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Morphometric characteristics of thyroid C cells in cattle, dogs, horses, and pigs

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Received 19.12.2024 *Accepted 27.02.2025*

Sokołowska J.

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Summary

The aim of the study was the morphometric assessment of C cells in cattle, dogs, horses, and pigs, which made it possible to complete the microscopic characteristics of C cells in these species. The material consisted of transverse sections of the left thyroid lobe (20 specimens/species), fixed in formalin, and subjected to standard histological processing. C cells were stained immunohistochemically with anti-calcitonin antibody. The following morphometric parameters were assessed: cell area (μm^2), perimeter (μm), length (μm), width (μm), and circularity. In each case, 40 C cells were measured, and the mean value of each parameter was calculated for each species. C cells of each animal species were of different shapes: spindle-shaped in pigs, oval in dogs, and polymorphic in horses and cattle. Bovine C cells had cytoplasmic processes. It was shown that the area of C cells was largest in horses (88.83 ± 37.24) and dogs (86.92 ± 34.29), significantly larger than in cattle (83.5 ± 37.78) and pigs (73.7 ± 43.12). Their perimeter was largest in pigs (46.56 ± 19.35), smaller in cattle (41.59 ± 13.34) and horses (41.81 ± 10.15), and smallest in dogs (37.01 ± 8.72). In horses vs. cattle and horses vs. pigs, the differences were not significant. The most extended C cells were found in pigs (19.55 ± 9.57) and shortest in dogs (12.67 ± 3.44). Their length in horses and cattle was comparable (15.29 ± 4.76 and 15.6 ± 6.61, respectively). Except for horses vs. cattle, these differences were significant. The width of C cells was highest in dogs (6.69 ± 1.59) and lower in horses (5.85 ± 1.72), cattle (5.46 ± 1.6), and pigs (3.78 ± 1.14). Except for horses vs. cattle, the differences were significant. The circularity of C cells was highest in dogs (0.78 ± 0.1), followed by cattle (0.63 ± 0.94), horses (0.64 ± 0.14), and pigs (0.46 ± 0.16). These differences were not significant only in horses vs. cattle. The results show that the most significant variation in the parameters examined characterizes porcine and canine C cells. Despite their morphological diversity, equine and bovine C cells are morphometrically similar. The results obtained can provide reference values for further studies on the behavior of C cells in various physiological and pathological conditions in these animal species. They can serve as an additional tool for diagnosing tumors originating from C cells and be used in comparative histological research.

Keywords: C cells, morphometry, cattle, dog, horse, pig

The parenchyma of the thyroid gland consists of two types of cells: thyrocytes (follicular cells) and C cells (parafollicular cells, C thyrocytes). C cells, however, constitute a small percentage of thyroid endocrine cells. The primary function of these cells is a synthesis of calcitonin, which, along with the parathyroid hormone released by the parathyroid glands, is responsible for maintaining calcium homeostasis. Moreover, they produce many regulatory peptides, including calcitonin gene-related peptide, katacalcin, serotonin, somatostatin, neuron-specific enolase, and ghrelin involved in the intrathyroidal regulation of thyrocyte activity, mainly in a paracrine manner (10).

It is widely known that many characteristics of C cells differ between species. These include the profile of secreted regulatory peptides, the exact location of C cells in relation to the follicle wall, their total number within the thyroid gland, and details of their morphology (3, 10, 16, 17, 33).

The microscopic appearance of thyroid C cells largely depends on the taxonomic group to which a given animal belongs. Hence, unrelated species are often characterized by a different morphology of C cells (3, 33), whereas, in closely related species, the morphology of C cells is similar. For example, studies conducted on some species belonging to the ruminant suborder, i.e., sheep, cattle, and European bison, have shown that the C cells of all these animals have a similar microscopic appearance (24, 32, 33). However, the number of shapes that animal cells can

take is limited. Thus, taxonomically distant species can also have C cells with similar morphology. One such example are dogs and guinea pigs (16).

A recently published study showed that the morphology of swine, canine, bovine, and equine C cells differs significantly (33). These differences are so pronounced that, based on the appearance of C cells, it is possible to recognize the species from which the thyroid gland was collected. However, the available literature lacks specific morphometric data. Therefore, the aim of the present study was the morphometric assessment of C cells in cattle, dogs, horses, and pigs, which would make it possible to complete the microscopic characteristics of C cells in these species.

The results obtained can provide a basis for establishing reference values for morphometric parameters of C cells. Such standards may be useful in future studies on the impact of various physiological and pathological factors on C cells morphology. They can also be helpful in C cell cancer research and studies in the field of comparative histology.

Material and methods

The study examined thyroid glands obtained from four domestic animal species: cattle, dogs, horses, and pigs, 20 glands/species. The cattle represented Polish Holstein-Friesian (11/20), Limousin (5/20), and Simmental (4/20) breeds. The dogs belonged to the following breeds: mixed breed (9/20), German Shepherd (3/20), Labrador Retriever (2/20), French Bulldog (2/20), Yorkshire Terrier (2/20), Border Collie (1/20), and German Pinscher (1/20). The horses comprised warmbloods (9/20), coldbloods (5/20), Polish Koniks (4/20), and ponies (2/20). All pigs included in this study were DanBred. Thyroid glands from all animal species except dogs were collected from individuals freshly slaughtered in qualified slaughterhouses. Canine thyroid glands were collected from dogs euthanized due to illnesses involving organs other than the thyroid gland. All thyroid glands were without any macroscopic or microscopic lesions. Tissue samples were collected from the medial part of the left thyroid lobe in the transverse section. The material was fixed in 10% buffered formalin for 24 hours. Then, tissue samples were processed by a common paraffin technique, i.e., dehydrated in a series of ethanol solutions (70% × 2, 96% × 2, and 99.8% × 2, 1 hour in each solution), cleared in xylene (2 × 1 hour), embedded in paraffin, and cut into 3 μm sections.

