Research Article

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A study on paranal sinus micromorphometrical parameters in dogs of different ages

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Abstract: This study determines the micromorphometric parameters of the main structural elements of the paranal sinus in male (n = 20) and female (n = 8) mongrel dogs of different ages. Pieces of about 1 cm3 were obtained from different parts of the organ and put in 10% neutral formalin. The tissue samples were further used for the preparation of paraffin sections that were stained with hematoxylin-eosin for light microscopy measurements. In the sinus wall, the thickness of the epithelium was measured, as was that of the subepithelial and subglandular connective tissue layers, the zona with the apocrine glands, and the entire sinus wall. The outer and inner diameters of the apocrine tubular glands and the height and number of glandular epithelial cells in the apocrine glands were also measured. Statistical data processing was done using Data Analysis tool and t-test by means of the StatMost for Windows software. For the period from the 1st month to the 2nd year, the thickness of the epithelial layer of the sinus wall remained almost identical (from 40.83 ± 9.37 µm during the 1st month to 47.79 ± 3.67 µm in the 2nd year). After the 2nd year, the thickness of the epithelial layer decreased. In the 8th year its thickness reached 35.72 ± 5.89 µm and in the 12th year it was 28.92 ± 6.53 µm. We measured the thickness of the subepithelial and subglandular connective tissue layers of the wall of the sinus, forming the organ's stroma. It was found that the thickness of these layers increased 3-fold for the period from the 1st month to the 12th year. Their thickness increased most intensively until the 5th month, followed by the period from the 5th month to the 2nd year, then from the 2nd to the 8th year, and changed the least up to the 12th year. Therefore, the increase in the thickness of the sinus wall (without its excretory duct) with age was due to the increase of the thickness of the subepithelial and subglandular connective tissue layers in particular. The results of the present study demonstrate that the period featuring the most active weight increase and growth of sinus macromorphometric parameters coincided with the period of increase in the number of cells and the active growth of the main structural elements of the organ.

Key words: Anatomy, morphometry, paranal sinus, dog

Introduction

The microstructure of the paranal sinus (Sinus paranalis, SP) in the dog was first studied in detail using light microscopy by Coquot et al. (1). Later, microscopic studies of the organ were completed by several other authors (2,3). A brief description of microstructure of the dog's paranal sinuses has been given by Getty (4). None of these authors have carried out micromorphometric studies of the paranal sinus in dogs, however. Detailed information about the paranal sinus of healthy dogs and the pathogenesis, diagnosis, and therapy of sinus impaction and sacculitis has been provided by Duijkeren (5).

A detailed study on the micromorphometric parameters and structural and histochemical features of the SP in cats was conducted by Greer and

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Colhoun (6). The lack of information in the literature on the micromorphometric parameters of the main structural elements of the paranal sinus in dogs of different ages, however, gave us reason to perform this investigation.

The aim of this study was to determine for the first time the micromorphometric parameters of the main structural elements of the paranal sinus in dogs of different ages.

Materials and methods

The study was carried out on material obtained from 28 healthy, immature male (n = 8) and female (n = 8) mongrel dogs aged 1 and 5 months, and 12 adult males aged 2 (n = 4), 8 (n = 4), and 12 (n = 4) years. They were euthanized with 5% thiopental solution (Biochemie, Austria) i.v., in accordance with Bulgarian laws.

Immediately after death, pieces of about 1 cm^3 were obtained from different parts of the organ and put in 10% neutral formalin. The tissue samples were further used for the preparation of paraffin sections (5-7 µm) that were stained with hematoxylin-eosin for light microscopy measurements. For this, 5 serial sections were used from each animal and 10 measurements were done on each section for each parameter.

In the sinus wall, the thickness of the epithelium, subepithelial and subglandular connective tissue layers, the zona with the apocrine glands, and the entire sinus wall were measured, as were the outer and inner diameters of the apocrine tubular glands and the height and number of the glandular epithelial cells in the apocrine glands. The outer diameter of the apocrine tubular glands was measured from basal membrane to basal membrane. Their inner diameter was measured as the distance between the luminal surfaces of the secretory cells' apical parts. The apocrine glands were counted per 1 mm^2. Data for number/1 mm^2 are given as mean ± SD.

