

RESEARCH ARTICLE

Open Access



# The morphology of the adrenal gland in the European bison (*Bison bonasus*)

Karolina Barszcz<sup>1\*</sup>, Helena Przespolewska<sup>1</sup>, Katarzyna Olbrych<sup>1</sup>, Michał Czopowicz<sup>2</sup>, Joanna Klećkowska-Nawrot<sup>3</sup>, Karolina Goździewska-Harłajczuk<sup>3</sup> and Marta Kupczyńska<sup>1</sup>

## Abstract

**Background:** The anatomy of the adrenal glands has been widely studied in many species of domestic and wild mammals. However, there are no available literature reports describing the morphology and morphometry of the adrenal glands of the European bison (*Bison bonasus*).

**Results:** The study was conducted on 97 European bison of both sexes. The growth of the adrenal glands corresponded to the growth of the whole body, with the largest increase in size occurring in the first 2 years of the animal's life, followed by a slower increase in size until the animal was 5–7 years old. There were no statistically significant differences between ipsilateral adrenal glands of males and females with respect to age. There was no statistically significant difference in weight between the left and the right adrenal gland. However, there was a difference in the length, width and thickness of the two glands. Reference intervals for adrenal gland size and weight were computed separately for two bison age groups (up to 2 years of age and older than two years). The adrenal gland consisted of a cortex and a medulla. The connective-tissue capsule contained two layers. It had a fibrous structure and abundant adipose tissue. The cortex was divided into three zones. The zona glomerulosa contained cells arranged in bundles and curves. Numerous apoptotic cells were observed among regular cells in the zona reticularis. There were vacuoles in the cells of both zona fasciculata and zona reticularis, which formed a foamy cytoplasm. The adrenal medulla was composed of large, dark cells with a highly basophilic cytoplasm in the superficial region and of smaller, lighter cells in deeper layers. Sinusoidal vessels were located in the central part of the medulla.

**Conclusions:** The left adrenal gland was significantly longer, narrower and thinner than the right one. There were no significant differences in the structure of the adrenal medulla and cortex of the European bison compared to other species of domestic and wild mammals. There was a thick layer of adipose cells at numerous locations in the adrenal capsule of the bison.

**Keywords:** European bison, Adrenal gland, Morphology, Histology

## Background

The European bison morphology has been studied since the 1920s. Although more than 100 original studies have been published focusing on the morphological structure of organ systems in the European bison [1, 2], few of them have analysed the endocrine glands in this species. Existing reports describe the morphology of the pancreas [3], thyroid gland [4–6] and thymus [7].

There are literature reports and figures showing the structure of the adrenal gland in various mammalian species, including domestic and wild animals. Studies on the adrenal gland have been carried out domestic animals: Nili-Ravi buffalo (*Bubalus bubalis*) [8], cattle [9], one-humped camel (*Camelus dromedarius*) [10–12], wild ruminants, such as in pampas deer (*Ozotoceros bezoarticus*) [13], as well as in other wild animals such as: mongoose (*Herpestes auro-punctatus*) [14], common shrew (*Sorex araneus*), Eurasian pygmy shrew (*Sorex minutus*), muskrat (*Ondatra zibethicus*), bank vole (*Myodes glareolus*), field vole (*Microtus agrestis*) [15], bottlenose dolphin (*Tursiops truncatus*) [16, 17] and common seal (*Phoca vitulina vitulina*) [18]. However,

\* Correspondence: karolina.barszcz@onet.eu

<sup>1</sup>Department of Morphological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, 159 Nowoursynowska, 02-776 Warsaw, Poland

Full list of author information is available at the end of the article



there are no available studies on the morphology of the adrenal gland in the European bison.

The European bison is an endangered species. More information on its anatomy is warranted as there are few published studies of this species [1–7]. This study is the first report on the morphology of the adrenal gland in the European bison. Given the anatomical similarity between the European bison and other ruminants, the results of this study can be applied to other species, including the endangered ones.

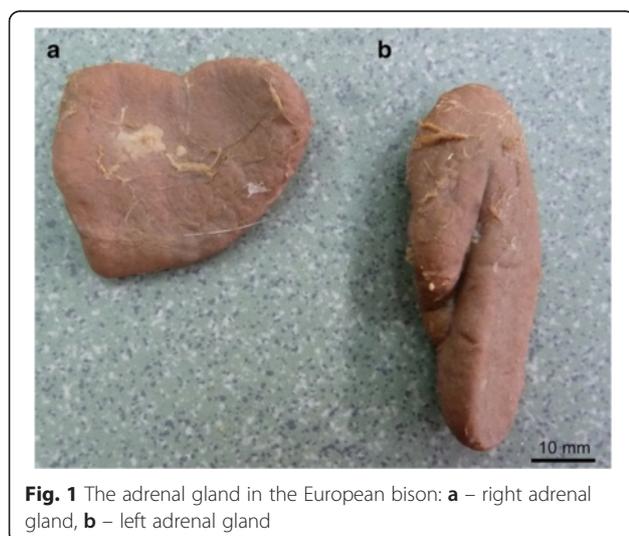
The aim of this study was to describe the location, macroscopic and microscopic structure of the adrenal glands in the European bison, as well as to contribute to the current knowledge of comparative anatomy of wild mammals.

## Results

### Gross anatomy

Our study showed that the adrenal glands were positioned intraperitoneally in the European bison. The left adrenal gland was located distally from the caudal extremity of the left kidney at the level of L1. It lay behind the cranial mesenteric artery, very close to the medial plane. Its lateral border was adjacent to the rumen, and its medial border was positioned next to the caudal vena cava. In all the studied European bison, the shape of the left adrenal gland resembled that of number “1” (Fig. 1).

The right adrenal gland was situated at the level of Th13-Th14, in front of the cranial extremity of the right kidney. Its dorsal surface adhered to the right crus of the diaphragm. The ventral surface adhered to the renal impression on the liver, close to the caudal vena cava. The right adrenal gland was triangular in shape (Fig. 1).



**Fig. 1** The adrenal gland in the European bison: **a** – right adrenal gland, **b** – left adrenal gland

### Statistical analysis

The growth of the adrenal glands seemed to correspond to the growth of the whole body, with the largest increase in size occurring within the first two years of the animal's life, followed by a slower increase in size until the animal was 5–7 years old (Fig. 2).

