WEIGHT EQUALITY IN EUTHYROID YOUNG HEAVY HORSES-A *POSTMORTEM* PILOT STUDY

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Abstract. This study aimed to investigate the thyroid weight asymmetry in 'Cavall Pirinenc Català' breed of horses. For this purpose, twenty-seven (27) young healthy animals of known breed and ages were selected, and the fresh weight of right and left thyroidal lobes were obtained. There were no detectable significant weight differences between the right and left sides, neither was fluctuating and directional asymmetry observed. The authors hope that this research, in addition to providing an overview as baseline data, will also stimulate research in both basic and clinical equine endocrinology as well as symmetric function of the thyroid gland in unilateral thyroidectomy.

Keywords: equine species, thyroid gland weight, 'Cavall Pirinenc Català', left–right symmetry, morphometry

Introduction. In bilateral symmetry along the left–right axis in vertebrate bodies, the asymmetry observed involves blood vessels and the viscera in particular (Stimec et al. 2006) (Komatsu & Mishina 2013), asymmetry is obvious in the heart, lungs, and liver and in the position of abdominal viscera (Barr 2001). This is a consequence of the development of the rostro-caudal axis, as lateralization deranges the initial midline symmetry (Stimec et al. 2006). The concentration of a nodal protein to the left side caused by clockwise rotation of cilia found in the organizer region leads to expression of a *nodal* gene in the lateral plate mesoderm on the left side of the embryo through the Pitx2 and FGF factors for left and right sides respectively in mammals (Gilbert 2010).

In humans, morphological, biochemical, physiological, and pathological asymmetries at different levels of the neuroendocrine system have been extensively reviewed (Yildirim et al. 2006), and several of paired viscera normally show a measure of weight asymmetry, e.g. the lungs, lobes of the liver, kidneys, adrenals, and gonads (Barr 2001). Thyroid scans frequently show asymmetry in human subjects with no evidence of thyroid disorders. It has been reported that the right lobe of the thyroid gland is generally larger and more vascularized than the left one (Anon 1968) (Yildirim et al. 2006) (Ying & Yung 2009). Moreover, the former is more likely to be affected by thyroid nodules and tends to enlarge more in diffuse hyperthyroid goiter (Yildirim et al. 2006) (Ying & Yung 2009).

In the equine species, the thyroid is located dorsal to the third through sixth tracheal rings and is composed of two lobes joined by a narrow fibrous isthmus (Daniel & Neelis 2014). The appearance of the normal gland is characterized by symmetrical uptake by paired thyroid lobes that are spherical to ovoid in shape (Daniel & Neelis 2014). The question of right-left asymmetry or otherwise of its lobes in this species has not been the subject of Veterinary research, at least to the best of our knowledge.

To resolve this question, a detailed *postmortem* pilot morphometric study was undertaken to study ponderal parameters for the thyroid gland in healthy horses belonging to the same breed. This study will not address the question of total thyroid weight deviation, only the deviation from the normal difference of weight between lobes.

Materials and Methods

Ethical statement- As animals were killed for commercial purposes not linked to this research, no ethical judgement was considered necessary.

The study was carried out on fresh carcasses from healthy horse breed; a local and rustical heavy breed that is raised extensively for meat, whose range is the Northern part of Catalonia (NE Spain), in the Pyrenees area. The animals were killed in a commercial slaughterhouse according to current European animal care protocols. Thyroid glands from these animals were harvested on the same day. A total of 27 complete glands were collected from young horses belonging to 'Cavall Pirinenc Català' breed, both sexes (14 males; mean age, 183-556 months; 13 females; age range, 198-422 months). These glands from dead animals were extracted separately and grouped both on side and sex bias. No cases with macroscopic pathologic changes or neck trauma were detected. Ponderal measurement was then performed using digital balances with fresh glands. The total thyroid weight was obtained by addition of the weights of both lobes. Statistical analysis of normality of distribution for both genders was evaluated by Shapiro-Wilk test for males and females respectively. A student-t test was also carried out to differentiate between pooled gender samples. Then a paired samples Wilcoxon test was used to test right-left individual differences. Furthermore an ANCOVA test (Analysis of Covariation) was used to evaluate the correlation between right and left weights and total thyroid weight using PAST v. 2.17c (Hammer et al. 2001) for Windows while the level of statistical significance was set at p=0.05.

