Evaluation of pulmonary function in European land tortoises using whole-body plethysmography


The aim of this study was to evaluate the use of whole-body plethysmography as a non-invasive method to determine the respiratory parameters and profiles in two tortoise species belonging to the genus *Testudo*. Pulmonary functions and volumetric parameters were determined in 10 adults of *Testudo hermanni* and in seven *Testudo marginata* animals, using whole-body plethysmography. A profile pattern was regularly observed: an inspiratory flow peak, an expiratory peak, an apnoea phase and a second expiratory peak, previous to the beginning of the next respiratory cycle. Positive and significant correlation was observed between the inspiratory time, weight and length of the tortoises. Larger tortoises showed a higher time of inhalation. The peak of inspiratory flow was correlated with the sex, being longer in the females. *T. marginata* had an inspiratory time longer than that of *T. hermanni*. In *T. hermanni*, differences related to the sex were observed in the tidal volume, peak inspiratory flow, peak expiratory flow, expiratory flow of 50 per cent and enhanced pause, which could be related to the smaller size of males. The results suggest that additional information on new technologies currently used in pet medicine or even in human medicine should be developed and adjusted as alternative ways to support the rehabilitation of turtles and tortoises.

Introduction

The Hermann’s tortoise (*Testudo hermanni*) and the marginated tortoise (*Testudo marginata*) are European chelonians legally protected throughout their range, and classified in the International Union for Conservation of Nature (IUCN) Red List as near-threatened due to the high mortality from fires and destruction of their habitat along the Mediterranean forests (van Dijk and others 2004). Each year, tortoises are found sick or burned, and are rescued and relocated to rehabilitation centres in many of the European countries. On the island of Majorca, Spain, more than 3500 tortoises died in a large fire that affected more than 200 ha (Serra 2010). The impact of fire and mechanical destruction of the habitat of *T. hermanni* populations have been studied in detail in northern Greece (Hailey 2000), France (Couturier and others 2010) and in Spain (Martínez-Silvestre and Soler Massana 2000, Franch and others 2001, Couturier and others 2010). To evaluate the effect of the smoke on the respiratory function of rescued tortoises, an understanding of normal respiration parameters is needed.

Compared with mammals, the respiratory profile in reptiles is unusual because they have irregular respiratory cycles due to variable pauses between each movement (White 1978, Perry and Sander 2004). This represents a great challenge for veterinarians because even the simplest method employed to verify respiratory function, such as assessing the breathing frequency, is not accurate in these circumstances (Hernandez-Divers and others 2002). Reptiles use buccopharyngeal movements for olfactory function and these can be misinterpreted as respiratory movements (Druzisky and Brainerd 2001). Currently, diagnosing respiratory problems in chelonians is carried out with the use of several methods, such as physical examination, cytology, microbial culture, radiography, CT and endoscopy (Hernandez-Divers and others 2005).

Whole-body plethysmography is a very sensitive technique that verifies lung measurements. It can be used to detect lung pathology that might have been missed with conventional pulmonary function tests. It is often employed in comparative physiology studies because it avoids excessive handling of the animals. In flow plethysmography, airway resistance is measured by two manoeuvres, and the record of the respiratory mechanics is obtained based on the interchange of air between the body and the chamber in which the animal is placed. The interchange induces pressure variation in the volume of air retained in the chamber, which is then measured.
The aim of this study was to evaluate the use of whole-body plethysmography as a non-invasive method to determine the respiratory parameters and profiles in two species belonging to the genus Testudo, T. hermanni and T. marginata.

Materials and methods
Pulmonary functions and volumetric parameters were determined in 10 Hermann’s tortoises (five males (150–172 mm long, bodyweight 593–830 g), five females (193–230 mm long, bodyweight 892–1718 g)) and seven marginated tortoises (five males (246–337 mm long, bodyweight 2250–5200 g), and two females (240 and 285 mm, and bodyweight 2450 and 3650 g, respectively) using whole-body plethysmography. A transparent chamber of plexiglass for plethysmography was used (Buxco Electronics Unrestrained WBP plethysmography as a non-invasive method to determine the respiratory parameters, and enhanced pause (Penh), were analysed.

