environment [15,16]. However, it is known that organs decompose at different rates, and the brain's physical decomposition starts minutes after death [17,18]; this process is relatively fast due to the brain's consistency and its high lipid, water, and enzyme content [19]. A liquefied brain from which *C. macellaria* larvae are collected may take only a few days to disappear [18]. *Cochliomyia macellaria* is a primary colonizer species [9], and the average persistence of a roadkill carnivore carcass is 9 days [20], and the minimum PIA was calculated as at least two days from the date of collection based on the 336 h that elapsed before the adults emerged (Fig. 1) under the climatic conditions in La Pintada and Medellín. The PMI based on the entomological evidence has been assumed to be more accurate than the taphonomic inference [21], according to which the roadkill dog might take approximately 37 days to turn into dry remains. Thus, the PIA*min* and the time estimated based on the brain's liquefied condition, taken together, allow for a reasonable assumption of a PMI of at least two days before the collection day, including the activity of

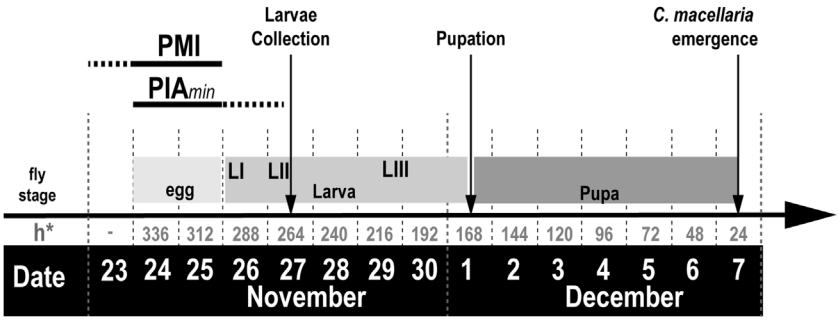

Fig. 2. Timeline proposed for events occurred before, during, and after the minimum period of insect activity in the roadkill dog. h* hours need for adult fly emergence; PIA*min*: Minimum period of insect activity; PMI: Postmortem interval. LI, LII, LIII larval instars.

vertebrate scavengers. These ideas adequately explain the assumption narrated above. Large scavengers may remove large quantities of flesh, accelerating or even eliminating decomposition stages [11]. In this study, the vulture scavenging witnessed before the skull inspection allows for the conclusion that the presence of larvae (*C. macellaria*) in the skeletal stage is due to the availability of soft (brain) tissue for food and the accelerating effect of vertebrate scavengers (Fig. 1b), not by the regular time elapsed under natural conditions out of the reach of vertebrate scavengers.

Consequently, the acceleration effected by the vertebrate scavengers of the decomposition stage duration was approximately 32 days; in other words, decomposition occurred approximately 7.4 times faster than it would have in the absence of scavengers. This data eventually could be extrapolated for human forensic cases. The roadkill dog carcass assessed here is possible evidence of this acceleration, explaining the presence of colonizing blow flies (shielded inside the skull) in the dry skeletal stage. In this case, other factors affecting the carcass persistence on the road, like traffic volume carcass destruction by vehicles, and position on the road [22] were not considered as critical. The occurrence of necrophagous blow flies (Diptera, Calliphoridae) in roadkill animals seems to be a common tendency [23]; this study was no exception and corroborated the importance of blow flies in the eventual calculation of a preliminary postmortem interval (PMI) under these circumstances of death. Nevertheless, adequate and exhaustive biological data sets of neotropical insects of forensic importance are still needed for more accurate inferences to be made in the forensic context.

Numerous wild fauna in Latin America are killed on roads [24]; however, wildlife forensic entomology has been neglected by conservation and wildlife enforcement officers and agencies [25]. Adequate knowledge of carrion insect fauna and successional and ecological studies on wild animal carcasses will provide essential information to support forensic case resolutions, especially those involving threatened or endangered species in jurisdictions where hunting and killing are punished. The same criteria used for human corpses and wildlife can be applied to roadkill fauna.

Authors' contributions

All authors conceived the idea of monitoring roadkill animals: EA proposed the use of forensic entomology legal field expertise and performed the calculations and analysis of the data; EA and AB performed carcass examination expertise in the field; AB reared the insects; and JCJ supported the logistics in the field. All authors drafted, reviewed, and approved the preliminary manuscript. The authors declare that they have no conflict of interest. This study was funded by the authors.

Declaration of Competing Interest

None.

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