For the purposes of this study, a light microscope (ZEISS Primo Star, Germany), camera (ProgRes, Capture 2.6, JENOPTIK), and software analysis program (Soft Imaging System GmbH) were used. Statistical data processing was done using Data Analysis tools and t-tests by means of the StatMost for Windows software.

Results

In the present study, micromorphometrical parameters were determined for the structural elements of the paranal sinus wall in dogs of different age groups (Tables 1 and 2).

The thickness of the sinus wall increased 3.5-fold from the 1st month to the 12th year, from 461.35 ± 159.58 µm in the 1st month to 1598.96 ± 462.43 µm in the 12th year. By 5 months of age the wall thickness had increased by 194.1 µm and during the second year this increase was observed at 502 µm; at 8 years the figure for increase was determined at 409.5 µm and at 12 years it remained almost unchanged, at 30 µm. Statistically significant difference ($P < 0.01$) in the values was found between animals aged 1 and 5 months. Statistically significant difference in measured values was also found between the 5th month and the 2nd year ($P < 0.001$) and between 2- and 8-year-old dogs ($P < 0.001$); no difference was determined in the values between 8- and 12-year-old dogs. The wall thickness increased most intensively until the 5th month, followed by the 2nd year and the period from the 2nd to the 8th year while the thickness was unchanged throughout the period from the 8th to the 12th year (Figures 1 and 2). Statistically significant difference between the sexes of sexually immature animals was not established.

For the period from the 1st month to the 2nd year of age, the thickness of the epithelial layer of the SP wall was almost unchanged (from 40.83 ± 9.37 µm during the 1st month to 47.79 ± 3.67 µm in the 2nd year) and demonstrated no statistically significant difference. By the 5th month the epithelial layer thickness had increased by 4.18 µm, and by the 2nd year it had increased by 2.78 µm. After the 2nd year the thickness of epithelial layer decreased. By the 8th year of age the thickness had decreased by 12.07 µm and by the 12th year it had further decreased by 6.8 µm. A statistically significant difference ($P < 0.001$) in the values of the measured parameter was observed between animals aged 2 and 8 years and between 8- and 12-year-old dogs. There was no statistically significant difference between sexes for sexually immature animals.

For the period from the 1st month to the 12th year of age, the thickness of the subepithelial connective tissue layer (SES) of the sinus wall increased 3-fold,

Table 1. Micromorphometric parameters (in μm) of the layers constituting the wall of the paranal sinus.

Age	Height of the SSCE without the stratum corneum	Thickness of the SES	Thickness of the SGS	Thickness of the zone with the apocrine glands	Thickness of the wall of the SP
1 month					
♂	40.83 ± 9.37	135.3 ± 103.1	150.89 ± 143.79	239.7 ± 110.1	461.35 ± 159.58
Limits of variation	28.29-59.2	21.2-358.2	32.3-450.4	110.5-410.0	210.3-788.3
♀	42.62 ± 9.042	135.5 ± 103.0	152.25 ± 138.15	238.9 ± 110.3	462.58 ± 155.88
Limits of variation	27.9-59.2	21.2-367.9	31.1-451.1	110.7-412.3	214.6 ± 803.1
5 months		***	*	***	**
♂	45.01 ± 12.35	216.2 ± 94.7	247.85 ± 161.33	357.2 ± 70.5	655.46 ± 214.29
Limits of variation	28.2 ± 66.45	12.4-401.3	25.9-492.8	147.4-468.0	385.0-1023.0
♀	45.09 ± 11.12	217.2 ± 95.5	242.95 ± 161.51	356.5 ± 70.0	660.40 ± 219.11
Limits of variation	29.3-66.3	12.4-401.4	24.6-489.3	147.1-468.3	364.2-1024.0
2 years		**	*	***	***
♂	47.79 ± 3.67	324.6 ± 118	329.41 ± 145.11	514.11 ± 163.1	1157.49 ± 182.39
Limits of variation	39.5-53.9	20.1-523.4	25.54-499.7	243.2-997.0	927.2-1416.0
8 years	***	*	*	**	***
♂	35.72 ± 5.89	399.28 ± 123.8	435.82 ± 199.17	613.03 ± 114.99	1566.95 ± 171.74
Limits of variation	29.0-50.4	22.9-602.2	49.37-623.8	275.0-850.1	1181.0 -1792.0
12 years	***			*	
♂	28.92 ± 6.53	421.75 ± 187.06	473.22 ± 188.55	673.33 ± 144.24	1598.96 ± 462.43
Limits of variation	18.24-43.3	25.2-630.0	32.6-678.5	262.0-809.1	693.0-1998.0