Fluctuating asymmetry (e.g. subtle random deviations from perfect symmetry) (Pither & Taylor 2000) and directional asymmetry (e.g. the case when one side's character value is consistently greater than the other) (Pither & Taylor 2000) are two of the three types of

asymmetry described. To detect their presence, an analysis of the distribution of signed right-left weight lobe differences was done for fluctuating asymmetry (studying the departure from the mean of 0, the skewness and kurtosis) (Pither & Taylor 2000), and the cumulative distribution graph was done for directional asymmetry (Kharlamova et al. 2010).

Results

Data evaluated presented a normal distribution for both genders, evaluated by Shapiro-Wilk test (W=0.955 and 0.9551, $p<0.05$ for males and females respectively). A random t- test demonstrated no differences between genders (t=-0.617, $p=0.542$) and pooled samples. The paired samples Wilcoxon test was used to test right-left individual differences. An ANCOVA test was used to evaluate the correlation between right and left weights and total thyroid weight.

The evaluation of the thyroid weight in both lobes, did not reveal any significant individual differences between the right (15.41 g±4.561) and the left (15.46 g±4.916) lobes ($p=0.718$) (Figure 1). These are the average weight cited by anatomical literature (Getty 1985).

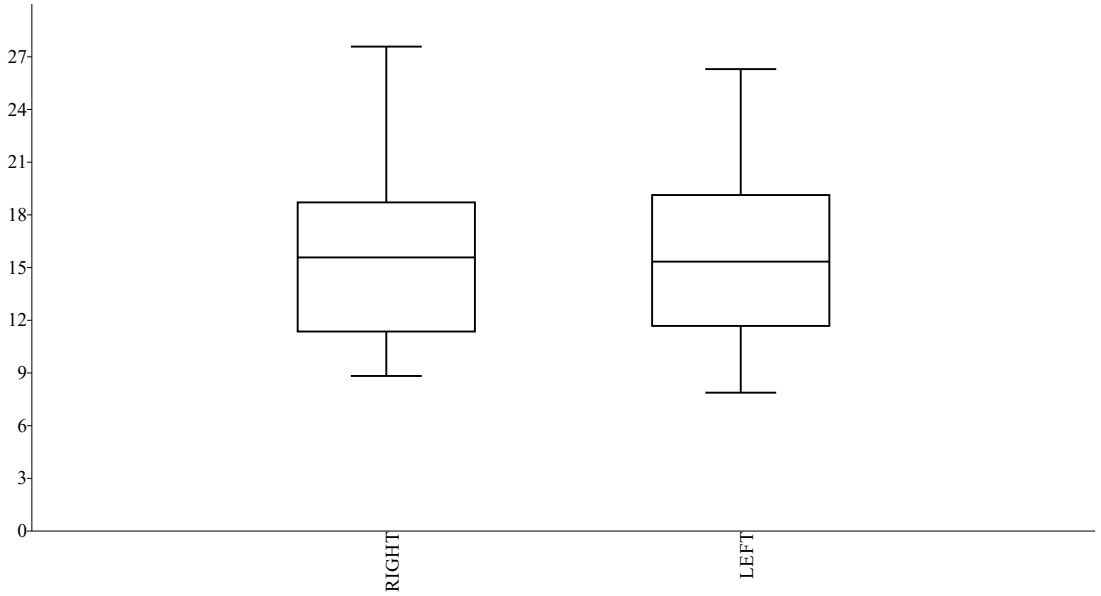

Figure 1. **Box plot for right (15.41 g±4.561) and left (15.46 g±4.916) lobes (n=27).** No significant individual differences between them ($p=0.718$). The median is shown with a horizontal line inside the box. The short horizontal lines were drawn from the top of the box up to the largest data point less than 1.5 times the box height from the box (the 'upper inner fence'), and similarly below the box. No outlier appeared.

On average the right lobe accounted for 50.11% of total thyroid weight, existing a similar correlation between the weight of the each lobe and the gland (F=1.116, $p=0.295$) (Figure 2). Signed right-left weight lobe difference presented a normal distribution ($p=0.267$), a negative skweness of -0.557, a kurtosis of 1.331 and a geometric mean of 0, revealing that differences were symmetrical, with no evidence of platykurtosis (Figure 3).

Cumulative distribution of scaled weight appears in Figure 4. One lobe side cannot be considered heavier and the opposite lighter.

