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ORIGINAL ARTICLE

Causes of morbidity and mortality of bats in a wildlife recovery center in Portugal

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ABSTRACT

Over the last decade, research on bats has been flourishing in many aspects of their biology. An increasing number of studies focussing on infectious agents in bats are being published, especially regarding zoonotic pathogens. However, there is only limited knowledge regarding the causes of death in these animals. In fact, when bat carcasses are found, or injured bats die in recovery centers, the *post-mortem* examination in this group of animals is not a routine procedure. The aim of this study is to report the main causes of morbidity and mortality in free ranging bats in Portugal using bats that were submitted to the Centre for Rehabilitation of Wild Animals of Gaia's Biological Park. During the period of three months, 20 animals were subjected to *post-mortem* examination. The main cause of morbidity and mortality was trauma, with lesions in the upper limbs, such as fractures and lacerations of the wing membrane. Although only a limited number of animals was included, this work intends to alert to the importance of studies concerning the causes of death in bats.

INTRODUCTION

The order Chiroptera, with >1300 species, constitutes around 20% of all mammalian species in the world (Van der Poel et al. 2006, Teeling et al. 2017). They inhabit diverse ecosystems and can be found in every continent except polar regions and a few isolated oceanic islands (Nowak 1994), and have an essential role in ecosystem health as plant pollinators or insect predators (Kunz et al. 2011). In continental Portugal there are 24 species of bats, some of them classified as endangered or vulnerable according to the Red Book of Vertebrates of Portugal (Cabral et al. 2005).

When bat carcasses are found, or injured bats die in recovery centers, the *post-mortem* examination in this group of animals is not a routine procedure, in comparison to other species of mammals. Reasons for this include the difficulty to access suitable samples, the small size and the fast decomposition after the animals´ death (Mühldorfer et al. 2011b).

Bats are reservoirs and vectors of numerous zoonotic pathogens, although they do not represent a generalized threat to human populations (Calisher et al. 2006, Van der Poel et al. 2006). A large number of viral, bacterial, fungal and parasitic agents have been identified in bat species, some with a potential zoonotic role, as for example *Pasteurella* spp., *Salmonella* spp., *Borrelia* spp., *Leptospira* spp. or *Hendra virus* (Van der Poel et al. 2006). One of the diseases more frequently studied in European bats in the last decades is rabies, caused by different Lyssaviruses (Racey et al. 2013). Bats are often healthy carriers of the virus and because of that, additional measures of safety during the necropsy proceedings are needed. Although there are many studies on infectious agents in bats, especially zoonotic pathogens, there is only limited knowledge regarding the causes of death in these animals (Mühldorfer et al. 2011b).

The aim of this study is to report the major causes of morbidity and mortality in free ranging bats in a wildlife recovery centre in Portugal.

METHODS

Between August and October 2015, 20 free-ranging bats were submitted to the Centre for Rehabilitation of Wild Animals of the Gaia's Biological Park, Avintes, Portugal. All bats originated from areas around the park. They were delivered to the wildlife centre either by general public or by the Portuguese service for nature and environmental protection (SEPNA). Most of the animals were found injured or moribund near roosts or humans habitations. Some died during veterinary treatment and others had to be euthanized for medical reasons. When it was not possible to immediately perform a necropsy, the carcass was stored cooled or frozen.

The *post-mortem* examinations were performed according to the established safety and hygiene procedures (Cardoso et al. 2016, Garcês & Pires 2017), and were always

made by the same technician. All the macroscopic findings were recorded in a written protocol and by photographs. For histopathological examination, small samples of several organs were collected and fixed in buffered 10% formalin solution. They were processed using standard methods described in Mescher (2013) and were stained with hematoxylin-eosin (HE). Histopathological analysis was performed at the Laboratory of Histology and Anatomic Pathology of UTAD.

RESULTS

Descriptive data

A total of 20 European bats were examined. The animals belonged to the family Vespertilionidae, i.e. one common serotine bat (*Eptesicus serotinus*) and eighteen common pipistrelle bats (*Pipistrellus pipistrellus*), and to the family Molossidae, namely one free-tailed bat (*Tadarida teniotis*). Regarding the age of the animals, 19 individuals were classified as adults and one was classified as neonate (no fur developed, milk teeth). The sex ratio was roughly equal with ten males and nine females and one animal with undetermined gender. At the time of admission to the wildlife recovery center sixteen animals were thin, presenting a "tucked-in" abdomen. Table 1 contains the details of the animals evaluated in this study.