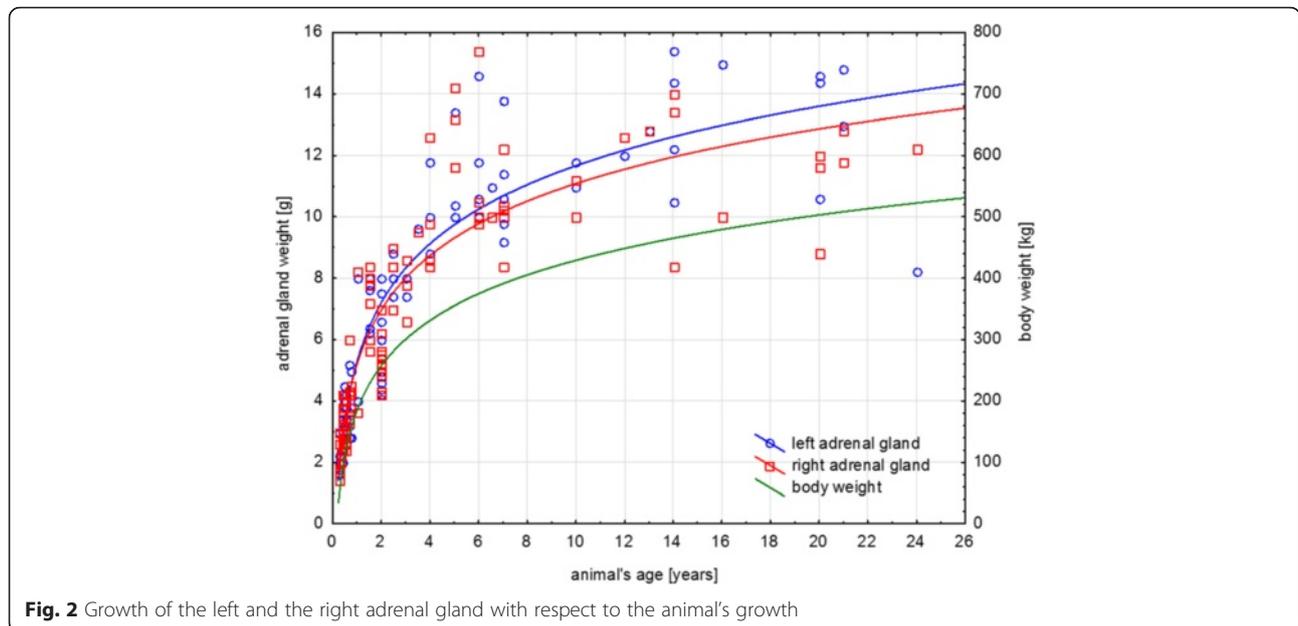
The weight and size of the adrenal glands are presented in Table 1. Taking into consideration the animals' age, there were no statistically significant differences in the size and weight of the adrenal glands between males and females.

The change in the weight and size of the left and right adrenal glands in the three consecutive age groups is presented in Table 2. Due to the fact that there were no statistically significant differences between males and females, those groups included both sexes. There was a statistically significant overall difference among the age groups with respect to all the analysed parameters ( $P_{ANOVA} < 0.001$ ). The only exception was relative weight, which remained unchanged in all the age groups and only slightly declined in the older animals. However, that effect was not statistically significant. There were significant differences between the youngest and the adolescent European bison with respect to all the analysed parameters, whereas only some parameters differed significantly between the adolescent and the adult European bison. There was no difference between the weight of the left and the right adrenal gland within the age groups ( $p = 0.123$ ). At the same time, all three size parameters differed between the left and the right gland. The left adrenal gland was significantly longer ( $p < 0.001$ ) but narrower and thinner (both  $p < 0.001$ ) than the right one in all the age groups. This corresponds to a different shape of the contralateral adrenal gland, as shown in the picture (Fig. 1).

Ninety-five percent reference intervals (RI) for the adrenal gland size and weight are presented in Table 3, separately for the youngest and for the adolescent/adult bison. A common RI was used for the adolescent and the adult bison due to a small size of the sample groups and insignificant differences in their parameters.

### Histological analysis

Our study showed that the adrenal gland of the European bison was composed of the cortex and medulla. It was covered by a thick connective tissue capsule (Fig. 3a), which had two layers (Fig. 3b). The outer layer was formed from collagen, elastin and smooth muscle fibers (Fig. 3c and d). Abundant adipose tissue (Fig. 3c and e) was observed between the inner and the outer layer. Sometimes, those fibers formed small irregular clusters. The inner layer was formed from tightly aligned collagen fibers (Fig. 3d). Looser connective tissue and an extensive network of well-developed blood vessels were found



on the border of those two layers (Fig. 3b). The internal layer of the capsule formed strands that penetrated the cortex (Fig. 3a). In turn, the cortex was divided into *zona glomerulosa s. zona arcuata*, *zona fasciculata* and *zona reticularis*. Collagen fibers formed numerous, irregular, thin strands in the *zona glomerulosa* that ran between cells. They formed large and thick trabeculae, which divided the *zona glomerulosa* and penetrated deep into the organ (Fig. 4a). The collagen fibers formed irregularly distributed clusters between cells in the deeper layers of the adrenal gland (Fig. 4b). Numerous blood vessels were observed at that level (Fig. 4c). Collagen fibers were arranged in thick circular layers around them (Fig. 4c).

Glandular cells of the *zona glomerulosa* formed bundles and arches (Fig. 5a). The bundles were located beneath the capsule and consisted of tightly and concentrically arranged cells. The cells were polygonal in shape (Fig. 5b). In contrast, the arches formed clear columns that penetrated the gland, forming a thin *zona fasciculata* (Fig. 5c). The cells of those structures were oval and were arranged in single or multiple arch-shaped strands (Fig. 5d). Connective tissue and numerous blood vessels

were present in both layers of the adrenal cortex. The *zona reticularis* was prominent and wide, and it was formed by irregularly arranged cells of various shapes (cubic, fusiform, oval, polygonal) (Fig. 6a). There were numerous apoptotic cells in the *zona reticularis* (Fig. 6b). That layer also contained numerous vessels (Fig. 6c). Cells with vacuoles formed a foamy cytoplasm in the *zona fasciculata* and *zona reticularis* (Fig. 6d).

The adrenal medulla consisted of larger dark cells and smaller, light ones (Fig. 7a). The dark cells were concentrated and formed elongated, dense and nest-like shapes. Those nest-like structures were situated directly beneath the adrenal cortex and had a ring-like distribution surrounding the medulla. The medulla was formed from light cells (Fig. 7a). The dark cells had an irregular, elongated shape and basophilic cytoplasm. The borders between those cells were ill-defined (Fig. 7b), and there were single, large and thick vessels between them (Fig. 7a). Light cells were located deeper within the adrenal medulla and were arranged in rows. They had a light, weakly basophilic, foamy cytoplasm (Fig. 7b). There were blood vessels of various diameters located between them, while sinusoidal vessels (single, double or triple) were located in