C cells were visualized immunohistochemically by the indirect immunoperoxidase method. Anti-calcitonin rabbit polyclonal antibody (Agilent Dako, Glostrup, Denmark, code no A0576) was used to visualize C cells. The cross-reactivity of this antibody for each species has been confirmed by the manufacturer and described in the literature (15, 19, 27, 31). All immunohistochemical procedures were carried out according to the manufacturer's protocols. Briefly, after tissue deparaffinization, rehydration, and inactivation of endogenic peroxidase with 3% hydrogen peroxide, slides underwent an unmasking procedure by microwaving (two cycles: 7 and 5 min, 700 W, in citrate buffer pH 6.0). After the slides were cooled, incubation with primary antibody was performed (dilution 1 : 400, 1 hour at room temperature). Then, the secondary antibody was placed for 30 min at room temperature, and after this, DAB chromogen solution was applied to visualize the reaction. The REAL EnVision Detection System, Peroxidase/DAB+, Rabbit/Mouse visualization system (Agilent Dako, code no K5007) was used for this purpose. Then, the sections were counterstained with Erlich's hematoxylin, dehydrated, cleared, and mounted. Negative controls were also performed by substituting the primary antibody with TBST buffer (Agilent Dako, code no S3006).

Morphometric analysis was performed under a Nikon Eclipse 80i microscope equipped with a Nikon DS-Ri1 camera (Nikon, Tokyo, Japan) using the NIS-Elements BR microscope imaging software (Nikon). Images were sampled randomly throughout the thyroid sections; any tissue areas with artifacts or nonspecific staining, as well as peripheral tissue regions, were excluded. The images were acquired with a resolution of 3840 × 3072 pixels (24-bit RGB, 8 bits/color) and stored in the TIF format. To distinguish C cells from the background, a color threshold was added to the RGB images by defining the limit values for all channels; this made it possible to differentiate the "blue" background pixels from the "brown" pixels of C cells. By carefully selecting the conditions and method of image acquisition, it was possible to distinguish between C cells and their background. The color threshold made it possible to convert the captured images into binary images. Then, the morphometric analysis was carried out. All measurements were performed automatically. Only the profiles of entire cells were analyzed. Cell projections were also included in the cell profile if visible in a given cell. C cell groups lacking clearly visible cell borders, incomplete cells or cell fragments (for example, sections by separated cytoplasmic processes), and swine C cells in cross-section were excluded from the analysis. The following parameters were calculated: cell area (μm^2), perimeter (μm), length (μm), width (μm), and circularity (the definitions of these parameters and formulas for their calculation are available in the NIS-Elements manual on www.nisoftware.net). In each case, forty C cells were measured at a microscope magnification of 400 ×, and the mean value of each parameter was calculated, first for each animal and then for each species.

The data obtained were analyzed using Statistica 13.3 for Windows (StatSoft, Kraków, Poland). All were presented as mean values ± SD. The Kolmogorov-Smirnov test indicated that the data were not normally distributed. Differences in morphometric parameters were calculated using the Mann-Whitney U-test. Morphometric characteristics were compared between species. $P \leq 0.05$ was considered significant, $P \leq 0.01$ highly significant, and $P \leq 0.001$ very highly significant.

Results and discussion

The C cells of the examined domestic species are characterized by different shapes. Porcine and canine C cells are the most uniform in shape, but they differ considerably from each other. In pigs, C cells are of a spindle shape, whereas in cross-section, the cells are

oval (Fig. 1). In dogs, they are oval or polygonal (Fig. 2). C cells in the other two species are characterized by polymorphism. However, in horses, they are oval, polygonal, irregular, or elongated (Fig. 3), whereas in cattle, C cells take an oval, polygonal, or slightly irregular shape. Moreover, a characteristic feature of bovine C cell morphology is the presence of long cytoplasmic processes (Fig. 4).

The morphometric analysis showed that the mean C cell area was highest in horses (88.83 ± 37.24, range 19.72--302.14) and dogs (86.92 ± 34.29, range 12.6-227.38). Significantly lower values were observed in cattle (83.5 ± 37.78, range 14.58-292.7) compared to dogs ($P \leq 0.05$) and horses ($P \leq 0.01$). The lowest value was found in pigs (73.7 ± 43.12, range 10.16-325.72, $P \leq 0.001$; Fig. 5).

The mean C cell perimeter was largest in pigs (46.56 ± 19.35, range 13.42-128.85), followed by cattle (41.59 ± 13.34, range 17.37-113.21) and horses (41.81 ± 10.15, range 18.24-81.28), in which this parameter was comparable, and then by dogs (37.01 ± 8.72, range 12.86-85.89). All differences were very highly significant, except for horses *vs.* cattle and horses *vs*. pigs (Fig. 5).

The mean length of C cells was largest in pigs (19.55 ± 9.57, range 3.88-59.97) and smallest in dogs (12.67 ± 3.44, range 4.57-37.06). Equine and bovine C cells demonstrated similar lengths: 15.29 ± 4.76 (range 5.6-33) and 15.6 ± 6.61 (range 5.57-53.19), respectively. All these differences were statistically very highly significant, except for horses *vs.* cattle (Fig. 5).

Regarding the mean width, lowest values were observed in pigs (3.78 ± 1.14, range 1.26-8.5), followed by cattle (5.46 ± 1.6, range 1.48-12.23), horses (5.85 ± 1.72, range 1.82-12.91) and dogs (6.69 ± 1.59, range 2.76-12.43). All these differences were statistically very highly significant, except for cattle *vs.* horses, with a significance of $P \leq 0.01$ (Fig. 5).