The main causes of admission to the recovery center were severe injuries and extreme weakness. The main cause of morbidity was trauma. The causes of death of these animals were euthanasia in fourteen individuals and six natural deaths due to disease or lesions complications.

Post-mortem findings

During external examination all animals revealed signs of dehydration and emaciation. Some of them presented severe emaciation and enteritis (six animals). In two bats, small areas of alopecia in the neck and in the abdomen were observed (Fig. 1). Two animals had ticks on neck, head and thorax (Fig. 2).

Ten animals had skeletal and/or skin lesions. They presented mild to severe traumatic injuries: wounds on the wing membranes (5), rupture in the wing membranes (10), exposed fractures of the humerus (2), fracture of the forelimb phalanges (2), and fracture of the forearm (3). They also presented subcutaneous hematomas associated with the fractured bones. One bat was suspected to have suffered from cerebral concussion. This animal did collide with the wall of a swimming pool. Fig. 3A, 3B & 3C depicts some examples of the detected lesions.

At the internal examination, the pectoral muscle was found to be haemorrhagic in four animals or had a pale colour in further two animals (Fig. 4).

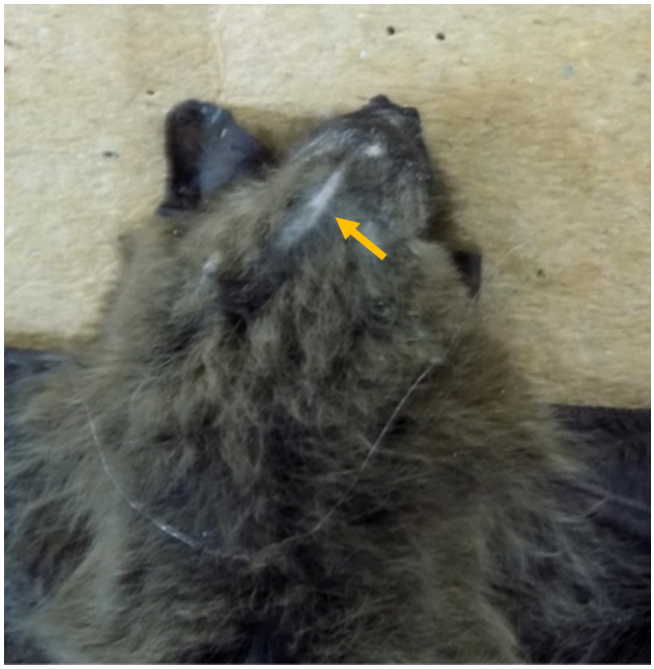

Fig. 1 - Area of alopecia in the lower jaw (→) in common pipistrelle (*Pipistrellus pipistrellus).*

Fig. 2 - Ticks in the neck (→) in a common pipistrelle (*Pipistrellus pipistrellus).*

Table 1 - Details on the species, gender, age, and body condition of the bats sampled in this study.

Family	Specie	Cases	Sex			Age		Body Condition	
		N	F	M	Unknown	Adult	Neonate	Thin	Normal
Vespertilionidae	*Eptesicus serotinus*	1	-	1	-	1	-	1	-
	Pipistrellus pipistrellus	18	8	9	1	17	1	14	5
Molossidae	*Tadarida teniotis*	1	1	-	-	1	-	1	-

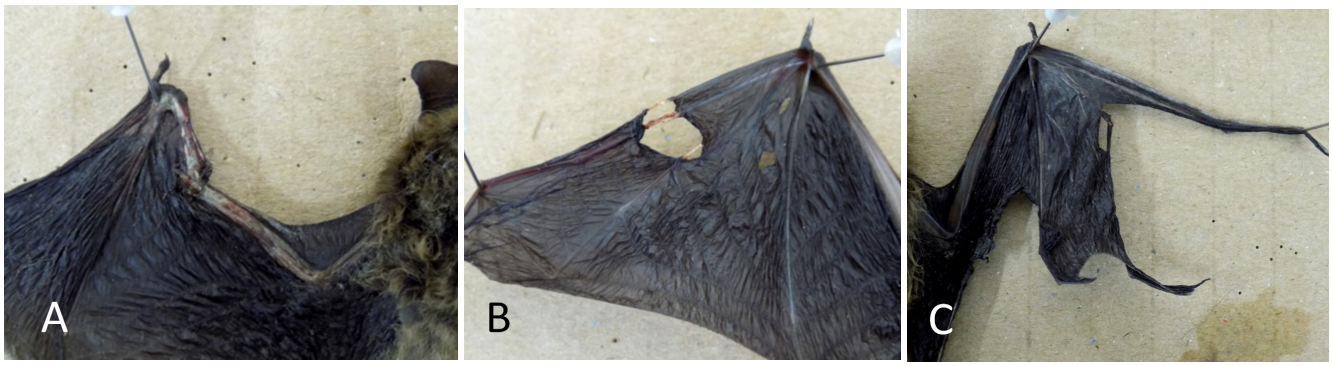

Fig. 3 - A- open fracture of the forearm in the right upper limb; B and C – lacerations of the wing membranes in a common pipistrelle (*Pipistrellus pipistrellus).*