**Table 1** Weight and size of the left and the right adrenal gland in males and females

	Left adrenal gland			Right adrenal gland		
	Female	Male	p ANCOVA	Female	Male	p ANCOVA
Length [cm]	54.03 ± 13.29	49.85 ± 11.19	0.360	39.60 ± 9.47	37.10 ± 8.90	0.262
Width [cm]	24.85 ± 5.60	21.75 ± 4.90	0.549	28.99 ± 7.45	27.03 ± 5.47	0.411
Thickness [cm]	9.47 ± 1.90	9.34 ± 1.79	0.186	11.42 ± 3.08	10.89 ± 2.37	0.286
Weight [g]	7.74 ± 4.34	5.84 ± 3.17	0.740	7.42 ± 3.97	5.76 ± 3.27	0.704
Relative weight	0.031 ± 0.009	0.032 ± 0.014	0.834	0.030 ± 0.010	0.032 ± 0.014	0.907

**Table 2** Weight and size of the left and the right adrenal gland in three age groups

Age group		Length [cm]	Width [cm]	Thickness [cm]	Weight [g]	Relative weight
Left adrenal gland						
1	The youngest bison – up to 2 years-old (n = 58)	44.52 ± 8.91	19.92 ± 3.26	8.39 ± 1.43	4.16 ± 1.84	0.033 ± 0.013
2	Adolescent bison – 3–5 years-old (n = 14)	59.21 ± 4.19	26.70 ± 3.34	10.96 ± 1.54	9.31 ± 1.72	0.025 ± 0.005
3	Adult bison – older than 5 years (n = 25)	66.50 ± 7.11	30.29 ± 2.32	10.94 ± 1.11	12.14 ± 2.04	0.028 ± 0.005
p Tukey test for unequal groups						
	class 1 vs. 2	<0.001 <sup>a</sup>	<0.001 <sup>a</sup>	<0.001 <sup>a</sup>	<0.001 <sup>a</sup>	0.090
	class 2 vs. 3	0.046 <sup>a</sup>	0.007 <sup>a</sup>	0.999	<0.001 <sup>a</sup>	0.681
Right adrenal gland						
1	The youngest bison – up to 2 years-old (n = 58)	32.82 ± 6.22	23.93 ± 4.34	9.75 ± 1.83	4.08 ± 1.73	0.034 ± 0.014
2	Adolescent bison – 3–5 years-old (n = 14)	45.56 ± 6.18	33.50 ± 3.52	12.28 ± 2.10	9.66 ± 2.34	0.026 ± 0.006
3	Adult bison – older than 5 years (n = 25)	48.00 ± 5.50	35.06 ± 4.55	13.94 ± 2.74	11.28 ± 1.80	0.027 ± 0.005
p Tukey test for unequal groups						
	class 1 vs. 2	<0.001 <sup>a</sup>	<0.001 <sup>a</sup>	0.006 <sup>a</sup>	<0.001 <sup>a</sup>	0.191
	class 2 vs. 3	0.536	0.603	0.098	<0.001 <sup>a</sup>	0.985

<sup>a</sup>Difference significant at α = 0.05

the central part of the medulla (Fig. 8a). Additionally, numerous ganglion cells were present. They were situated between the light cells and formed oval plexuses surrounded by loose connective tissue and veins (Fig. 8b, c and d). Single, large pink polygonal multipolar nerve cells were seen within the plexus. Light or

dark-orange polygonal ganglion cells predominated and formed numerous oval shaped clusters (Fig. 8e).

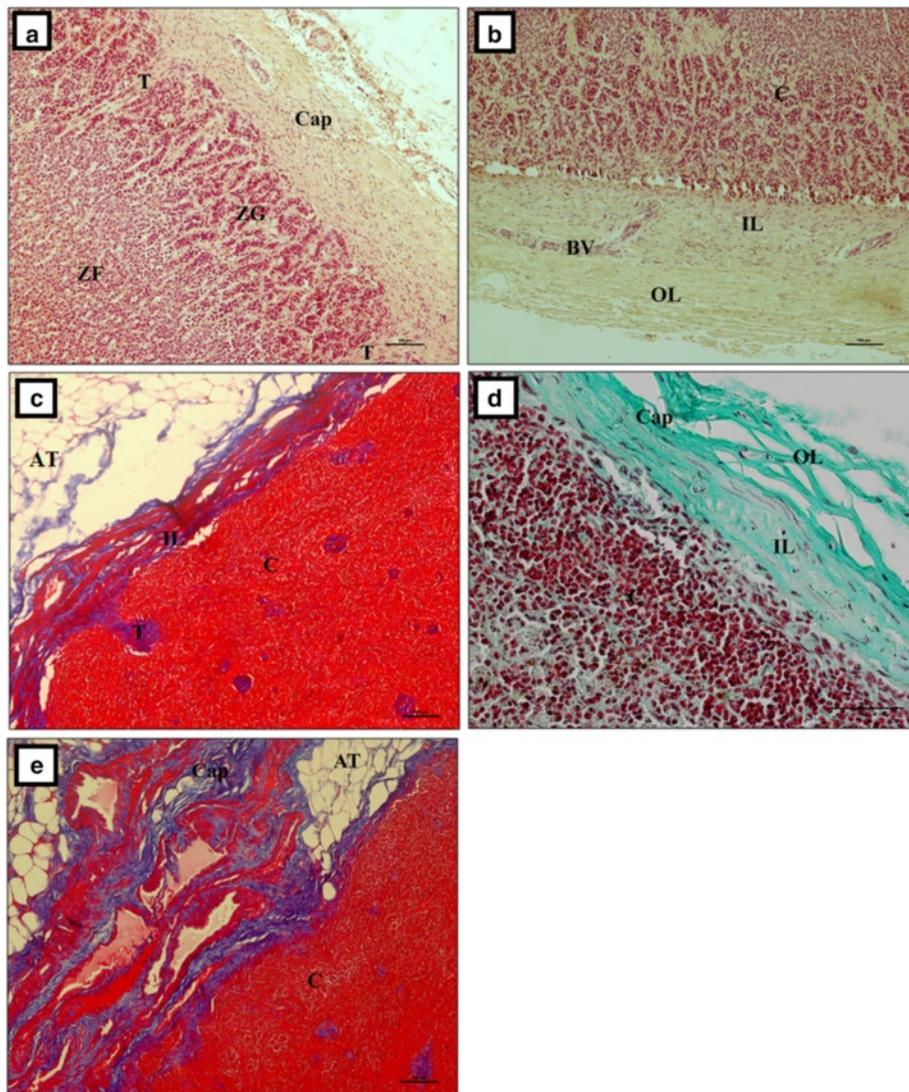
**Discussion**

Numerous studies on the shape of the adrenal gland were carried out in various wild and domesticated

**Table 3** Reference intervals (RIs) of the weight and the size of the left and the right adrenal gland of the European bison