Discussion

Developmental brain disorders and endemic goiter caused by severe iodine deficiency may seriously deteriorate overall health status and economic performance (Laurberg et al. 2004) in equine species. Though symmetry breakage in vertebrates remains controversial (Blum et al. 2007), a complete absence of one organ of a bilateral pair—absence asymmetry—is rarely found in any animal clade (Kipling et al. 2005) and in paired organs of paired origin, it has been postulated that the less-developed lobe is compensated contra-laterally one, but this seems not to be the case in young healthy horses, at least in the present investigation. It has been postulated, too, that asymmetry of paired organs may be associated with the presence and the size of their adjacent organs, the oesophagus in this case, but this is not confirmed in horse thyroid. Age related developmental asymmetries' in the thyroid of young equine species may represent syndromic hereditary manifestations of Gilbert (2010).

A limitation of our study is that we have worked with young horses rather than the small sample size. Therefore, further study with wider aged population is suggested, because weight changes may be expected with aging. A number of studies indicate that moderate and mild iodine excess (median urinary iodine >220 μ g per 24 hours) are associated with a more frequent occurrence of hypothyroidism, especially in elderly subjects. New questions would arise if possible asymmetries of the left and right thyroid lobes appear in sexually mature animals, as there is asymmetry of the neuroendocrine system (Gerendai & Halász 1997), as well as to consider right/left a/symmetries including accessory thyroid glands. Also it would be interesting to establish a/symmetries considering shape and biochemical properties, as well as in case of pathological thyroid cases.

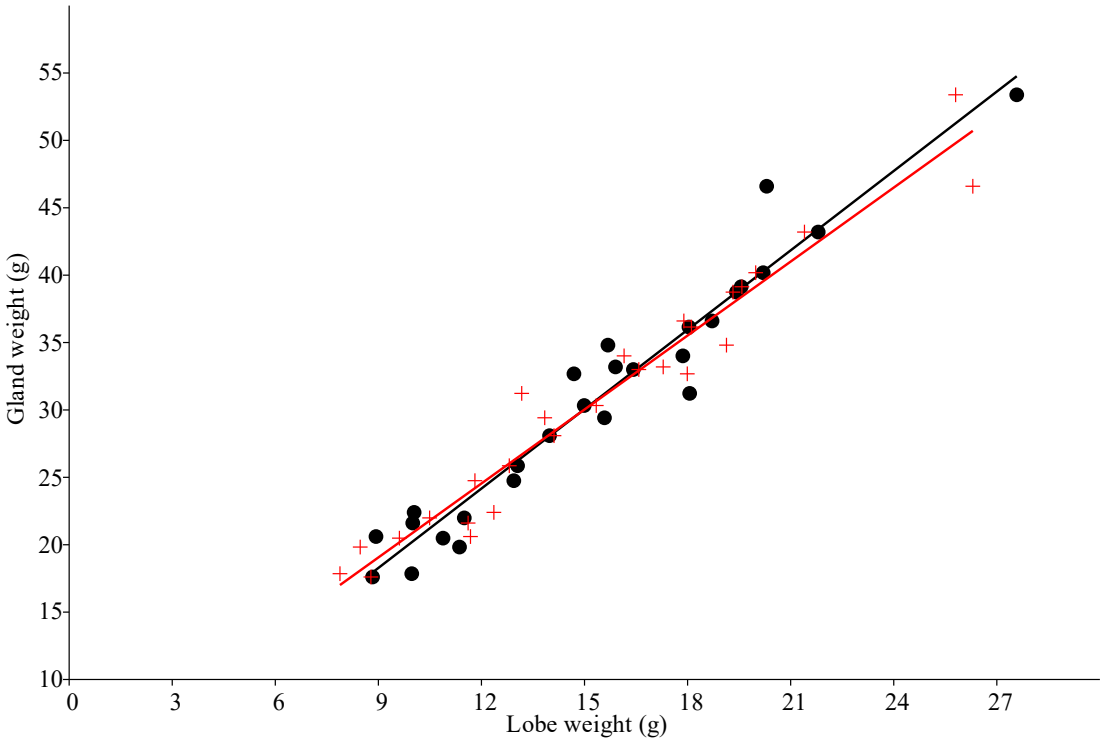

Figure 2**. ANCOVA test between the weight of the each lobe and the total weight of the entire thyroid gland (n=27).** No differences appeared between lobes (F=1.116, *p*=0.295).