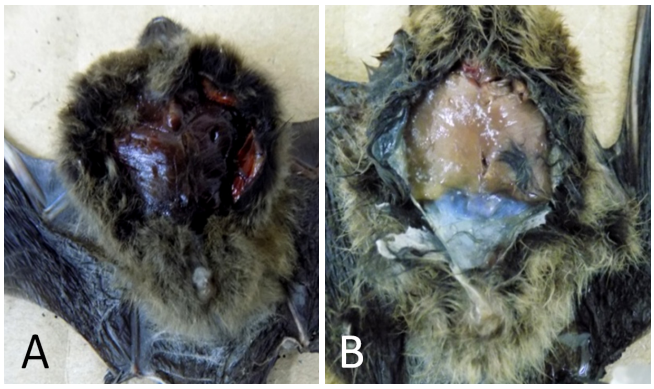

Fig. 4 - The pectoral muscle presented haemorrhage (A) or pallid coloration (B) in two common pipistrelles (*Pipistrellus pipistrellus).*

The internal organs of nine animals presented congestion and three had some free sanguineous liquid in the thoracic and abdominal cavities. In two cases the liver had a yellowish coloration. In one animal the intestinal wall was thick with transmural whitish coloration. Two females were pregnant (Fig. 5A), each with a foetus in an advanced status of development (Fig. 5B).

For the remaining seven individuals it was not possible to investigate any lesions of the internal organs because two carcasses were mummified and five were too autolytic. Histopathological examinations were mostly impaired by severe autolysis and it was only possible to confirm haemorrhage in lungs and kidneys and congestion in liver and heart in six individuals. A transversal section of one of the foetuses showed the internal anatomy of the foetus (Fig. 6).

DISCUSSION

The present study describes the first investigation on a limited number of carcasses of free-ranging bats from Portugal. Traumatic injuries have been described in many reports as the major cause of death in free-ranging European bats (Mühldorfer 2011a, Mullineaux et al. 2003, Simpson 2000). The same was observed in our study with more than

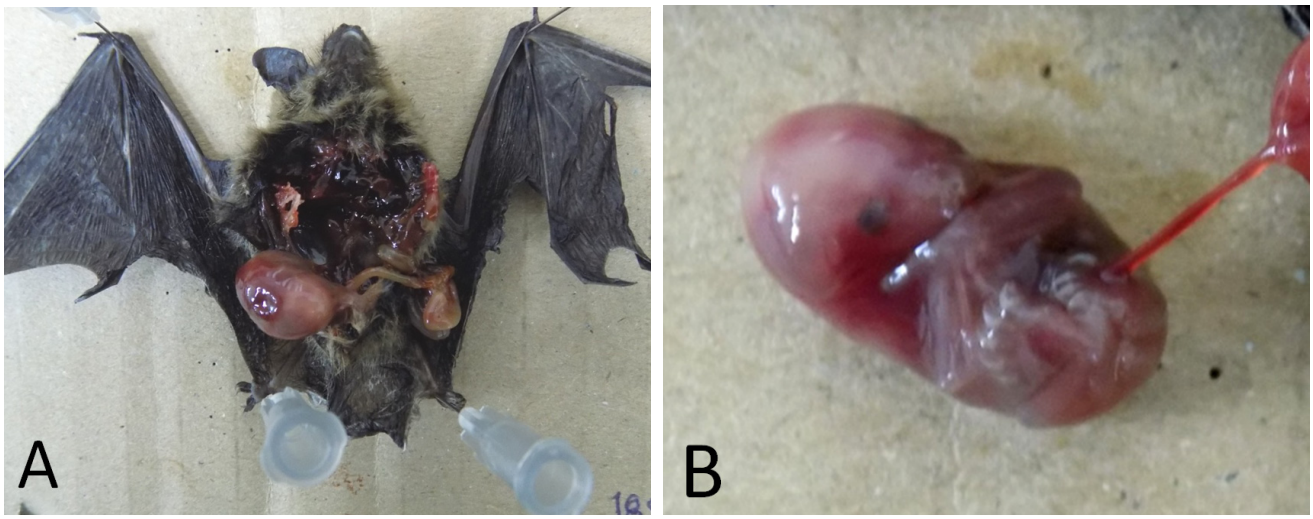

Fig. 5 - Uterus dilated (A) and foetus after extraction from the uterus (B), in a common pipistrelle (*Pipistrellus pipistrellus).*