	Parameter	95 % reference interval (RI)					
		Youngest bison (n = 58)		Adolescent and adult bison (n = 39)		Overall (n = 97)	
		Lower limit (CI 90 %)	Upper limit (CI 90 %)	Lower limit (CI 90 %)	Upper limit (CI 90 %)	Lower limit (CI 90 %)	Upper limit (CI 90 %)
Left adrenal gland	Length [cm]	28.1 (25.9–32.3)	67.0 (59.1–69.5)	48.4 (46.4–52.5)	78.6 (75.1–81.6)	30.9 (25.9–33.6)	76.7 (72.1–81.5)
	Width [cm]	13.1 (11.5–15.4)	26.7 (25.6–27.0)	22.5 (21.1–24.4)	35.9 (34.6–37.6)	14.8 (11.5–16.0)	32.9 (32.2–35.5)
	Thickness [cm]	5.5 (5.5–6.1)	11.7 (10.5–12.1)	8.4 (7.8–9.1)	13.6 (13.1–14.3)	5.8 (5.5–6.6)	13.0 (12.3–13.1)
	Weight [g]	1.6 (1.6–2.0)	8.0 (7.8–8.0)	6.1 (5.4–7.1)	16.0 (14.8–17.0)	1.8 (1.6–2.1)	14.9 (14.4–15.4)
	Relative weight	0.019 (0.018–0.020)	0.083 (0.054–0.091)	0.017 (0.015–0.019)	0.038 (0.036–0.040)	0.019 (0.018–0.020)	0.067 (0.050–0.091)
Right adrenal gland	Length [cm]	23.1 (22.7–23.9)	47.4 (43.3–49.5)	34.8 (32.5–38.0) <sup>a</sup>	54.7 (57.3–61.9)	23.7 (22.7–24.9)	55.4 (54.0–56.0)
	Width [cm]	15.7 (15.5–17.1)	32.7 (31.0–33.2)	25.3 (23.2–27.9)	42.6 (40.4–45.0)	16.2 (15.5–18.3)	43.7 (38.2–46.2)
	Thickness [cm]	6.2 (6.0–7.0)	13.0 (12.4–13.0)	8.0 (6.9–9.1)	18.7 (17.5–19.9)	6.7 (6.0–7.0)	18.0 (16.5–21.1)
	Weight [g]	1.5 (1.4–2.0)	8.3 (7.6–8.4)	6.2 (5.4–7.1)	15.2 (14.1–16.2)	1.7 (1.4–2.2)	14.1 (13.3–15.4)
	Relative weight	0.017 (0.016–0.020)	0.089 (0.058–0.091)	0.015 (0.013–0.017)	0.037 (0.034–0.040)	0.018 (0.016–0.019)	0.076 (0.049–0.091)

The NPAR method was used to calculate all RIs in the youngest bison. The RUD method was used to calculate virtually all RIs in the adolescent and adult bison except for one case (<sup>a</sup>), in which the RTD method was used



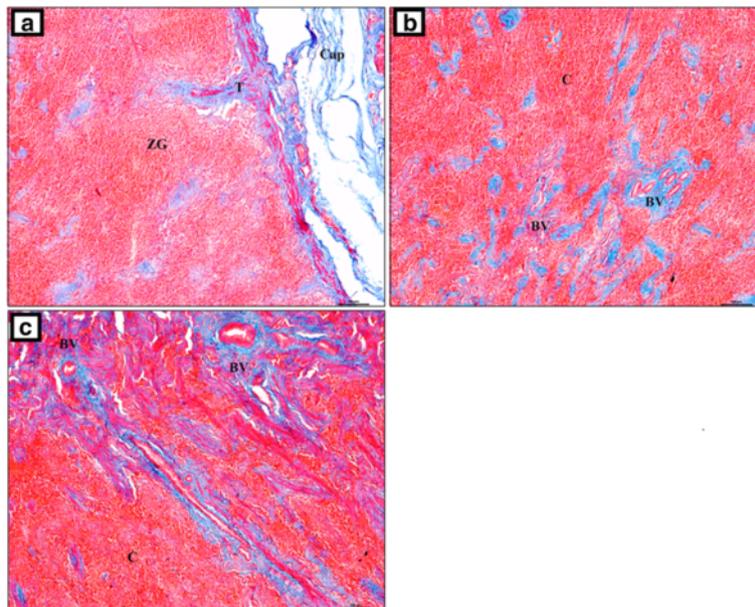
**Fig. 3** The adrenal gland in the European bison. **a** Thick connective tissue capsule, Stain = H&E, Bar = 100  $\mu$ m; **b** The inner and outer layer of the connective tissue capsule, Stain = H&E, Bar = 100  $\mu$ m; **c** Adipose and inner layer of connective tissue capsule formed from collagen fibers, Stain = Azan trichrome, Bar = 100  $\mu$ m; **d** Inner layer of connective tissue capsule with thick and regular strands of elastic fibers. Stain = Masson-Goldner trichrome. Bar = 50  $\mu$ m; **e** Collagen fibers arranged in a mesh. Stain = Azan trichrome. Bar = 100  $\mu$ m. Cap – capsule, OL – outer layer, IL – inner layer, AT – adipose tissue, T – trabeculae, ZG – zona glomerulosa, ZF – zona fasciculata, C – cortex, BV – blood vessels

animal species [9–11, 14–16, 19, 20]. The shape of the adrenal glands may differ not only between species but also between animals of the same species. In all the examined European bison, the left adrenal gland resembled the shape of number “1”, while the right adrenal gland was triangular. A similar adrenal shape was described in domestic cattle and small ruminants [19]. Studies carried out in the Nili-Ravi buffalo showed that the left adrenal gland had a V-shape, while the right gland was C-shaped [8].

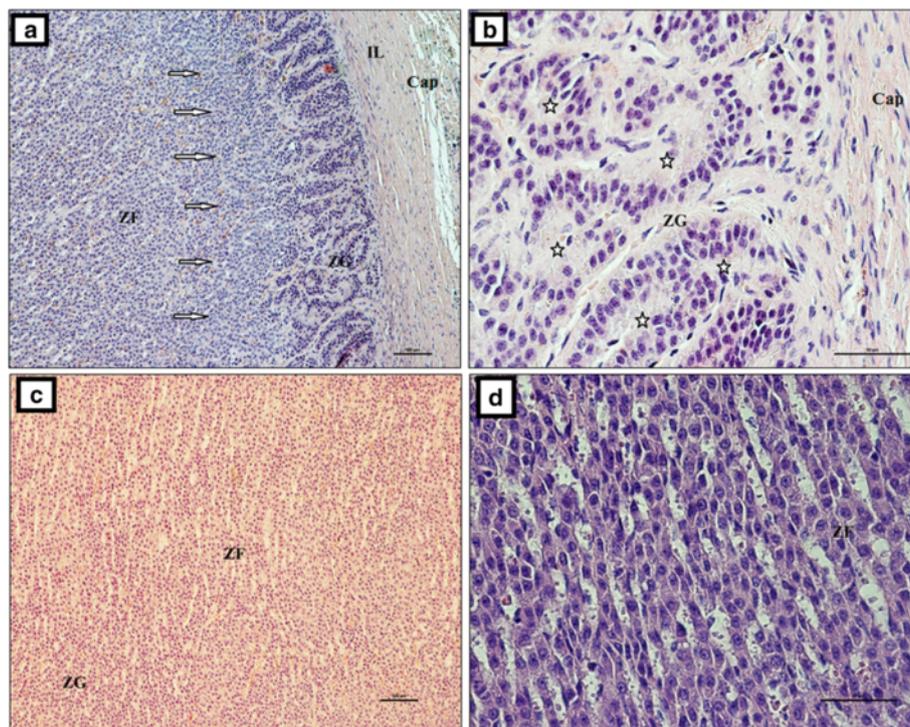
Erdoğan and Pérez [13] described the shape of the adrenal glands in the pampas deer and found inter-individual differences in the shape of the left gland. Two

of the ten studied deer had a V- or heart-shaped left gland, while the remaining eight animals had an oval shaped one. The right gland was oval-shaped in all the studied deer.

