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VOLATOLOMIC ANALYSIS APPLIED TO FARM ANIMALS

I. VOLATILE COMPOUNDS IN RESPIRATION OF CATTLE

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Abstract

Volatolomics opens new possibilities for the study of biological systems. Volatility distribution of highly- and semi-volatile organic compounds emitted through respiration by cattle was studied. Samples were collected from farm animals in three regions of Romania, namely Râmnicu Vâlcea, Bistrița Năsăud and Constanța. Special procedures for sample collection, storage, transportation and analysis were developed. The volatile compounds were distributed mainly in the boiling point range of n-C₇ – n-C₁₄ normal paraffins (69-254°C), with higher concentration of about 60-70% around n-C₁₀ and n-C₁₂ (151-174°C and 196-216°C, respectively). Some differences were observed among samples collected from different geographical regions.

Keywords: *volatolomics, cattle, respiration*

Introduction

Breath samples are characterized by the presence of exogenous and endogenous compounds, as well as microbiome associated metabolites. The study of metabolites (metabolomics) produced by organisms can provide information on biological functions from the scale of organisms up to the scale of ecosystems (Goulitquer et al., 2012) and can indicate the presence and the metabolic activity of organisms in environment, e.g. pathogens (Lee et al., 2012). The exhaled breath has a large chemical diversity, with around 1800 identified volatile organic compounds (VOCs), besides the aerosols or exhaled breath condensate, consisting of water-soluble volatiles and nonvolatile compounds. The VOCs in exhaled breath are related to food consumption, medication, exposure to contaminants, etc., or are produced by the microbiome (Amann et al., 2014(a); Amann et al., 2014(b); Lawal et al., 2017; Berg et al., 2011). The study of volatile metabolites (volatolomics) in

exhaled air is regarded as a very important tool in clinical applications, for evaluation of health status and for diagnostic and prognostic of pathophysiological conditions, especially those with respiratory or gastrointestinal origin (Kuban & Foret, 2013; Priego-Capote & Luque de Castro, 2019).

The application of breath sample analysis could be extended to farm animals as non-invasive, rapid and cost-effective tool for monitoring their health status. The present paper focused on the respiration of cows, as a first part of a study on the volatility distribution of the VOCs emitted by farm animals from different locations in Romania. The next two papers will consider the VOCs from faeces and those released through the skin of the cows.

Materials and methods

10 healthy cows from three cattle farms across Romania, namely 2 from Bistrița Năsăud, 4 from Râmnicu Vâlcea and 4 from Constanța counties were selected. A special designed mask was placed on the head of the animal. The mask was connected through Tygon tube to a small, portable and settable vacuum pump that forced the gases to pass through a biological PTFE filter then through a Tenax tube that captures the VOCs – Figure 1. The Tenax tubes contain a porous polymer sorbent that is able to absorb compounds with medium to high boiling points, has a low background and is hydrophobic, so that it does not absorb humidity, which is very high in exhaled breath air of animals.



Figure 1. *Sampling of VOCs from respiration of farm cows*

The Tenax tubes were stored and transported at low temperature of 4°C. The volatile organic compounds trapped in the tubes were thermally desorbed in a laboratory-designed system. This consists of a small electric furnace connected to a temperature controller, which allowed precise programming of the heating rate and

of the final temperature. The VOCs in breath have low concentration; therefore, pre-concentration techniques such as solid-phase microextraction (SPME) are often used (Vogt et al., 2008; Zhang et al.). The volatile compounds desorbed from the Tenax tubes were concentrated on a solid phase microextraction (SPME) fibre, from which they were thermally released in the injection port of the gas chromatograph, for analysis. A 6890N gas chromatograph (GC) from Agilent, with a HP5-MS column (30 m x 250 mm x 0.25 mm), was used for chromatographic separation of volatile organic compounds.