The mean circularity value was lowest in pigs (0.46 ± 0.16, range 0.13-0.96). Higher values were noted in horses (0.64 ± 0.14, range 0.25-0.96) and cattle (0.63 ± 0.94, range 0.13-0.94). The highest

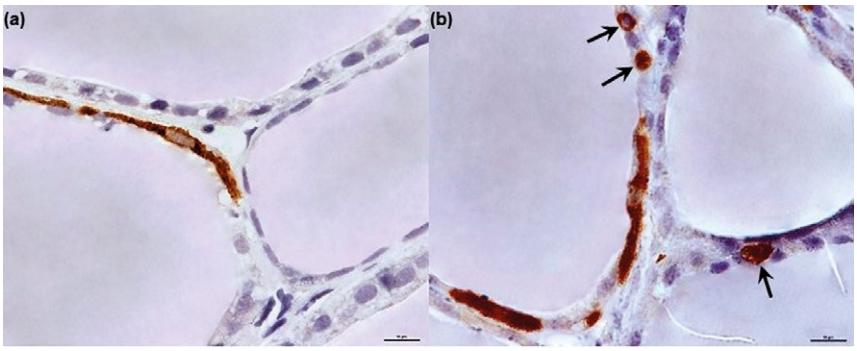

Fig. 1. Typical morphology of porcine C cells. In longitudinal sections, porcine C cells are elongated spindle-shaped cells (a), whereas in cross-section, they are oval (arrows) (b). Immunoperoxidase staining with anti-calcitonin antibody, scale bars = 10 µm

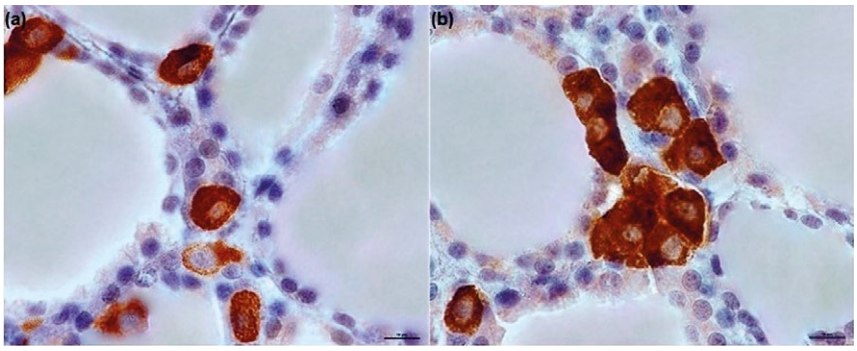

Fig. 2. Typical morphology of canine C cells. Canine C cells are characterized by oval or polygonal shape (a, b). Immunoperoxidase staining with anti-calcitonin antibody, scale bars = 10 µm

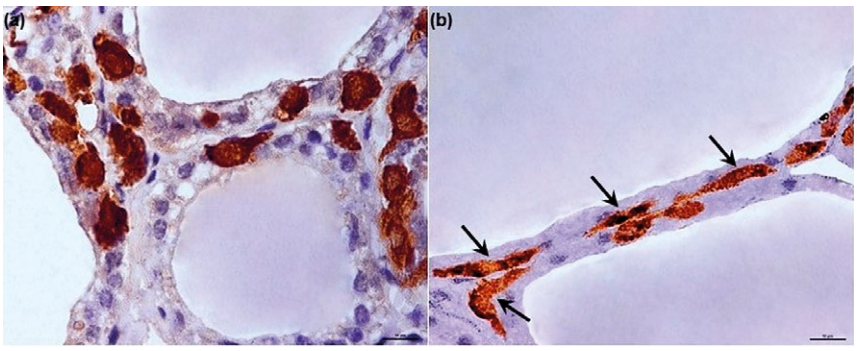

Fig. 3. Typical morphology of equine C cells. Equine C cells are polymorphic, with the cell shape ranging from oval or polygonal to irregular (a) or even elongated (arrows) (b). Immunoperoxidase staining with anti-calcitonin antibody, scale bars = 10 µm

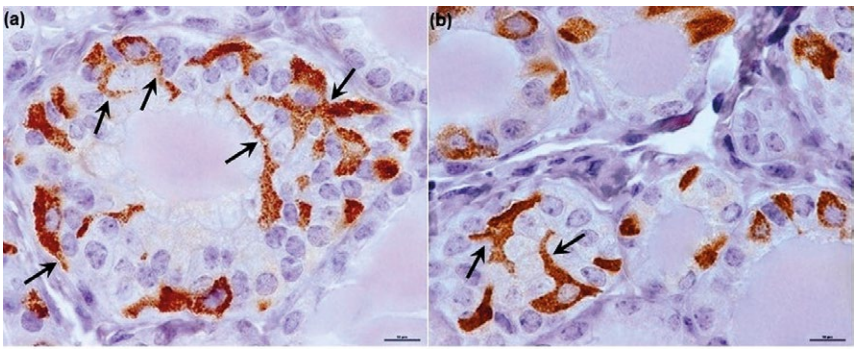

Fig. 4. Typical morphology of bovine C cells. Bovine C cells are polymorphic and take a polygonal, oval, or slightly irregular shape. A characteristic feature of bovine C cell morphology is the presence of long cytoplasmic processes (arrows) (a, b). Immunoperoxidase staining with anti-calcitonin antibody, scale bars = 10 µm