The chamber and plethysmography system, which was designed for cats and other small mammals, was sensitive to gas changes in the tortoises when calibrated to 50 ml. The tortoises did not show any signs of discomfort inside the chamber and usually remained quiet. A tryphasic profile was regularly observed: a PIF, a PEF, an apnoea phase and a second PEF, previous to the beginning of the next respiratory cycle (Fig 1). The interval between the first inspiratory movement and the second one was considered one respiratory cycle. Another profile of short and irregular respiration was seen in the three smallest tortoises (T. hermanni males). A period of apnoea as long as two minutes and 10 seconds was recorded in a female tortoise. The mean values of each studied parameter for each species are presented in Tables 1 and 2.

Results
The chamber and plethysmography system, which was designed for cats and other small mammals, was sensitive to gas changes in the tortoises when calibrated to 50 ml. The tortoises did not show any signs of discomfort inside the chamber and usually remained quiet. A tryphasic profile was regularly observed: a PIF, a PEF, an apnoea phase and a second PEF, previous to the beginning of the next respiratory cycle (Fig 1). The interval between the first inspiratory movement and the second one was considered one respiratory cycle. Another profile of short and irregular respiration was seen in the three smallest tortoises (T. hermanni males). A period of apnoea as long as two minutes and 10 seconds was recorded in a female tortoise. The mean values of each studied parameter for each species are presented in Tables 1 and 2.
A positive and significant correlation was observed between the inspiratory time, the weight (Table 1). Due to the low number of females of inspiratory time, the weight (Table 1).

When data were compared between species, only the inspiratory time showed significant differences. *T. marginata* had a Ti higher than *T. hermanii* (Table 1). Considering only the males and females of *T. hermanii*, differences related to their sex were observed in the TV, PIF, Penh and EF50 (Table 2). Due to the low number of females of *T. marginata* (n=2), the mean by sex of this species was not statistically compared.

**Discussion**

Most previous studies to check the ventilatory drive in tortoises were performed at least 25 years ago. Some of them used invasive techniques and did not have clinical application (Gans and Hughes 1967, Benchetrit and Dejours 1980). The mechanism of lung ventilation in the tortoise *Testudo graeca* is known in some species of reptiles (Randall 1967). The authors verified the changes of intrapulmonary pressure during the respiratory movements, and verified that pressures changes were triphasic in form, consisting of an initial increase in pressure followed by a fall to a level of 7 mmH2O below atmospheric pressure, and again returning to atmospheric level or usually slightly above. During the pause between individual ventilation cycles, any overshoot gradually declined to the baseline. Controversially, the respiratory rhythm does not appear to be composed of brief periods of ventilation activity followed by prolonged pauses, as supposed by many authors, and it was attributed to differences in the species investigated. In the present study, the authors have identified an inspiration–expiration–apnoea phase, and a second expiration after the apnoeic plateau being different from the profiles described previously for Russian tortoises, *Testudo hermanni* (Benchetrit and Dejours 1980) and for the leopoard tortoise, *Testudo pardalis* (currently Stigmochelys pardalis) (Glass and others 1978).

In both papers, the authors describe an expiratory phase immediately followed by the inspiratory one and then by an apnoeic plateau. The authors considered apnoea when there was no movement of the muscles of respiration, and the volume of the lungs remained unchanged. The second peak expiratory described in the present study, which was considered to belong to a same respiratory cycle, was not cited by previous authors. Unlike that described for *T. hermanni* in the present work, the expiratory phase was always longer than the inspiratory phase, and a second expiration after the apnoeic plateau being different from the profiles described previously for Russian tortoises, *Testudo hermanni* (Benchetrit and Dejours 1980) and for the leopoard tortoise, *Testudo pardalis* (currently Stigmochelys pardalis) (Glass and others 1978).
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References

Appendix

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breath holding that could be associated with different methodologies to access the respiratory function in chelonians. In sea turtles, it is known that there is a good correlation between breath-hold length and the end-inspiratory lung volume over a range of 35% per cent of resting lung volume (Milson and Janssen 1975). As a consequence of a progressive decline in respiratory quotient in the lungs during the apneic period, true tidal volume not excised via extrapulmonary routes must be eliminated. The second peak expiratory described in the present study may account for Shaw and Baldwin's (1935) observation that agree with a respiratory cycle terminated by a partial 'exhalation'.