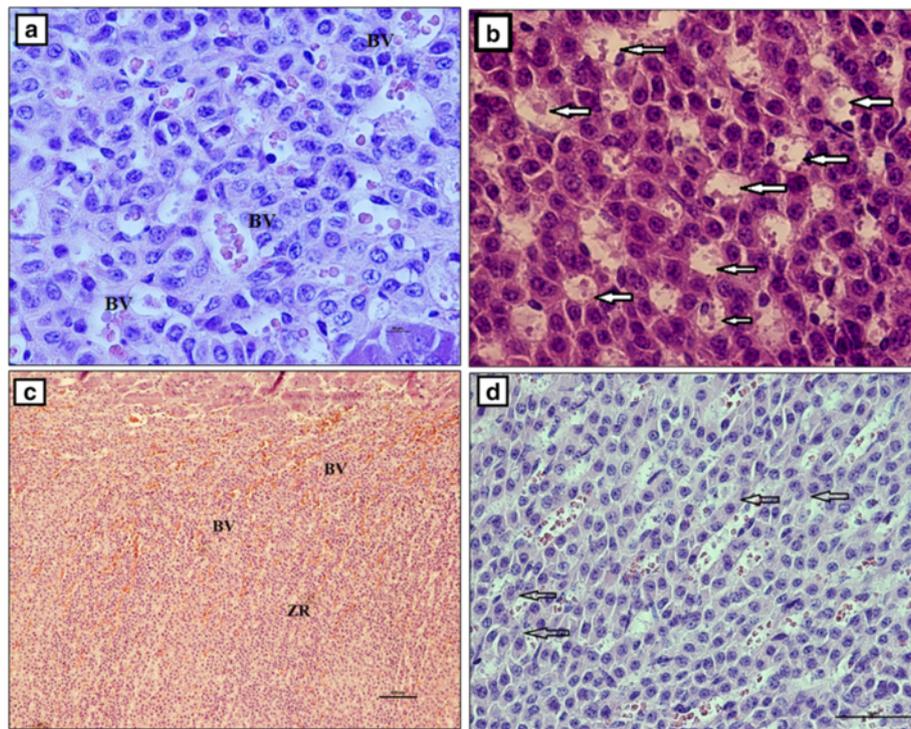
There are several morphometric studies of the adrenal gland in literature. They describe the relative and absolute weight, length, width, thickness and circumference of each gland. However, our study is the first to report reference intervals for size and weight of the adrenal glands which may prove useful to clinicians, in particular if abdominal sonography is planned as a diagnostic method in sedated European bison.



**Fig. 4** The adrenal gland in the European bison. Stain = Azan trichrome. Bar = 100  $\mu$ m. **a** Inner layer of the connective tissue layer forming thick connective tissue trabeculae that penetrate the zona glomerulosa; **b** Numerous blood vessels in the adrenal cortex; **c** Numerous blood vessels surrounded by collagen fibers forming thick circular layers in the adrenal cortex. Cap – capsule, T – trabecule, ZG – zona glomerulosa, C – cortex, BV – blood vessels



**Fig. 5** The adrenal gland in the European bison. Stain = H&E. **a** Glandular cells of the zona glomerulosa are arranged in the form of bundles and arches (white arrow), Bar = 100  $\mu$ m; **b** A concentric arrangement of the cells of the zona glomerulosa (white star), Bar = 50  $\mu$ m; **c** Arches that form columns creating the zona fasciculata, Bar = 50  $\mu$ m; **d** Oval cells are arranged in strands of arches, Bar = 50  $\mu$ m. Cap – capsule, IL – inner layer, ZG – zona glomerulosa, ZF – zona fasciculata



**Fig. 6** The adrenal gland in the European bison. Stain = H&E. **a** Cells of the zona reticulata of various shape and irregularly arranged form a network, Bar = 10  $\mu$ m; **b** Numerous apoptotic cells (white arrow), Bar = 10  $\mu$ m; **c** Blood-filled vessels, Bar = 100  $\mu$ m; **d** Cells of the zona fasciculata with vacuoles (black arrow), Bar = 50  $\mu$ m. ZR – zona reticularis, BV – blood vessels

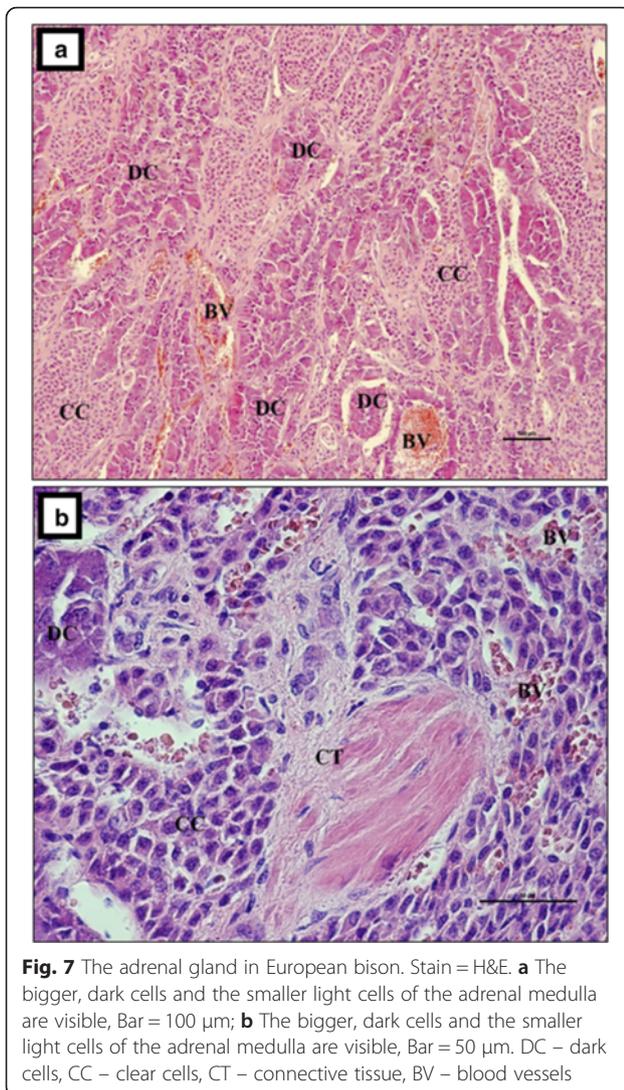
The studies carried out in the pampas deer by Erdoğan and Pérez [13] revealed that the right adrenal gland mean weight was  $0.59 \pm 0.09$  g, and the mean weight of the left adrenal gland was  $0.64 \pm 0.1$  g. The mean length and width of the left adrenal gland ( $18.04 \pm 0.94$  mm,  $11.83 \pm 0.81$  mm) was larger than the right gland ( $17.3 \pm 1.58$  mm,  $10.11 \pm 0.42$  mm). The mean thickness of the right adrenal gland ( $5.11 \pm 0.45$  mm) was greater than that of the left gland ( $4.59 \pm 0.31$  mm).