SSCE - stratified squamous cornified epithelium; SES - subepithelial connective tissue (connective tissue layer between the stratified squamous cornified epithelium and the apocrine glands);

SGS - subglandular connective tissue (connective tissue layer between the apocrine glands and the muscles); ♂- males; ♀- females.

Asterisks indicate statistically significant difference against the previous age group of dogs (Student t-test):
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 2. Some micromorphometric parameters (in μm) of the apocrine glands and their structural elements.

Age	Outer diameter of AG	Diameter of the anal gland's lumen	Number of AG/mm^2	Number of SC in 1 AG	Height of SC
1 month					
♂	77.93 ± 14.49	55.03 ± 17.49	27.56 ± 3.35	32.75 ± 5.91	11.43 ± 1.24
Limits of variation	54.3-99.7	41.8-92.9	21-36	23.0-44.0	10.09-14.60
♀	77.83 ± 14.33	54.39± 17.85	27.95 ± 2.61	30.37 ± 7.17	11.45 ± 1.18
Limits of variation	54.3-99.7	42.87-91.35	22-36	20.0-45.0	10.09-14.62
5 months	**	**	*	***	***
♂	94.8 ± 26.4	70.89 ± 20.16	19.03 ± 1.14	8.0 ± 10.06	12.55 ± 0.76
Limits of variation	70.3-190.6	53.5-108.7	17-21	31.0-63.0	11.1-14.0
♀	95.33 ± 25.36	69.47 ± 2041	19.1 ± 1.34	49.13 ± 9.83	12.53 ± 0.78
Limits of variation	70.2-190.5	50.3-105.9	17-22	32.0-66.0	11.1-13.9
2 years	***	***	***	**	**
♂	115.65 ± 21.94	89.84 ± 47.58	12.27 ± 2.14	57.7 ± 947	13.17 ± 0.92
Limits of variation	83.2-201.5	58.21-178.3	9-17	42.0-71.0	11.6-15.1
8 years	***	*	*	**	***
♂	184.03 ± 82.29	138.37 ± 50.30	9.68 ± 1.02	74.07 ± 23.18	11.2 ± 1.56
Limits of variation	86.82-425.8	59.18-378.6	8-12	31.0-103.0	8.9-13.2
12 years	***	***	***		***
♂	272.7 ± 97.5	189.66 ± 56.62	6.58 ± 1.24	76.67 ± 19.59	9.05 ± 0.85
Limits of variation	117.9-501	94.8-482.1	5-9	39.0-102.0	7.6-10.8

AG - apocrine glands; SC - secretory cells; ♂- males; ♀- females.

Asterisks indicate statistically significant difference against the previous age group of dogs (Student t-test):
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

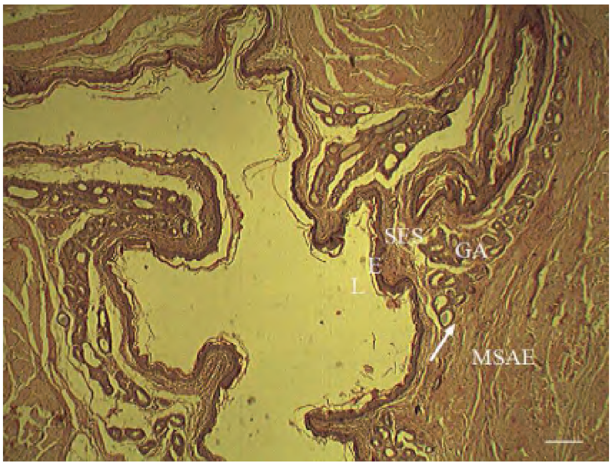

Figure 1. A cross section of the paranal sinus wall of a dog aged 1 month.