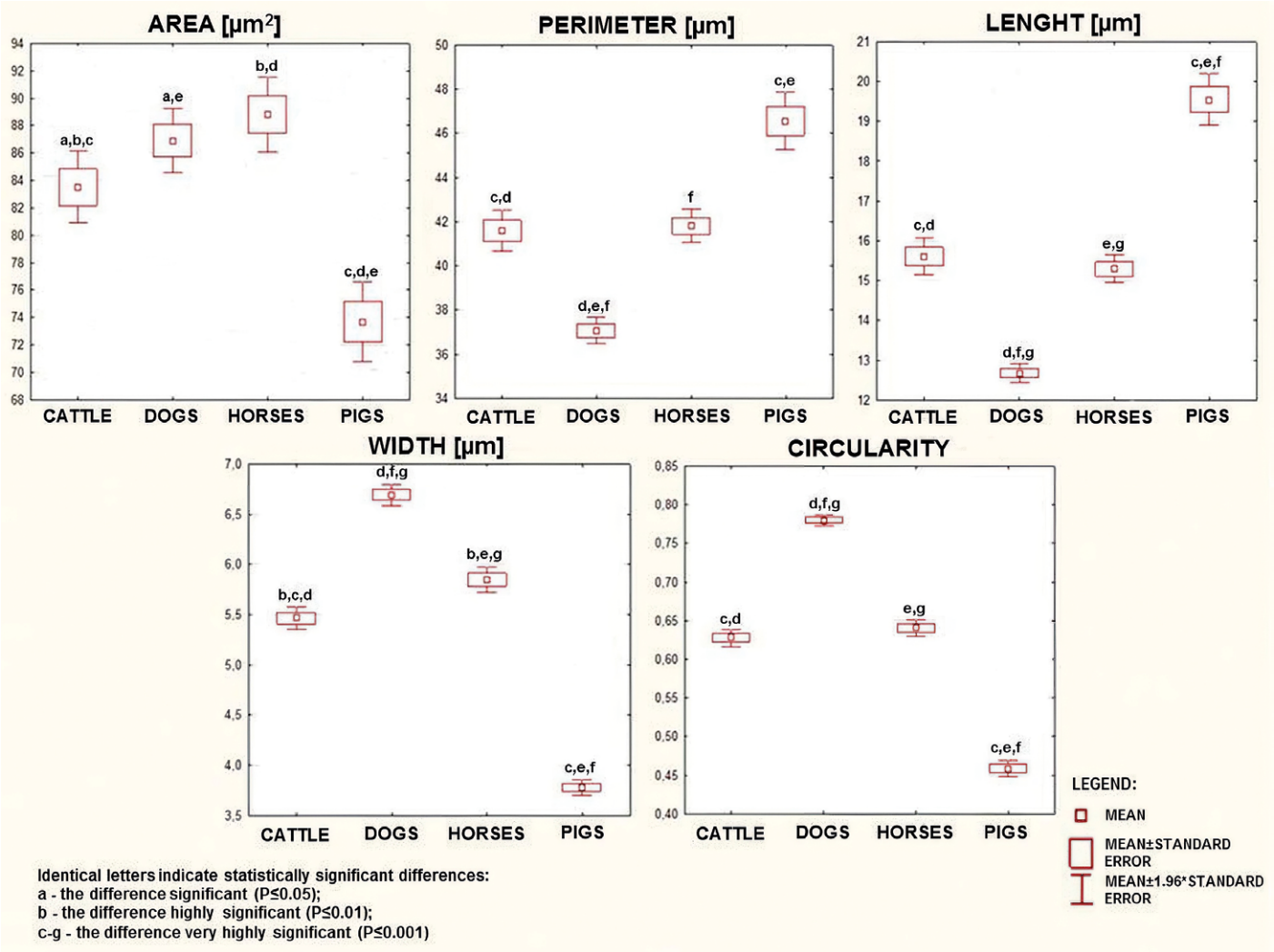

Fig. 5. Comparison of C cell morphometric parameters in cattle, dogs, horses, and pigs

value was measured in dogs (0.78 ± 0.1, range 0.4-1). All these differences were statistically very highly significant, except for horses *vs.* cattle (Fig. 5).

Detailed data regarding all morphometric parameters for each species are given in Tables 1-4.

The morphometrical analysis results confirmed the diversity of C cell morphology in cattle, dogs, horses, and pigs described in the available literature (3, 33). It was found that the most pronounced differences in all parameters existed between dogs and pigs. This was due to the markedly different morphology of C cells in these species, where the C cells of dogs are oval, while porcine C cells are elongated. In addition, C cells constitute a monomorphic cell population in both species. In contrast, some of the C cell parameters, i.e. their perimeter, length, and circularity, did not differ significantly between cattle and horses. This may result from the polymorphism of equine and bovine C cells. In both species, C cells are characterized by mostly similar shapes, oval and irregular, but in horses, some C cells are elongated. This characteristic is not observed in cattle. The presence in both species of similarly shaped C cells in different mutual proportions and the fact that these cells were randomly selected for morphometric analysis can explain the lack of statistical differences between the mean values of some parameters. Interestingly, the cytoplasmic extensions in bovine C cells did not significantly influence the values of parameters such as perimeter, length, or width, which may be due to the fact that only a certain percentage of C cells in the sections had clearly visible cytoplasmic processes.

The main limitation of this study is related to the fact that cells are three-dimensional structures, whereas the current study analyzed only their two-dimensional profiles. Moreover, the shapes of cell profiles strongly depend on the section plane and their orientation. To minimize this bias, cell fragments, visually incomplete C cell profiles, and cross-sected porcine C cells were excluded from the analysis. The exclusion was based on subjective selection and thus only partially corrected this bias. However, it can be assumed that at least some future studies that may use the morphological parameters presented here as reference values, will be based on assessing two-dimensional profiles of C cells in histological sections.

Cellular morphology is a function of the dynamic interactions and the balance between forces occur-

ring between the cytoskeleton, cell membrane, and adhesion complexes that interface with the extracellular matrix, often via the actions of regulatory signal transduction systems. Traditional approaches to cell analysis have relied on visual observation and subjective assessment of its appearance (21). Morphometric image analysis was developed and introduced into biomedical research to increase the objectivity and reproducibility of microscopic cell examinations.

Because morphometry offers the possibility of a more precise characterization of cells or organ structure, it is used in histological research, including comparative histology (5, 12, 13). To date, however, the current study is the only one with such comprehensive morphometric characteristics of C cells in various animal species. Only one study on this subject exists in the available literature. It was conducted on European bison (32). A comparison of its results with the values of morphometric parameters obtained in this study for bovine C cells indicates that these parameters were very similar in both species. However, bovine C cells appear to be slightly larger. This suggests that C cells in related species are characterized not only by similar morphology, but also by comparable cell size. However, further research is needed to confirm this observation.