Results and discussions

The gas chromatographic analysis showed the presence of about 100 organic compounds in the samples collected from the respiration of cows. About 40-45% from the total area of chromatograms is represented by two main compounds, one with retention time of about 7.6 min, and another with retention time of about 7.7 min (compounds marked with “*” in Figure 2). These are accompanied by a small number of compounds with peak area above ~1%, while most of other compounds were found in very small amounts, making their correct identification to be difficult. Figure 2 shows the chromatograms of three samples, one from Râmnicu Vâlcea, one from Bistrița Năsăud and one from Constanța. Although the chromatograms look similar, some differences could be observed between samples. For example, the compounds with retention time of about 8.1, 8.7 and 10.0 min. (compounds marked with “+” in Figure 2 were found in higher amounts in the sample collected from Bistrița Năsăud (b), while the compounds with retention time of 12.0 and 12.5 min. (compounds marked with “-” in Figure 2 where found in much lower amounts in the sample from Constanța (c) compared with the samples from Râmnicu Vâlcea (a) and Bistrița Năsăud (b). Differences in the relative amount of various compounds along the chromatograms were also observed.

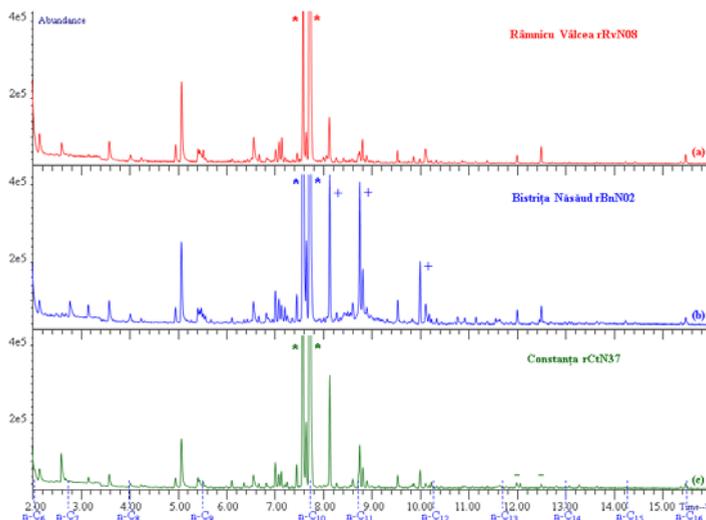


Figure 2. *The chromatograms of several breath air samples collected from farm cows in three regions on Romania*

Figure 2 also shows the position in the chromatogram of normal paraffins from n-C₆ (hexane) up to n-C₁₆ (hexadecane). This is helpful to describe the volatility of organic compounds in the samples by comparing their retention time with that of normal paraffins. Under identical analysis conditions, the organic volatile compounds with low polarity leave a non-polar chromatographic column in the order of their increasing boiling point (the order of decreasing volatility). That is, lighter, smaller, more volatile compounds leave the column earlier than heavier, bulkier, less volatile compounds. The normal paraffins can be used as references of the boiling point range, which is a measure of compound volatility. The volatility range of compounds, based on the boiling points of normal paraffins, is presented in Table 1.

Table 1

Volatility ranges based on boiling point of normal paraffins

Carbon number	Volatility range (°C)	Carbon number	Volatility range (°C)	Carbon number	Volatility range (°C)
n-C ₆	36-69	n-C ₁₀	151-174	n-C ₁₄	234-254
n-C ₇	69-98	n-C ₁₁	174-196	n-C ₁₅	254-271
n-C ₈	98-126	n-C ₁₂	196-216	n-C ₁₆	271-287
n-C ₉	126-151	n-C ₁₃	216-234	n-C ₁₇	287-302

Under reasonable approximation, the compounds leaving the chromatographic column before a normal paraffin n-C_x, but after the previous normal paraffin n-C_{x-1}, have the boiling point, and consequently the volatility, in the range of the two normal paraffins, and are regarded as having the carbon number “x”. The sum of peak area percentage of all compounds in the n-C_{x-1}–n-C_x range of retention time is graphically represented versus the carbon number, “x”. The obtaining curve – Figure 3 is called np-gram (np – standing for normal paraffins) and is an easy way to globally describe the volatility distribution of compounds in a mixture.

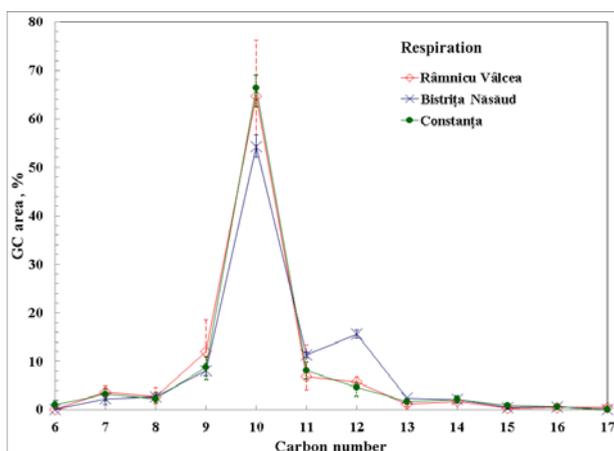


Figure 3. *The volatility distribution of organic compounds emitted through respiration of farm cattle*