Hussain et al. [8] presented the absolute and relative weight of the adrenal glands in the Nili-Ravi buffalo. The absolute weight was  $23.70 \pm 1.12$  g, and the relative weight was  $0.06 \pm 0.001$  % in young animals. In old animals, the absolute and relative weights were  $33.85 \pm 1.17$  g and  $0.05 \pm 0.001$  g, respectively. The authors provided the length and width of both the left and the right adrenal glands. The length and width of the right adrenal gland in young animals were  $5.03 \pm 0.05$  cm and  $3.84 \pm 0.06$  cm, and  $6.69 \pm 0.09$  cm and  $4.13 \pm 0.0015$  cm in adult animals, respectively. In the left adrenal gland, those measurements were  $5.17 \pm 0.08$  cm and  $2.85 \pm 0.05$  cm in young animals and  $6.85 \pm 0.100$  cm and  $4.82 \pm 0.22$  cm in adult animals, respectively. The mean length of the adrenal glands was significantly greater in adults than in young individuals. However, the difference

in the width of the right adrenal gland between young and adult buffaloes was non-significant [8].

In the studied European bison population, there was no difference in the weight of adrenal glands with respect to age. However, all three size parameters differed between the left and the right gland. The left adrenal gland was significantly longer, narrower and thinner than the right one in all the age groups.

In the European bison, like in other mammalian species, the capsule consisted of an inner and outer layer, which differed in the amount of connective tissue [19–21]. Interestingly, we found a substantial layer of adipose cells between the inner and the outer layer of the capsule, which may be presumed to be characteristic of wild animals. There are no literature reports describing a similar finding in domestic animals. According to Nabipour [22], the adrenal capsule in camels (*Camelus dromedarius*) has dense irregular connective tissue. Al-Bagdadi [10] reported that the adrenal capsule in camels (*Camelus dromedarius*) consists of two layers, and the inner layer contains cellular elements. Similarly to domestic animals, the adrenal capsule in the European bison contained a lot of collagen, elastin and smooth muscle fibers [19–21]. Vuković et al. [17] found mainly collagen fibres and a few elastin fibres.



In the European bison, similarly to domestic animals and camels, the adrenal capsule was well developed and formed connective tissue trabeculae that penetrated the cortex and rarely entered the medulla [10, 19–22]. In contrast, Vuković et al. [17] reported that in marine mammals (dolphins) the thick connective tissue trabeculae did not divide the cortex into pseudo-lobes.

Similarly to other land and marine mammals, the adrenal gland in the European bison consisted of the cortex and medulla. The cortex contained three layers – the zona glomerulosa, zona fasciculata and zona reticularis.

In the zona glomerulosa of the European bison, the cells formed numerous bundles and arches, similarly to domestic ruminants, horse and carnivores [19–21, 23]. The cells of the zona glomerulosa in the European bison were polygonal in shape as in domestic mammals, camels and marine mammals [9, 10, 22].

In the European bison, as in humans and domestic ruminants, the cells in the zona fasciculata were predominantly arranged in bundles [24]. They were oval and arranged in single or multiple arch-shaped strands. On the other hand, in the zona fasciculata of the marine mammals (dolphin), the arches passed into columns and contained one or two cell rows in each cord [17]. Those cells were large and polygonal in marine mammals. Our research showed numerous apoptotic cells in the zona reticularis. A similar finding was described in cattle [9]. In the European bison and camel (*Camelus dromedaries*), many cells in the zona fasciculata and zona reticularis contained vacuoles, which formed a foamy cytoplasm and indicated the presence of lipids [12]. In the European bison, the structure of the adrenal medulla was similar to that of domestic animals [9, 19–21]. In the superficial layer of the adrenal medulla, there were large dark cells with a strongly basophilic cytoplasm. Small, lighter cells were present in the deeper layer of the medulla. According to Jelinek and Konecny [9], both types of cells were intertwined in cattle. According to Dellman [21], the medulla was divided into two parts in domestic animals (horses, cows, sheep and pigs). The outer part was composed of strongly staining epinephrine-secreting cells, while the inner part was formed from cells with a weaker stain affinity (norepinephrine-secreting cells). A similar structure of the adrenal medulla was reported in marine mammals by Vuković et al. [17].