Morphometric parameters can also be used to assess changes in cell morphology and, indirectly, their function that occur under various physiological or pathological conditions (8, 29, 35). In the available literature, very few studies focus on the influence of various conditions on the morphometric characteristics of C cells. One of them concerns C cells in humans (23), and another describes C cells in European bison (32). In both papers, the morphometric characterization of C cells

Tab. 1. Morphometric characteristics of bovine C cells in particular cases and overall for the species

Case No.	Cell area (μm²)		Cell perimeter (μm)		Cell length (μm)		Cell width (μm)		Cell circularity	
	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD
1	30.95-158.41	79.22 ± 32.96	21.49-82.14	43.57 ± 15.02	7.39-37.39	17.11 ± 7.73	2.70-7.06	4.82 ± 1.14	0.23-0.86	0.57 ± 0.17
2	20.84-235.03	102.12 ± 55.15	18.63-106.74	48.19 ± 20.36	5.57-51.05	18.93 ± 10.02	2.32-10.11	5.51 ± 1.75	0.13-0.90	0.60 ± 0.19
3	33.47-191.33	74.35 ± 32.15	26.52-71.72	39.54 ± 10.97	8.01-29.34	14.68 ± 5.51	3.02-8.53	5.14 ± 1.28	0.33-0.86	0.61 ± 0.13
4	27.41-167.97	82.16 ± 32.99	24.28-90.82	41.38 ± 14.38	7.89-41.35	15.48 ± 7.28	2.23-9.37	5.50 ± 1.41	0.23-0.92	0.64 ± 0.17
5	26.65-192.5	85.44 ± 36.14	20.52-84.17	41.01 ± 13.15	7.61-36.86	15.31 ± 6.30	3.19-8.22	5.61 ± 1.34	0.33-0.91	0.66 ± 0.15
6	36.61-173.1	83.12 ± 31.53	23.69-67.79	39.65 ± 8.89	7.98-27.63	14.34 ± 3.86	3.14-8.08	5.75 ± 1.36	0.47-0.93	0.66 ± 0.13
7	41.11-126.54	82.01 ± 21.42	28.20-64.10	39.96 ± 8.58	7.76-27.44	14.50 ± 4.55	2.88-8.02	5.84 ± 1.27	0.31-0.94	0.67 ± 0.15
8	55.43-182.02	102.1 ± 32.25	28.31-65.28	42.46 ± 9.11	9.61-27.45	15.15 ± 4.27	4.33-10.07	6.83 ± 1.58	0.42-0.91	0.72 ± 0.13
9	18.87-181.54	76.94 ± 35.24	17.37-72.65	36.70 ± 10.38	6.82-31.24	13.00 ± 4.36	2.77-12.23	5.82 ± 1.73	0.38-0.87	0.71 ± 0.12
10	39.17-181.15	83.17 ± 31.16	23.28-73.48	41.79 ± 12.43	8.14-32.64	15.58 ± 6.35	2.97-9.45	5.54 ± 1.39	0.26-0.91	0.63 ± 0.16
11	55.64-292.7	126.9 ± 51.46	32.45-113.21	51.22 ± 16.55	11.12-50.85	19.29 ± 8.41	2.89-10.53	6.80 ± 1.70	0.21-0.91	0.64 ± 0.17
12	29.27-156.76	70.4 ± 27.68	27.48-64.41	41.40 ± 9.17	8.59-28.13	16.27 ± 4.82	1.48-8.02	4.49 ± 1.59	0.20-0.85	0.53 ± 0.16
13	26.84-172.24	93.55 ± 32.00	19.78-111.95	42.87 ± 13.88	7.39-53.19	15.57 ± 7.13	2.78-10.09	6.20 ± 1.53	0.15-0.87	0.67 ± 0.14
14	17.36-180.47	66.85 ± 35.46	20.84-73.95	38.54 ± 14.89	6.23-33.73	14.89 ± 7.42	2.08-7.76	4.61 ± 1.25	0.25-0.89	0.60 ± 0.18
15	23.37-185.3	73.07 ± 33.63	23.02-109.21	41.39 ± 15.41	7.16-50.97	16.16 ± 7.78	1.61-8.29	4.65 ± 1.33	0.20-0.87	0.57 ± 0.16
16	45.73-217.42	98.77 ± 42.17	27.79-74.54	42.99 ± 11.35	8.35-30.03	15.27 ± 5.42	2.90-9.84	6.46 ± 1.51	0.32-0.89	0.67 ± 0.12
17	30.02-193.58	91.49 ± 38.37	25.54-89.54	42.98 ± 13.85	7.87-39.92	15.82 ± 6.92	3.07-9.45	5.91 ± 1.58	0.25-0.83	0.64 ± 0.15
18	32.92-133.46	68.2 ± 24.54	25.11-64.00	37.81 ± 10.52	8.04-29.52	14.20 ± 5.33	2.28-6.84	4.93 ± 1.05	0.21-0.87	0.63 ± 0.15
19	29.03-131.93	62.84 ± 23.93	24.73-68.55	39.70 ± 10.82	8.22-31.92	15.68 ± 5.89	1.86-7.45	4.26 ± 1.50	0.20-0.86	0.54 ± 0.18
20	14.58-149	67.4 ± 31.69	18.14-83.82	38.71 ± 15.13	6.98-38.86	14.85 ± 7.54	2.09-8.07	4.62 ± 1.25	0.21-0.93	0.60 ± 0.16
Total	14.58-292.7	83.5 ± 37.78	17.37-113.21	41.59 ± 13.34	5.57-53.19	15.60 ± 6.61	1.48-12.23	5.46 ± 1.60	0.13-0.94	0.63 ± 0.94

Tab. 2. Morphometric characteristics of canine C cells in particular cases and overall for the species