The np-grams in Figure 3 show the average of the values determined for the samples in each region in Romania, that is 2 samples from Bistrița Năsăud, 4 from Râmnicu Vâlcea and 4 from Constanța, as well as the range of these values (minimum and maximum) for each region. It was found that the respiration of cows contained compounds distributed in the range of n-C₆–n-C₁₇, which corresponds to boiling point range of 36-302°C. All three np-grams had a peak at n-C₁₀, which, according to Figure 2 and to Table 1, corresponds mainly to the compounds marked with “*” at about 7.6 and 7.7 min in the chromatograms, that have the volatility in the range of 151-174°C. Besides, higher amounts of compounds, of about 5-10%, were found and n-C₉, n-C₁₁ and n-C₁₂, having the volatility in the range of 126-151, 174-196 and 196-216°C respectively. Some significant differences could be observed among samples collected from various regions in Romania. The amount of compounds at n-C₁₂ is higher for the samples collected in Bistrița Năsăud, compared with the samples collected in Constanța. This comes mainly from the compounds marked with “+” at retention times of around 8.1, 8.7 and 10.0 min. in Figure 2. On the other hand, the samples from Bistrița Năsăud have lower amount of about 54 % of the main compounds at n-C₁₀, compared with about 66% for the other two samples. The samples collected from Râmnicu Vâlcea and Constanța gave similar results, with only small differences at n-C₉, n-C₁₁ and n-C₁₂. A greater variation among the collected samples was found in Râmnicu Vâlcea, as shown by the dotted red segments representing the range of calculated values, especially at n-C₁₀, n-C₉ and n-C₁₁.

Conclusions

1. Respiration samples were collected from 10 cows in farms from three regions in Romania.
2. Around 100 organic compounds were found in respiration samples from cows, with volatility in the range of boiling points from 36 to 302°C. Two main compounds were found at n-C₁₀, with boiling point in the 151-174°C range.
3. Some differences were found between samples collected in different regions of Romania.

Acknowledgements

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THE VARIATIONS OF PHYSIOLOGICAL PARAMETERS STRESS-SENSITIVE LANDRACE PIGLETS

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Abstract

The study was conducted on 24 piglets Landrace, male and female, aged 90 days (average weight 35 kg), under conditions of intensive growth. In these piglets were recorded for two weeks individual variations of the pulse, breathing rate, rectal temperature and body surface temperature according to ambient temperature. Significant variations were found in 8 Landrace piglets, considered sensitive to temperature stress (SP) and the remaining 16 Landrace piglets, considered adapted to temperature stress (SN), the variations were insignificant. Circadian variation of 10°C in the air generated changes in the body surface temperature cycle of 4.4°C in SN piglets and 9.8°C in SP piglets. Diurnal variation in the rectal temperature was 0.8°C in SN piglets and de 1.7°C in SP piglets. Piglet growth rate was also monitored during the experimental period and it was noticed that the average daily gain was 0.4 Kg lower in SP piglets compared to SN piglets

Keywords: *physiological parameters, stress, Landrace piglets*

Introduction

In the intensive pig breeding and exploitation system there are frequent stress phenomena leading to significant reductions in production. The accelerated growth period, which characterizes the age range of 3-6 months, when the daily weight gain can exceed 1 Kg (if energy and protein factors in ration are balanced), represents a physiological state that increases the sensitivity of the organisms to the stressors. In this period young animals become more demanding in particular for the quality of the protein in ration and the housing conditions (Whittemore, 1998). The ability to adapt pigs to environmental conditions varies according to their heritage and individual behavioural traits (Kick et al., 2010). Repeated exposure to high temperature variations, restraint of resting space or changes in the social life of piglets at the weaning age would cause chronic activation of the hypothalamic-pituitary-adrenal axis, which leads in time to adrenal fatigue with decreased cortisol response to stress and changes in leukocyte status (Huynh et al., 2005; Niekamp et al., 2006).