L - lumen of the sinus parnalis; E - epithelial layer of the sinus wall; SES - subepithelial connective tissue layer; Arrow - subglandular connective tissue layer; GA - glandulae apocrinae; MSAE - musculus sphincter ani externus. Hematoxylin-eosin staining. Bar = 80 μm.

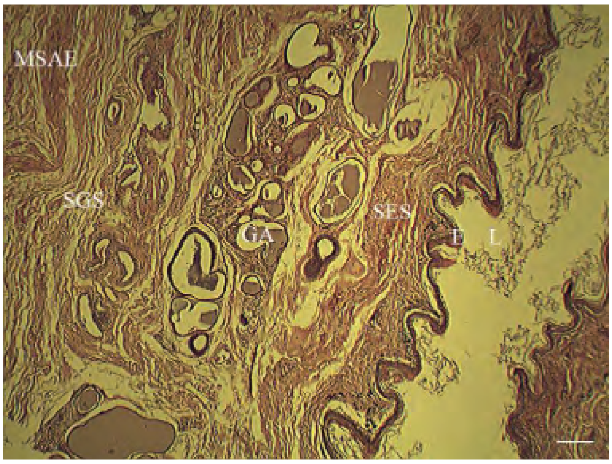

Figure 2. A cross section of the paranal sinus wall of a dog aged 12 years.

L - lumen of the sinus parnalis; E - epithelial layer of the sinus wall; SES - subepithelial connective tissue layer; SGS - subglandular connective tissue layer; GA - glandulae apocrinae; MSAE - musculus sphincter ani externus. Hematoxylin-eosin staining. Bar = 80 μm.

from 135.3 ± 103.1 μm for the 1st month to 421.75 ± 187.06 μm for the 12th year. By the 5th month of age the thickness of this layer had increased by 80.9 μm, at the age of 2 years the measure had increased by 108.4 μm, at 8 years the increase was determined at 74.6 μm, and at 12 years of age the thickness of this layer had further increased by 22.5 μm. Statistically significant difference ($P < 0.001$) in the values of the measurements was observed between animals at the age of 1 and 5 months and between 5-month- and 2-year-old dogs ($P < 0.001$). In animals aged 2 and 8 years, the statistically significant difference was at $P < 0.05$; between those aged 8 and 12 years, there was no significant difference. The thickness of the layer increased most intensively until the 5th month, followed by the period from the 5th month to the 2nd year and from the 2nd to the 8th year; the value changed the least from this point up to the 12th year. Statistically significant difference was not established between sexes in sexually immature animals.

For the period from the 1st month to the 12th year, the thickness of the subglandular connective tissue layer (SGS) of the sinus wall increased 3-fold, from 150.89 ± 143.79 μm in the 1st month to 473.22 ± 188.55 μm in the 12th year. At 5 months the thickness of this layer had increased by 96.9 μm, at 2 years increase was determined at 81.6 μm, at 8 years the figure had increased by 106.42 μm, and at 12 years by 38 μm. Statistically significant differences ($P < 0.05$) were found between animals aged 1 and 5 months, between the 5th month and the 2nd year, and between 2- and 8-year-old dogs. Statistically significant difference also existed between the values for 8- and 12-year-old dogs. The most intensive increase in the thickness of the layer was observed by the 5th month, followed by the period from 5 months to 2 years, the period from the 2nd to the 8th year, and finally by the period ending in the 12th year. Statistically significant difference between sexes of sexually immature animals was not established.

From the 1st month to the12th year, the thickness of the zona with the apocrine glands (AG) increased 2.8-fold, from 239.7 ± 110.1 μm in the 1st month to 673.33 ± 144.24 μm in the 12th year. By 5 months of age the thickness of this zone had grown by 117.5 μm, in the 2nd year the increase was observed at 156.9 μm, in the 8th year it had increased by 98.9 μm, and in the 12th year by 60.3 μm. Statistically significant difference ($P < 0.001$) in the measured values was found between animals aged 1 and 5 months, between the 5th month and the 2nd year, and between 2- and 8-year-old dogs ($P < 0.01$). A slight statistically significant difference (P < 0.05) was found between 8- and 12-year-old dogs. The thickness of the glandular zone increased most intensively by the 5th month followed by the 2nd and the 8th year and, least intensively, during the 12th year. Statistically significant difference was not established between sexes in sexually immature dogs.