Case No.	Cell area (μm²)		Cell perimeter (μm)		Cell length (μm)		Cell width (μm)		Cell circularity	
	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD
1	38.90-202.68	89.14 ± 36.22	23.40-80.43	36.99 ± 12.25	7.83-34.31	13.04 ± 5.57	4.86-9.66	6.70 ± 1.60	0.40-0.95	0.81 ± 0.12
2	41.14-177.23	89.95 ± 27.76	24.75-72.07	39.77 ± 8.28	8.59-30.16	14.04 ± 3.71	4.12-8.31	6.40 ± 1.08	0.43-0.87	0.72 ± 0.11
3	44.68-177.86	84.41 ± 27.87	26.33-56.61	37.46 ± 7.04	9.08-23.39	12.59 ± 2.88	4.88-11.5	6.68 ± 1.41	0.45-0.90	0.75 ± 0.09
4	32.93-105.89	63.53 ± 17.75	22.34-40.75	30.99 ± 4.49	6.79-14.37	10.74 ± 1.66	3.47-8.45	5.84 ± 0.97	0.61-0.95	0.82 ± 0.09
5	12.60-128.44	61.88 ± 25.28	12.86-45.36	30.40 ± 48.02	7.47-15.32	10.23 ± 2.60	2.76-10.97	5.79 ± 1.76	0.48-1.00	0.81 ± 0.10
6	27.93-227.38	112.36 ± 56.81	25.63-84.31	40.49 ± 17.09	7.96-35.80	14.17 ± 6.52	4.86-12.17	7.08 ± 2.79	0.40-1.00	0.78 ± 0.13
7	27.93-194.15	58.03 ± 28.84	19.34-53.85	28.81 ± 6.23	4.57-19.96	10.29 ± 2.31	3.43-9.72	5.43 ± 1.28	0.68-0.96	0.84 ± 0.06
8	52.60-217.94	112.58 ± 34.49	28.58-85.89	42.87 ± 9.38	8.74-37.06	14.54 ± 4.49	5.23-10.33	7.75 ± 1.30	0.37-0.93	0.77 ± 0.11
9	38.17-160.35	83.99 ± 25.05	24.66-59.77	38.05 ± 6.76	8.68-25.72	13.05 ± 3.12	2.80-10.15	6.53 ± 1.64	0.38-0.92	0.73 ± 0.13
10	35.02-225.99	91.78 ± 41.65	23.46-59.43	39.55 ± 8.65	8.15-21.46	13.64 ± 3.29	3.63-11.40	6.58 ± 1.95	0.45-0.88	0.71 ± 0.11
11	35.07-159.33	100.25 ± 29.73	22.72-52.98	38.83 ± 6.19	7.90-18.15	13.02 ± 2.38	4.20-9.66	7.55 ± 1.22	0.69-0.94	0.82 ± 0.07
12	34.32-200.67	90.72 ± 28.99	23.62-57.15	37.78 ± 5.74	6.65-18.22	12.85 ± 2.11	4.98-12.43	6.95 ± 1.32	0.63-0.93	0.78 ± 0.08
13	44.98-191.13	112.82 ± 39.64	26.49-58.04	42.70 ± 8.10	8.35-19.30	13.82 ± 3.09	4.69-11.29	8.02 ± 1.63	0.56-0.90	0.76 ± 0.07
14	32.26-125.92	73.35 ± 20.49	20.77-42.49	32.73 ± 4.75	7.00-15.37	11.32 ± 1.65	4.53-8.90	6.39 ± 1.04	0.67-0.94	0.85 ± 0.06
15	41.30-165.64	91.52 ± 28.57	23.91-49.94	36.87 ± 5.87	8.55-17.61	12.84 ± 2.23	4.81-9.40	7.00 ± 1.22	0.58-0.94	0.83 ± 0.07
16	39.79-162.69	82.59 ± 29.41	25.61-50.18	36.07 ± 6.40	8.88-17.86	12.10 ± 2.36	3.22-10.01	6.69 ± 1.35	0.52-0.92	0.78 ± 0.08
17	34.84-158.73	91.9 ± 35.21	25.58-58.77	40.07 ± 8.07	7.71-22.25	14.12 ± 3.43	2.89-10.81	6.43 ± 1.58	0.47-0.93	0.71 ± 0.12
18	36.81-158.55	84.15 ± 28.43	25.63-58.81	37.66 ± 8.22	7.05-23.98	13.19 ± 3.57	3.19-10.62	6.37 ± 1.44	0.45-0.91	0.75 ± 0.12
19	28.09-155.89	76.09 ± 24.53	21.64-49.06	34.89 ± 6.19	6.49-18.65	11.79 ± 2.69	4.33-8.66	6.40 ± 1.15	0.59-0.94	0.78 ± 0.10
20	44.48-159.43	87.11 ± 23.38	25.70-51.23	37.05 ± 5.07	8.05-16.68	12.37 ± 1.88	4.65-10.67	6.98 ± 1.26	0.59-0.93	0.79 ± 0.08
Total	12.60-227.38	86.92 ± 34.29	12.86-85.89	37.01 ± 8.72	4.57-37.06	12.67 ± 3.44	2.76-12.43	6.69 ± 1.59	0.40-1.00	0.78 ± 0.10

was the basis for assessing their function related to calcium homeostasis. The former compared the characteristics of C cells in healthy individuals and in those with calcium disorders (23). In the latter study, the level of calcitonin synthesis in C cells of European bison during spring-summer and autumn-winter periods was compared based on the intensity of immunohistochemical staining (32). Another study evaluated the influence of dulaglutide on monkey C cells. However, it only assessed total C cell volume rather than individual cells parameters (34). Thus, the results of the current study can serve as a basis for similar research in the future, especially related to the evaluation of calcitonin levels and calcium metabolism in various physiological and pathological states.

One of the most clinically significant applications of morphometry is in oncological research. Morphometric parameters have been used to distinguish between neoplastic and non-neoplastic lesions and to predict the behavior, malignancy, and disease outcome of cancer cells (21). It has been used widely in both human (6, 9) and veterinary medicine (25, 26).

The utility of morphometric analysis has also been confirmed in diagnosing and classifying human thyroid tumors (18, 22). Unfortunately, very few morphometric studies of human thyroid cancer cells also encompass single cases of C cell neoplasms (28), and there is a complete lack of such research on animals. This may be attributed to C cell-derived neoplasms being remarkably rarer than tumors originating from thyrocytes (2, 30).