The paper looked at the individual reactivity of Landrace pigs aged 90 days at daily diurnal temperature variations according to their sensitivity to common stressors: changing the shelter, changing food, animal transport, animal weighing and the animal density in the stalls.

Material and methods

Researches were performed on 24 piglets, 90 days of age (average weight 35 kg) of the Landrace breed (both sexes) after their transfer from the nursery to the fattening sector. Piglets were raised in boxes with floorboards and grills, ad libitum fodder, average temperature – 21°C, humidity – 65%, airflow speeds between 0.2-0.5 m/sec. These animals were observed over a period of two weeks, variations occurring in the values of certain physiological parameters depending on the circadian temperature of ambient air.

The following physiological parameters were determined: surface temperature and body temperature by thermometry, breathing by auscultation method, heart rate by palpation method.

Data recording was based on the daytime schedule and the ambient temperature variation in the shelter (table 1).

Table 1

Daytime schedule and ambient temperature

hour	6.00	9.00	11.00	14.00	16.00	19.00	22.00	2.00	4.00
°C	16.5	16.6	18.2	22.4	24.9	26.8	23.6	17.5	17.2

At the same time, weight gain was recorded during individual daily weighing. The data obtained were statistically processed for the elaboration of average values and statistical significance using the Student test.

Results and discussions

Analyzing the data of the physiological parameters followed in the group of 24 piglets, we found significant variations only in 8 Landrace piglets; they are considered sensitive to ambient temperature stress or stress-positive (SP) group. The remaining 16 Landrace piglets were considered the adapted to temperature stress or stress-negative (SN) group, (figures 1 and 2), because the variations of the physiological parameters observed were insignificant.

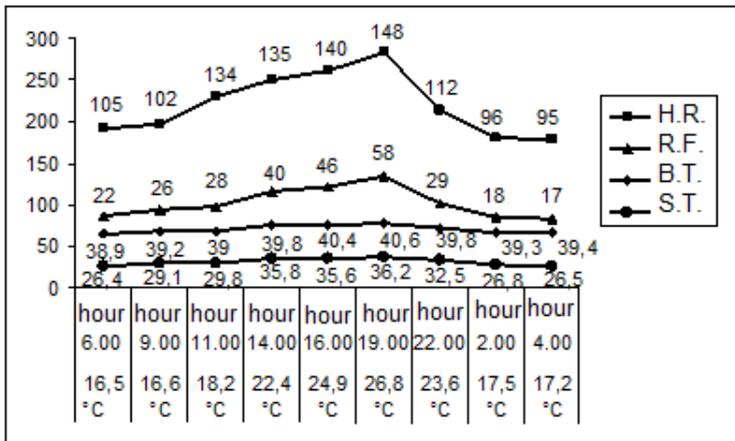


Figure 1. Dynamics of pulse (H.R.), respiration (R.F.), body temperature (B.T.) and surface temperature (S.T.) to the piglets considered stress-positive (SP)

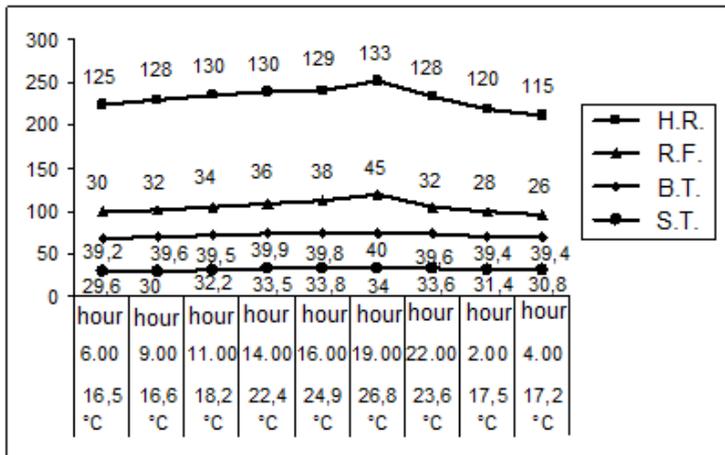


Figure 2. Dynamics of pulse (H.R.), respiration (R.F.), body temperature (B.T.) and surface temperature (S.T.) to the piglets considered stress-negative (SN)

In the daytime schedule it was observed that the ambient temperature recorded at 6.00 in the morning was 16.5°C and that recorded at 19.00 was 26.8°C (figures 1 and 2).