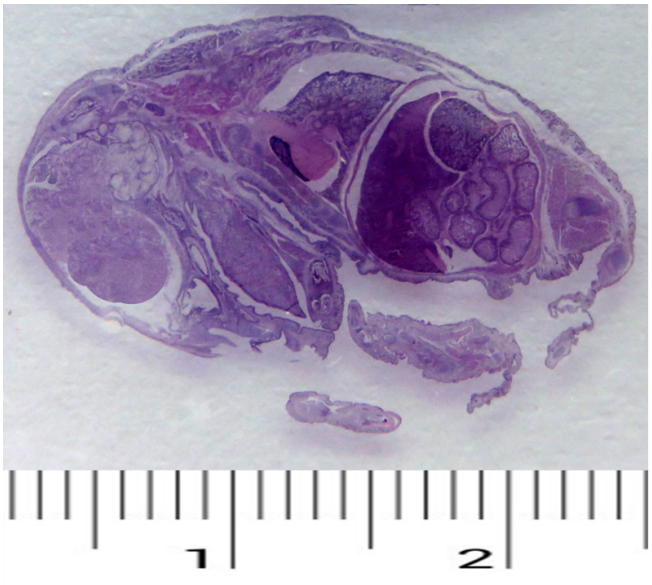

Fig. 6 - Microscopic image of a transversal section of a common pipistrelle foetus (*Pipistrellus pipistrellus)* (HE).

50% of the animals presenting signs of trauma. Fractures of the upper limbs and laceration of the wing membranes were the most common lesions. The wings are the most affected area due to their large size and delicate structure (Bexton & Couper 2010). All these injuries, even if minor, (or any potential infectious disease) could have been the reason why an animal was found on the ground presenting signals of dehydration and starvation. Most of the bats had trauma lesions associated with haemorrhages and congestion of the internal organs and pectoral muscles. Domestic cats are described as a major cause for similar traumatic lesions in Chiroptera (Ancillotto et al. 2013, Rocha et al. 2015). A total of 60% of injuries to bats were reported to be caused by cat attacks (Simpson 1994). The fact that in our study the animals were mostly of the species *Pipistrellus pipistrellus*, known to roost in buildings (Mühldorfer 2011b) and have been found near houses, suggests that a high proportion of the traumatic injuries could have been caused by cat predation. However, some of them might also have been entangled/caught in fine garden netting or similar materials as described by other authors (Bexton & Couper 2010). Other direct attacks to bats in urban areas have been reported such as predation events by genet *(Genetta genetta*) (Mas et al. 2015), fine garden raptors (Baker 1962) or barn owl (*Tyto alba*) (Ruprecht 1979).

Most bats are known to harbor one or two ectoparasite species, which are usually of little significance for their health (Mullineaux et al. 2003). Similarly, we found a limited number of ticks in two individuals, although it seems very likely that the other animals also carried some ectoparasites, but these might have abandoned the carcasses before our examination (Bexton & Couper 2010).

Finding pregnant females in August was unexpected considering their life cycle. However, females of some bat species have the ability to control the timing of pregnancy and birth depending on the environmental conditions. Many species can store sperm cells in their reproductive tract for several months during hibernation until favourable conditions prevail (Altringham 1998, Schober 1996). This explains why females pregnant with end-term foetuses could be found in early August, quite late for the species (Dietz et al. 2009).

The present study provides first information on causes of mortality and morbidity on free-ranging bats in Portugal.

CONCLUSIONS

Based on this study we conclude that the main cause of morbidity in bats could be trauma, which lead to euthanasia as the irreversible lesions would have affected the animals' quality of life. In the future is important to perform more similar studies and to extend them with complementary investigations like bacteriology, virology and parasitology. This data will aid the identification of bat diseases including zoonotic agents. Additionally, these investigations would contribute to the recognition of direct human impact on bat populations, and help to design better impact mitigation initiatives. Understanding the pathogenesis of pathologic entities can provide essential information for the treatment of animals in recovery centres.

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