Among C cell neoplasms, C cell adenomas have been described in cattle and horses (19, 31), while medullary carcinomas have been reported in many species of animals, both wild (11, 14) and domestic (4, 7, 20). They can be spontaneous or represent part

of multiple endocrine neoplasia syndromes (1, 7, 31). Thus, morphometric analysis can support the histopathological diagnosis of C cell tumors, mainly medullary carcinomas. Especially morphologically, some types of tumors originating from thyrocytes (e.g., follicular cell thyroid carcinoma of compact type) are very similar to medullary carcinomas (2). Moreover, more recent data have indicated that medullary carcinomas in dogs occur much more frequently than previously assumed (4). Therefore, C cell shape analysis can be an additional tool supporting routine tumor diagnosis, especially in cases with less obvious morphology. The results of the current study can be useful for this purpose.

In conclusion, this study is the first to present morphometric characteristics of C cells in four domestic animal species: cattle, dogs, horses, and pigs. It was shown that the most significant variation in the parameters examined characterizes porcine and canine C cells. Despite their morphological diversity, equine and bovine C cells are morphometrically similar. The results obtained in this study can be used as reference values in further studies on the behavior of C cells under various experimental and environmental factors as well as pathological conditions. They can serve as an additional tool for diagnosing neoplastic lesions and can be used in comparative histological research. Further morphometrical studies on a larger number of individuals are needed to provide more precise reference values. Geometric morphometric analysis of C cells will make it possible to characterize their size and shapes very precisely. Morphometric three-dimensional measurements will finally dispel doubts about C cell morphology in individual animal species described on the basis of traditional microscopic examination of tissue sections.

Tab. 3. Morphometric characteristics of equine C cells in particular cases and overall for the species

Case No.	Cell area (μm²)		Cell perimeter (μm)		Cell length (μm)		Cell width (μm)		Cell circularity	
	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD
1	19.72-121.94	68.21 ± 19.51	18.24-67.16	38.99 ± 8.48	5.60-31.32	14.81 ± 4.55	2.26-8.79	4.77 ± 1.35	0.20-0.83	0.58 ± 0.14
2	28.51-158.47	66.63 ± 28.28	21.29-67.11	35.44 ± 9.65	7.26-28.80	12.85 ± 4.46	2.53-7.80	5.18 ± 1.22	0.38-0.96	0.67 ± 0.15
3	38.92-118.56	74.13 ± 20.41	25.21-66.05	39.02 ± 7.70	8.38-30.11	14.32 ± 4.11	2.52-8.04	5.36 ± 1.40	0.25-0.91	0.63 ± 0.15
4	41.91-127.66	74.42 ± 19.41	25.75-54.50	38.51 ± 6.74	9.03-22.46	14.08 ± 3.36	3.34-7.51	5.37 ± 1.08	0.37-0.87	0.64 ± 0.12
5	50.72-122.88	81.24 ± 19.83	29.84-63.58	41.30 ± 7.77	7.67-27.96	15.28 ± 4.36	2.98-8.31	5.54 ± 1.42	0.30-0.85	0.62 ± 0.14
6	34.41-138.00	72.95 ± 21.07	25.68-54.43	39.30 ± 7.06	8.14-24.05	14.72 ± 3.85	3.06-8.95	5.11 ± 1.47	0.32-0.87	0.61 ± 0.14
7	33.27-127.46	79.44 ± 25.67	22.77-48.14	37.04 ± 6.69	7.26-17.64	12.87 ± 2.62	3.72-9.63	6.12 ± 1.36	0.52-0.95	0.72 ± 0.10
8	61.84-194.90	115.18 ± 33.39	35.88-70.59	49.69 ± 8.98	9.95-30.65	18.59 ± 4.72	3.98-10.39	6.35 ± 1.68	0.36-0.84	0.59 ± 0.12
9	32.29-151.06	67.77 ± 25.20	24.04-67.98	38.86 ± 8.85	7.60-29.46	14.77 ± 4.58	1.82-9.55	4.74 ± 1.53	0.27-0.92	0.58 ± 0.15
10	28.84-200.25	99.16 ± 38.78	23.14-62.99	45.78 ± 10.12	7.94-27.36	17.03 ± 5.09	2.31-11.29	5.91 ± 1.86	0.26-0.85	0.60 ± 0.13
11	32.19-147.99	75.32 ± 27.96	24.75-62.80	39.67 ± 8.93	7.02-25.62	15.06 ± 4.19	2.36-8.10	5.03 ± 1.35	0.35-0.87	0.61 ± 0.15
12	22.90-196.41	101.33 ± 40.86	19.61-72.70	43.89 ± 12.86	5.97-31.63	15.81 ± 6.18	3.80-9.77	6.46 ± 1.58	0.35-0.88	0.67 ± 0.14
13	53.63-302.14	138.24 ± 56.60	31.40-81.28	53.56 ± 11.31	9.26-32.64	20.05 ± 5.40	3.86-12.91	6.93 ± 2.18	0.35-0.87	0.60 ± 0.13
14	52.50-227.26	113.06 ± 39.12	27.92-70.15	46.36 ± 8.78	9.18-26.50	16.33 ± 4.20	4.14-12.1	7.00 ± 1.77	0.48-0.90	0.66 ± 0.11
15	30.23-182.90	93.94 ± 35.68	20.37-63.95	41.49 ± 9.76	7.51-26.36	14.53 ± 4.46	2.83-12.67	6.54 ± 1.95	0.39-0.92	0.69 ± 0.14
16	41.41-236.74	104.39 ± 39.31	24.51-80.34	43.69 ± 10.74	8.08-33.00	15.34 ± 5.01	4.32-10.89	6.83 ± 1.54	0.42-0.88	0.69 ± 0.12
17	39.60-119.13	76.3 ± 23.24	24.26-53.11	38.31 ± 7.78	9.27-23.46	13.99 ± 3.69	3.03-9.06	5.54 ± 1.48	0.32-0.90	0.67 ± 0.15
18	49.39-199.71	104.77 ± 42.42	28.85-64.09	42.22 ± 12.62	8.83-23.58	14.82 ± 4.75	4.99-8.97	6.67 ± 1.94	0.57-0.89	0.71 ± 0.10
19	48.00-143.29	82.64 ± 26.39	27.54-50.99	38.42 ± 6.82	9.85-22.07	13.65 ± 2.99	3.43-9.08	6.14 ± 1.73	0.37-0.96	0.71 ± 0.15
20	40.40-118.42	82.65 ± 24.35	25.77-65.85	40.64 ± 9.61	9.45-28.82	15.03 ± 4.99	3.88-7.76	5.64 ± 1.27	0.34-0.86	0.65 ± 0.15
Total	19.72-302.14	88.83 ± 37.24	18.24-81.28	41.81 ± 10.15	5.60-33.00	15.29 ± 4.76	1.82-12.91	5.85 ± 1.72	0.25-0.96	0.64 ± 0.14