The outer diameter of the apocrine glands increased 3.2-fold from the 1st month to 12th year, from 77.93 ± 14.49 μm in the 1st month to 272.7 ± 97.5 μm in the 12th year. By the 5th month the diameter had increased by 16.9 μm, at 2 years it had increased by 20.3 μm, by the 8th year the increase was 68.5 μm, at 12 years it was determined at 71.3 μm. Statistically significant difference in the values of the measured parameter was established with age as follows: animals aged between 1 and 5 months, P < 0.01; between 5 months and 2 years, P < 0.001; between 2 and 8 years, P < 0.001; and between 8 and 12 years, P < 0.001. The outer diameter increased most intensively up to the 5th month, followed by a period from the 8th to the 12th year, then between the 5th month and the 2nd year; the slightest change occurred during the period from the 2nd to the 8th year. Statistically significant difference between sexes of sexually immature dogs was not established.

The diameter of the apocrine glands' lumen increased proportionally to the outer diameter of the glands. From the 1st month to the 12th year, the gland lumen diameter increased by 3.4-fold, from 55.03 ± 17.49 μm in the 1st month to 189.66 ± 56.62 μm in the 12th year. At the 5th month the diameter had increased by 15.9 μm, at 2 years by 20.4 μm, at 8 years by 48.5 μm, at 12 years by 51.29 μm. Statistically significant difference in the values of measurements was established with age as follows: animals aged between 1 and 5 months, P < 0.01; between 5 months and 2 years, P < 0.001; between 2 and 8 years, P < 0.001; and between 8 and 12 years, P < 0.001. The diameter of the gland lumen increased most intensively by the 5th month, followed by the period from the 8th to the 2nd year, then between 5 months and 2 years of age, and only slight change was observed between the 2nd and the 8th year. Statistically significant difference could not be established between the sexes in our examination of sexually immature animals.

The number of apocrine glands per 1 mm^2 decreased 4.2-fold from the 1st month to the 12th year (n = 27.56 ± 3.35 in the 1st month, n = 6.58 ± 1.24 in the 12th year). By 5 months of age the number of glands had decreased by 8.5, at 2 years the decrease was 6.8, at 8 years the figure for decrease was found to be 2.6, and at 12 years the number had decreased by 3.1. Statistically significant difference in the values of the measured parameter was established with age as follows: animals aged between 1 and 5 months, P < 0.001; between 5 months and 2 years, P < 0.001; between 2 and 8 years, P < 0.001; between 8 and 12 years, P < 0.001. The most intensive reduction of the glands was found at 5 months, followed by the period from the 5th month to the 2nd year, the period between the 8th and the 12th years, and between the 2nd and the 8th years. Statistically significant difference between sexes of sexually immature dogs was not established.

The number of the secretory cells in the apocrine glands increased 2.4-fold from the 1st month to the 12th year, from n = 32.75 ± 5.91 in the 1st month to n = 76.67 ± 19.59 in the 12th year. At 5 months the number of secretory cells had increased by 15.3, at 2 years of age it had increased by 9.7, at 8 years by 16.3, and at 12 years by 2.7. Statistically significant difference in the measured values was established with age as follows: animals aged between 1 and 5 months, P < 0.001; between 5 months and 2 years, P < 0.01; between 2 and 8 years, P < 0.01; between 8 and 12 years age, a statistically significant difference was not determined. The most intensive increase in the number of the secretory cells was found in the 5th month, followed by the period from the 5th month to the 2nd year, then the period from the 2nd to the 8th year, and the least change was observed from the 8th to the 12th year. Statistically significant difference between sexes of sexually immature animals was not established.