## Conclusions

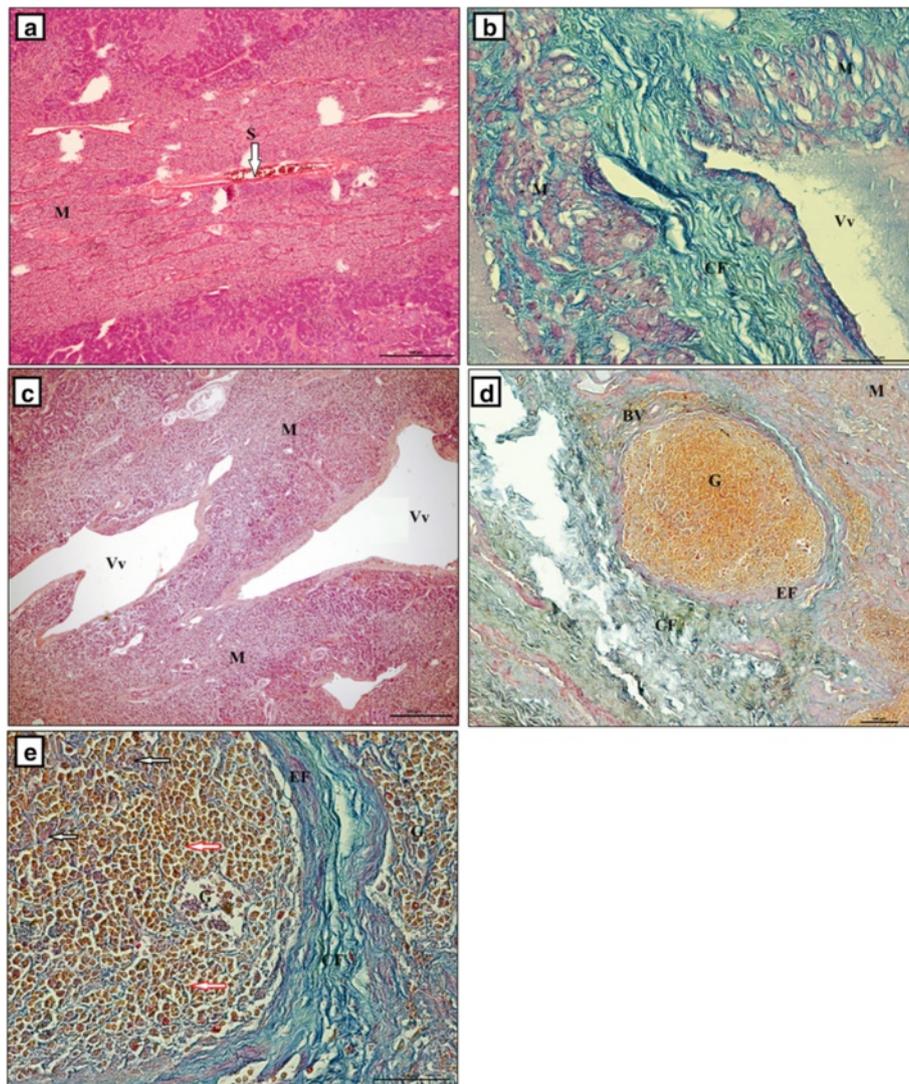
The left adrenal gland in the European bison was significantly longer, narrower and thinner than the right one in all the age groups. There were no significant differences in the structure of the adrenal medulla and cortex of the European bison compared to other species of domestic and wild mammals. There was a thick layer of adipose cells at numerous locations in the adrenal capsule, which had not been described in land and marine mammals.

## Methods

### Animals and gross anatomy

The study material comprised adrenal glands from the 97 European bison (*Bison bonasus*) of both sexes (40 males and 57 females), aged 3 months to 24 years (median of 24 months, IQR from 7 to 72 months), inhabiting the Białowieża Forest (Białowieża National Park, Poland) (Additional file 1). The females were significantly older than the males ( $p = 0.006$ ) with a median (IQR) of 24 (7–84) and 18 (5–30) months, respectively.

The animals were divided into three groups depending on their age: 3 months to 2 years old (group I), 2 to 5 years old (group II), and older than 5 years (group III). The animals were also classified based on data on their



**Fig. 8** The adrenal gland in the European bison. **a** Single sinusoidal vessels in the central part of the adrenal medulla, Stain = H&E, Bar = 100  $\mu$ m; **b** Veins surrounded by loose connective tissue, Stain = Masson-Goldner trichrome, Bar = 50  $\mu$ m; **c** Veins in the adrenal medulla, Stain = H&E, Bar = 50  $\mu$ m; **d** Nerve ganglion and vein in the adrenal medulla, Stain = Mallory trichrome, Bar = 100  $\mu$ m; **e** Nerve ganglion in adrenal medulla, Stain = Mallory trichrome, Bar = 50  $\mu$ m. M – medulla, G – ganglion, CF – collagenous fibres, EF – elastic fibres, black arrow – large polygonal cells stained pink, red arrow – polygonal cells stained light - or dark orange, BV – blood vessels, EF – elastic fibres, Vv – Venous vessels, S – sinusoid

growth and development [25–27]. The information on the animals' age was drawn from the Białowieża Nature Reserve Record Book.

The weight of each animal was determined under field conditions after legal culls were performed. After the abdominal cavity was opened, the holotomy, skeletotomy and syntopy of the adrenal glands were recorded. The adrenal glands were then removed, and the shape and mass of the left and the right gland were recorded using the AXIS AD2000 laboratory weighing scale, accurate to the nearest 0.01 g. Next, an electronic (TESA – CAL IP67) caliper, accurate to the nearest 0.01 mm, was used to measure the length, width and thickness of each gland.

The Białowieża National Park was responsible for the culling of the European bison. The animals were not killed for the purpose of this study. Population control, bone fractures and car accidents were the most common reasons for culling the animals. The culling was carried out with the permission of the Ministry of Environment and the General Director for Environmental Protection in Poland (decision number: DOP-OZGIZ. 6401.06.7.2012.ls, DOP-OZ.6401.06.7.2012.ls.1 and DLP-III-4102-459/36490/14/ZK). The adrenal glands and other organs were collected by the authors (veterinarians) during dissection. The autopsy protocols are available in Białowieża National Park. According to the Polish law, tests on tissues obtained

*post-mortem* do not require an approval of the Ethics Committee (Parliament of the Republic of Poland, 1997).

The terminology used in the manuscript is in accordance with prevailing veterinary nomenclature [28].

### Statistical analysis

Numerical variables are presented as mean  $\pm$  standard deviation (SD) except for the animal age, which is presented as the median and interquartile range (IQR). A Mann-Whitney U test was used to compare the age of the males and females. Since there was a significant difference in age between sexes, the female and male adrenal gland mass and size were compared, using the analysis of covariance (ANCOVA), with the animals' age as a covariate. One-way analysis of variance (ANOVA) followed by the Tukey post-hoc test for unequal groups were used to compare the adrenal gland mass and size in the three age groups. ANOVA for paired samples was applied to compare the weight and size of the left and the right adrenal glands. For all the statistical tests, a significance level ( $\alpha$ ) of 0.05 was assumed. The analyses were performed using Statistica 10 software (StatSoft Inc.).

A 95 % reference intervals (RI) together with their 90 % confidence intervals (CI 90 %) were computed for adrenal gland weight and size (i.e. length, width and height) using either a nonparametric (NPAR) or robust method with or without Box-Cox transformation (RTD and RUD, respectively) depending on the results of the Anderson-Darling normality test and symmetry test. Given the small number of animals in the adolescent and adult bison groups (14 and 25, respectively), those classes were merged into one category ( $n = 39$ ) for the needs of reference interval calculation. Moreover, the overall reference intervals were computed for all 97 bison. All the calculations of RIs were performed using the Reference Value Advisor [29].

### Histological study

The research material was directly fixed in 4 % buffered formaldehyde for 72 h, rinsed in running water for 24 h, processed in a vacuum tissue processor – ETP (RVG3, INTELSINT, Italy), embedded in paraffin and cut on a Slide 2003 (Pfm A.g., Germany) sliding microtome into 3–4  $\mu\text{m}$  sections. The samples were then stained with haematoxylin and eosin (H&E), Azan trichrome, Masson-Goldner trichrome and Mallory trichrome to observe the general structure of the adrenal glands. The H&E, Azan trichrome, Masson-Goldner and Mallory trichrome staining scoring system was based on a standard protocol previously described [30]. All the obtained slides were examined using the Nikon Eclipse 80i light microscope (NIKON, Tokyo, Japan) for histological description.