Tab. 4. Morphometric characteristics of porcine C cells in particular cases and overall for the species

Case No.	Cell area (μm²)		Cell perimeter (μm)		Cell length (μm)		Cell width (μm)		Cell circularity	
	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD	Range of values	Mean ± SD
1	18.85-169.37	58.27 ± 36.35	19.18-120.80	39.53 ± 20.14	6.00-57.45	16.14 ± 10.04	1.94-8.50	3.70 ± 1.48	0.15-0.96	0.51 ± 0.18
2	23.27-137.80	67.08 ± 26.67	20.22-74.82	44.00 ± 12.18	6.57-33.27	18.36 ± 6.03	1.86-6.09	3.68 ± 0.97	0.20-0.81	0.45 ± 0.13
3	27.20-267.20	79.14 ± 46.89	23.91-128.85	45.33 ± 19.80	8.12-59.97	18.33 ± 9.86	2.39-7.70	4.33 ± 1.16	0.20-0.77	0.51 ± 0.14
4	10.98-181.34	51.50 ± 33.44	13.42-92.79	37.78 ± 18.33	3.88-42.08	15.63 ± 9.08	1.89-5.05	3.26 ± 0.72	0.19-0.77	0.49 ± 0.16
5	10.66-107.71	55.81 ± 24.97	13.54-70.82	40.93 ± 13.79	4.28-32.05	17.13 ± 7.00	1.91-6.88	3.33 ± 1.13	0.25-0.77	0.45 ± 0.16
6	20.84-170.06	58.41 ± 38.55	18.51-95.56	38.66 ± 19.19	6.22-43.91	15.62 ± 9.58	2.04-6.80	3.81 ± 1.01	0.22-0.93	0.54 ± 0.19
7	14.61-131.65	48.24 ± 28.08	17.44-79.87	35.61 ± 13.88	5.19-36.31	14.69 ± 6.74	1.46-5.51	3.29 ± 1.01	0.17-0.84	0.51 ± 0.18
8	22.46-115.18	59.94 ± 22.96	17.99-64.34	36.35 ± 10.50	7.16-28.53	14.03 ± 5.07	2.65-7.21	4.31 ± 0.98	0.32-0.90	0.59 ± 0.15
9	32.20-230.20	77.54 ± 47.03	22.64-121.48	40.86 ± 20.23	7.64-57.08	15.70 ± 10.06	2.50-7.92	5.09 ± 1.38	0.18-0.90	0.64 ± 0.19
10	14.00-98.94	47.09 ± 17.94	15.94-60.39	31.81 ± 10.02	5.35-26.46	11.97 ± 5.17	2.61-5.99	4.07 ± 0.91	0.30-0.90	0.61 ± 0.15
11	19.63-214.46	92.52 ± 48.68	19.70-104.02	54.60 ± 19.47	7.07-47.73	23.43 ± 9.48	2.24-6.36	3.86 ± 0.96	0.21-0.64	0.40 ± 0.12
12	27.77-200.12	88.24 ± 40.95	25.02-89.33	54.77 ± 16.09	9.62-41.21	23.70 ± 7.87	2.04-6.40	3.69 ± 1.07	0.21-0.62	0.38 ± 0.11
13	42.71-325.72	105.20 ± 54.02	30.80-127.56	59.48 ± 19.15	11.77-58.18	25.70 ± 9.36	2.55-8.00	4.04 ± 1.11	0.19-0.59	0.38 ± 0.10
14	39.74-213.64	108.24 ± 40.88	28.22-92.82	57.97 ± 15.00	9.13-41.25	24.54 ± 7.52	2.75-6.98	4.44 ± 1.10	0.23-0.72	0.42 ± 0.12
15	19.15-189.81	75.89 ± 40.36	21.58-101.63	49.33 ± 19.03	7.86-47.21	21.09 ± 9.42	1.97-5.23	3.57 ± 0.82	0.18-0.72	0.42 ± 0.13
16	27.85-307.58	100.24 ± 61.03	25.13-113.35	59.54 ± 23.82	9.69-50.60	26.00 ± 11.66	2.16-7.52	3.77 ± 1.14	0.16-0.65	0.37 ± 0.13
17	29.74-179.07	80.92 ± 33.56	25.63-84.06	52.27 ± 15.88	8.90-37.66	22.54 ± 7.9	2.26-5.75	3.59 ± 0.82	0.20-0.67	0.39 ± 0.12
18	19.69-149.95	68.96 ± 34.02	22.75-101.40	48.43 ± 18.41	9.25-48.54	20.93 ± 9.14	1.88-5.02	3.28 ± 0.84	0.13-0.63	0.39 ± 0.11
19	10.16-222.02	72.59 ± 49.42	18.65-122.78	50.96 ± 23.24	7.24-57.53	22.31 ± 11.41	1.26-5.84	3.17 ± 1.11	0.15-0.71	0.37 ± 0.16
20	21.63-180.19	75.93 ± 40.69	23.01-100.29	49.78 ± 18.45	8.13-46.29	21.37 ± 9.09	1.61-6.09	3.52 ± 1.08	0.18-0.77	0.40 ± 0.13
Total	10.16-325.72	73.70 ± 43.12	13.42-128.85	46.56 ± 19.35	3.88-59.97	19.55 ± 9.57	1.26-8.50	3.78 ± 1.14	0.13-0.96	0.46 ± 0.16

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