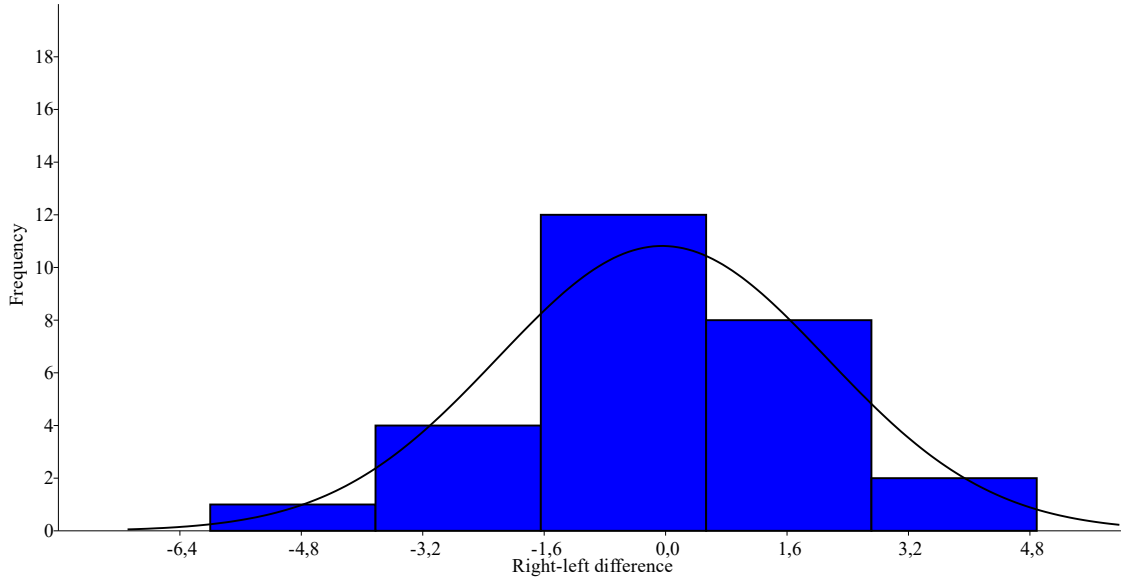

Figure 3. **Histogram for signed right-left weight differences of thyroid lobes (n=27).** It presented a normal distribution (*p*=0.267), a negative skweness of -0.557, a kurtosis of 1.331 and a geometric mean of 0, revealing that differences were symmetrical, with no evidence of platykurtosis. The "fitting normal" draws a fitted normal distribution (Parametric estimation, not Least Squares).

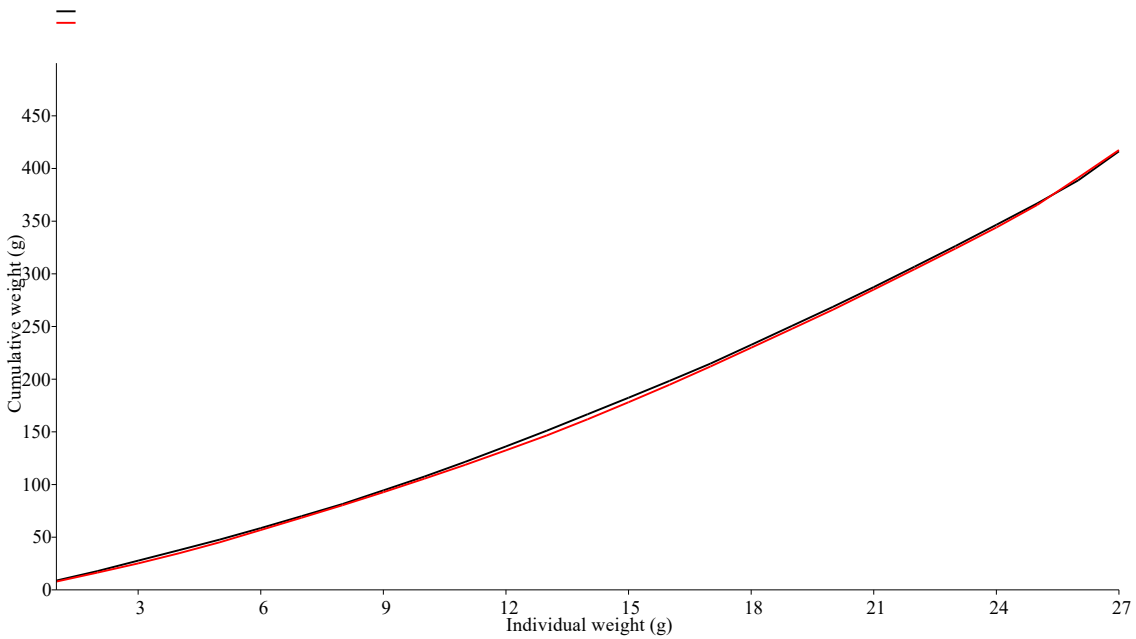

Figure 4. **Cumulative distribution of scaled weight for both thyroidal lobes (n=27).** One lobe side cannot be considered heavier and the opposite lighter.

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