From the 1st month to the 2nd year the height of the secretory cells in the AG increased slightly (1.2 times), from 11.43 ± 1.24 μm during the 1st month to 13.17 ± 0.92 μm in the 2nd year. By 5 months of age the height of secretory cells had grown by 1.12 μm and at 2 years growth had further increased by 0.6 μm. After the 2nd year of age, the height of the cells decreased and at 8 years it reached a value of 11.2 ± 1.56 μm, similar to that at observed at 1 month of age. During the period from the 8th to the 12th year, the height of the secretory cells decreased by 2.15 μm. Statistically significant difference in the measured values was established by years as follows: animals aged between 1 and 5 months, $P < 0.001$; between 5 months and 2 years, $P < 0.01$; between 2 and 8 years, $P < 0.001$; and between 8 and 12 years, $P < 0.001$. The height of the secretory cells increased most intensively until the 5th month, followed by the period from the 5th month to the 2nd year. Then, during the period from the 2nd to the 8th year, the height of the cells decreased by 1.97 μm and between the 8th and the 12th year the height decreased even further, by 2.15 μm. Statistically significant difference was not established between sexes in the sexually immature dogs examined.

Discussion

The results of our research on the structural features of the stratified squamous cornified epithelium of the paranal sinus using light microscopy correlate with studies on SP by several authors performed not only on dogs (7) but also on cats (6). As in the reports by these authors, we also found that the basal layer contained cubic to columnar epithelial cells located on the basal membrane. The outer layers were represented by improperly ordered polygonal cells with larger nuclei compared to the basal layer. The last layer contained flat epithelial cells, forming a thick layer of keratin. Unlike other authors, however, we conducted the first morphometric study to determine the thickness of the epithelial layer (without the stratum corneum) of the sinuses in dogs of different ages. For example, for the period from the 1st month to the 2nd year, the thickness of the epithelial layer of the sinus wall remained almost identical (from 40.83 ± 9.37 μm during the 1st month to 47.79 ± 3.67 μm in the 2nd year). After the 2nd year, the thickness of the epithelial layer decreased. In the 8th year, its thickness reached 35.72 ± 5.89 μm, and by the 12th year it was measured at 28.92 ± 6.53 μm.

A similar morphometric study was undertaken by Greer and Colhoun (6) on the sinus of the cat. They found that the height of the stratified squamous cornified epithelium in the lowest part was 11 μm. In our study the smallest height of the epithelium varied depending on the age of the animals—from the 1st month to the 2nd year this height varied from 28.29-59.2 μm to 39.5-53.9 μm while in 12-year-old dogs it ranged between 18.24 and 43.3 μm. These results indicate that the sinus epithelium in the dog is significantly taller (by 2-3 times) than that observed in the cat.

For the first time, we measured the thickness of the subepithelial and subglandular connective tissue layers of the wall of the sinus that form the organ's stroma. It was established that the thickness of these layers increased 3-fold for the period from the 1st month to the 12th year. Their thickness increased most intensively to the 5th month, followed by the 2nd year, then the period from the 8th to the 12th year, and it changed the least between the 2nd and 8th year. Therefore, the increase in the thickness of the sinus with age was due to the increase in the thickness of the subepithelial and subglandular connective tissue layers in particular. The results of this study showed that the thickness of the subglandular connective tissue layer (from 24.6 to 678.5 μm) in the wall of the sinus in the dog is significantly greater than that of the same layer in the sinus of the cat (from 12 to 174 μm) (6). It is therefore our opinion that the thickness of the subglandular layer that separates the zone of the apocrine glands from the external anal sphincter is extremely important in paranal sinus surgery. Some authors have reported that during careless sinus extirpation part of the external anal sphincter muscle can be removed together with the sinus, leading to disruption of the muscle function (8,9). On the other hand, unexcised fragments of the sinus wall are a frequent cause of surgical failure and may result in severe chronic infection with multiple fistulous tracts (3).

The results of the present study demonstrate that the period of the most active weight increase and growth of sinus macromorphometric parameters coincided with the period of increase in the number of cells and the active growth of the main structural elements of the organ. The increase in the outer diameter of the apocrine gland was directly related to the increase in the diameter of the gland lumen. There was a correlation between the increase in area and in diameter of the apocrine glands on one side and the increase in the thickness of the gland zone with age. The number of glands gradually decreased over the period of the study.

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