## Additional file

**Additional file 1:** Measurements of the adrenal glands in the studied European bison population. (DOC 275 kb)

### Acknowledgements

None.

### Funding

None.

### Availability of data and materials

The datasets supporting the conclusions of this article are included within the article (and its Additional file 1).

### Authors' contributions

KB, HP, KO, JKN, KGH carried out the dissection of the cadavers, participated in the data analyses and wrote the manuscript. MC participated in the design of the study and in the statistical analyses. MK participated in the design of the study and helped to draft the manuscript. All the authors have read and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

### Consent for publication

Not applicable.

### Ethics approval and consent to participate

The culling was carried out with the permission of the Ministry of Environment and the General Director for Environmental Protection in Poland (decision number: DOP-OZGIZ. 6401.06.7.2012.Is, DOP-OZ.6401.06.7.2012.Is.1 and DLP-III-4102-459/36490/14/ZK).

### Author details

<sup>1</sup>Department of Morphological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, 159 Nowoursynowska, 02-776 Warsaw, Poland. <sup>2</sup>Laboratory of Veterinary Epidemiology and Economics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland. <sup>3</sup>Department of Animal Physiology and Biostructure, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Kozuchowska 1/3, 51-631 Wrocław, Poland.

Received: 15 December 2015 Accepted: 20 July 2016

Published online: 03 August 2016

### References

1. Kita J, Anusz K. Heath threats for the European Bison particularly in free-roaming populations in Poland. Warszawa: The SGGW Publishers; 2006.
2. Kupczyńska M, Barszcz K, Olbrych K, Polguj M, Wysiadecki G, Topol M, Klećkowska-Nawrot J. Coronary arteries of the European bison (*Bison bonasus*). *Acta Vet Scand.* 2015;57:82.
3. Pytel S, Wojnowska B. Morphology of the pancreas In the European bison. *Acta Theriol.* 1980;25:141–50.
4. Sawicki B, Siuda S, Kasacka I. Microscopic structure of the thyroid gland in the European bison. *Acta Theriol.* 1992;37:171–9.
5. Węgrzyn M, Makowiecka M. Thyroid arterie of European bison (*Bison bonasus* Linnaeus, 1758). *Ann. Warsaw Agricult. Univ.-SGGW, Vet. Med.* 1983;11:17–21.
6. Serwatka S. Morphology of the thyroid gland in European Bison. *Folia Morphol.* 2001;60:158.
7. Kobryń H. Morphology of the thymus in the European bison. *Acta Theriol.* 1975;20:161–4.
8. Hussain R, Qureshi AS, Shahid RU, Rahman SU. Age related changes in the morphometric parameters of the heart, kidneys and adrenal glands of Nili-Ravi buffalo (*Bubalus bubalis*). *Pakistan Vet J.* 2006;26:59–62.
9. Jelinek F, Konecny R. Adrenal glands of slaughtered bulls, heifers and cows: a histological study. *Anat Histol Embryol.* 2011;40:28–34.
10. Al-Bagdadi FAK. The adrenal gland of the camel (*Camelus dromedarius*). A study of the comparative anatomy and lipoids. *Zentralbl Veterinarmed A.* 1969;16:354–64.

11. Abdalla MA, Ali AM. Morphometric and histological studies on the adrenal glands of the camel *Camelus dromedarius*. *Acta Morphol Neerl Scand*. 1988–1989;26:269–81.
12. El-Nahla SM, Imam HM, Moussa AE, Elsayed AK, Abbott LC. Prenatal development of the adrenal gland in the one-humped camel (*Camelus dromedarius*). *Anat Histol Embryol*. 2011;40:169–86.
13. Erdoğan S, Pérez W. Arterial vascularization and morphological characteristics of adrenal glands in the Pampas deer (*Ozotoceros bezoarticus*, Linnaeus 1758). *Anat Histol Embryol*. 2014;43:369–74.
14. Tomich PQ. Weight variation in adrenal glands of the mongoose in Hawaii. *Pac Sci*. 1965;19:238–43.
15. Pankakoski E, Tähkä KM. Relation of adrenal weight to sex, maturity and season in five species of small mammals. *Ann Zool Fennici*. 1982;19:225–32.
16. Clark LS, Pfeiffer DC, Cowan DF. Morphology and histology of the atlantic bottlenose dolphin (*Tursiops truncatus*) adrenal gland with emphasis on the medulla. *Anat Histol Embryol*. 2005;34:132–40.
17. Vuković S, Lucić H, Živković A, Duras Gomerčić M, Gomerčić T, Galov A. Histological structure of the adrenal gland of the bottlenose dolphin (*Tursiops truncatus*) and the striped dolphin (*Stenella coeruleoalba*) from the Adriatic Sea. *Anat Histol Embryol*. 2010;39:59–66.
18. Bragulla H, Hirschberg RM, Schlotfeldt U, Stede M, Budras KD. On the structure of the adrenal gland of the common seal (*Phoca vitulina vitulina*). *Anat Histol Embryol*. 2004;33:263–72.
19. König HE, Liebich HG. *Veterinary anatomy of domestic mammals*. Germany: Schattauer; 2007.
20. Dyce KM, Sack WO, Wensing CJG. *Textbook of Veterinary Anatomy*. 3rd ed. Philadelphia: Saunders; 2010.
21. Dellman HD. Endocrine system. In: Dellmann HD, editor. *Textbook of Veterinary Histology*. 4th ed. Philadelphia: Lea and Fabiger Records; 1993. p. 270–84.
22. Nabipour A, Khanzadi S, Behdegani A. Gross and histological study of adrenal gland in camels (*Camelus dromedarius*). *J Camel Pract Res*. 2008;15:121–5.
23. Yamauchi S. A histological study of the adrenal gland in aged cows. *Okajimas Fol Anat*. 1965;40:393–407.
24. Iki T. Zur Struktur und Funktion der Nebenniere bei den Haussäugetieren. München: Inaugural-Dissertation; 2005.
25. Kobryńczuk F, Kobryń H. Postembryonic growth of bones of the autopodia in the European bison. *Acta Theriol*. 1973;18:309–11.
26. Kobryńczuk F, Kobryń H. Growth rate of bones in the postembryonic development of the European bison. *Acta Theriol*. 1975;20:151–7.
27. Kobryńczuk F, Kobryń H. Growth rate of selected parameter of the European bison sully. *Folia Morphol*. 1980;39:69–77.
28. World Association of Veterinary Anatomist. *Nomina Anatomica Veterinaria*. Gent: 2012. p. 64.
29. Geffre A, Concordet D, Braun JP, Trumel C. Reference Value Advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel Vet. *Clin Pathol*. 2011;40:107–12.
30. Burck HCh. *Technika histologiczna*. Warszawa: PZWL; 1975. pp. 139–140, 143–145.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)