Most lung physiological studies in turtles and tortoises have determined the pulmonary volume (Milson and Janssen 1975, Crawford and others 1976, Vitalis and Milsom 1986). In these studies, anatomical dead space was estimated, but tidal volumes were not usually presented due to missing an accurate method (Funk and others 1986). Although not using whole-body plethysmography, tidal volume is known in T.papillosa. It ranged from 4.7 to 10 ml/kg in tortoises weighing around 2.9 kg (Glass and others 1976). Crawford and others (1976) verified lung volume, pulmonary blood flow, and CO-diffusing capacity during pump-ventilation in an aquatic turtle, Chelydromys (formerly Pseudemys) scripta elegans and in a terrestrial turtle, T. graeca. The mean resting lung volumes, determined by argon dilution, were similar in both species, being 160 ml/kg and 170 ml/kg, respectively. The dead space averaged 0.6 ml/kg in T. scripta elegans and 2.6 ml/kg in T.graeca. The values reported by those authors were lower than those described in the present study, which, except for the methodological questions, could be related to differences due to species, or even because of the small sample size used in those works.

Druzisky and Brainerd (2001), studying the buccal oscillation and lung ventilation in four specimens of big-headed turtles (Pseudemys scripta elegans), recorded TV between 2.513 and 10.482 ml/kg and respiratory frequency of 65–90 movements. TV around 6.9 ml/kg and respiratory frequency as low as two breaths/minute were described for other semi-aquatic turtles, Pseudemys (currently Trachemys) scripta (Vitalis and Milsom 1986). Compared with these data, the present study has reported higher TV and respiratory frequencies, which could be justified as differences in the physiological mechanisms due to the usage of aquatic environment and methodological differences in the studies.

Gans and Hughes (1967) documented pauses in the breathing drive in T.graeca that lasted between four seconds and 23 minutes. These authors related the great oscillation on the apnoea timing to the level of disturbance that the animals had been subjected to. Three tortoises that moved constantly in the chamber providing an inaccurate record were excluded. Tortoises used in the present study remained very quiet; the maximum time of apnoea recorded occurred in one female T. hermanii, and was approximately two minutes. The higher value of Ti in T.margaritata could be associated with the larger size of the species, which have a very compact carapace, with the posterior end having a saw-like formation, flanged outward like a bell. Differences in the TV, PIF, PEF and EF50 observed between male and female T. hermanii could be also justified by sexual dimorphism in the species, in which males are smaller than females.

TV is usually used in mammals and calculated by means of volume of air exchanged and weight of the animal. In tortoises, this parameter could be influenced by the disproportional weight, density and shape of the plastron and carapace in relation to the whole body of the animal. Therefore, in chelonia, TV should be interpreted with caution mainly when comparing between species with differences in size and shape of the carapace.

According to Bates and Irvin (2003), Penh is a unit-less index of airway hyper-reactivity. This parameter is not a pure measure of resistance, but a mathematical artifice that has been criticised by some authors (Lundblad and others 2002, Mitzner and Tankersley 2003). Plethysmograph manufacturers claim, however, that it is the only commercial option for studying a wide range of pulmonary parameters in conscious, unrestrained animals. Interestingly, this test could have importance in case of tortoises experiencing smoky conditions, in which the bronchial and tracheal oedema could be responsible for changes in the standard values.

Comparing the parameters recorded in the present study with those verified in healthy cats (Garca-Guash 2008), the differences in the respiratory patterns are clear. Cats, like other mammals, due to their homeothermic condition, present a relatively higher respiratory frequency, although similar values of tidal volume as tortoises. The most notable difference observed is in the Ti, Te, PIF and PEF parameters. In T. hermanii in cats, all these parameters are very close, while in tortoises, Te is approximately 10 times higher than Ti, characterised by a long exhalation period. The values of Penh in cats are lower than in tortoises, which could be attributable to the differences in the morphology of the lungs and low respiratory tract between mammals and reptiles, especially the S-shaped trachea present in chelonians belonging to the suborder Cryptodira. Ancillary diagnostic tests in reptilian medicine have been improved in the last decade, mainly concerning imaging diagnostic methods (Valente and others 2006a, 2007, 2008) and clinical pathology (Delgado and others 2011). In conclusion, the authors would encourage the development and evaluation of this technique as a major benefit to tortoises and turtles in pet ownership, rehabilitation and rescue centres, zoos and other institutions.

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