The difference of 10°C in the ambient temperature recorded at the two moments of the day determined different adaptive physiological reactions at the animals from the experimental study.

Thus, in piglets considered stress-positive (SP), there was a variation in the surface temperature value of 9.8°C (significant difference) and in pigs considered stress-negative (SN), only 4.4°C (figure 1 and 2).

The large difference, recorded in surface temperature variation in SP pigs group, indicates a poor thermoregulation compared to SN pigs group, in which adaptive mechanisms were more efficient.

In piglets, heat loss through conduction, convection and sweating has a minor role in thermoregulation; the most important being the mechanisms of evaporative heat loss through perspiration and thermal polypnea.

Daily diurnal variation in body temperature was 1.7°C in SP piglets group and 0.8°C in SN piglets group. No significant variations in body temperature were observed in the piglets in the two groups, because the high number of thermal receptors in the skin causes the ambient temperature changes to be received before they change the central temperature.

It was found that at around 19.00 the thermal polypnea phenomenon was manifested in both groups of animals, 45 respirations per minute in the SN group and 58 respirations per minute in the SP group as an adaptive response to increased ambient temperature (figure 1 and 2).

Adrenergic stimulation also caused tachycardia. Thus, in pigs in the SP group there were significant increases of the heart rate with 11% compared to those in the SN group (figure 1 and 2).

In the dynamics of surface temperature and body temperature variation, the lowest values were observed during the first hours of the morning (2.00-6.00) and the highest values in the afternoon, between 16.00 and 19.00. In the rate of breathing and heart rate there were increasing trends, between 06.00 and 19.00, followed by successive decreases between 22.00 and 04.00 (figure 1 and 2).

Changes in the dynamics of the physiological parameters studied can be correlated with the times of the day: feeding, periods of digestion and rest or periods of agitation of the animals in the shelters.

Following the growth rate by weighing the piglets in the two groups over a two-week period, the average daily weight gain was assessed (figure 3).

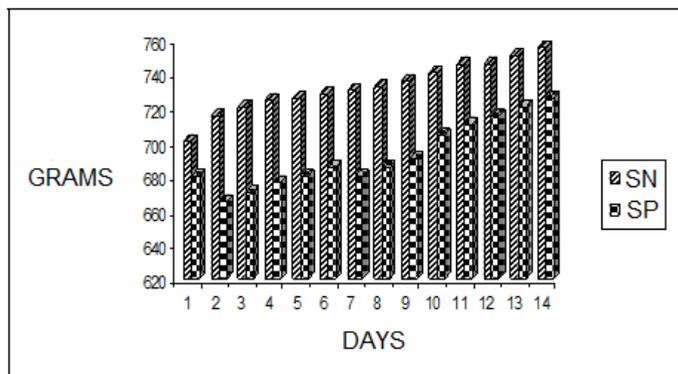


Figure 3. Average variations in weight gain in pigs from the two experimental groups

The average live weight that piglets initially had was 35 Kg and, at the end of the experimental period, the piglets of the SN group had an average weight of 45.2 Kg and the piglets of the SP group had an average weight of 44.8 Kg.

The difference in the average value of the two weights was 0.4 Kg. In the first 10 days of the analyzed period, a pig weight gain of 20 to 50 grams was recorded in piglets in the SP group, compared to the weight gain recorded in piglets of the SN group (figure 3). In the following days of the follow-up period, the decrease in the weight gain in piglets of the SP group compared to the SN group was only 30-35 grams, which allowed later recovery of body weight as an adaptive response to stress factors which acts during the first 10 days of transfer of the piglets from the nursery to the fattening sector. In this age category (90 days), the temperature variation in the shelter was an important factor that influenced the daily average gain of the animals.

Conclusions

The difference of 10°C in the ambient temperature recorded at the two times of the day (6.00 in the morning and 19.00 in the evening), generated significant changes in the value of the surface temperature only in the piglets from the SP group.

The dynamics in the surface and body temperature values, directly depended on the variations of the ambient temperature, while the dynamics in the breath and heart rate values were influenced by the circadian biorhythms.

The growth rate is influenced by the temperature of the environment and at the same time depends on the ability of animals to adapt to stressors.

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**MALE PSEUDOHERMAFRODITISM IN A TOMCAT,
ACCIDENTALLY DISCOVERED IN A CLINIC IN BUCHAREST
– A CASE STUDY**

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Abstract

The present work brings to light an extremely rare case of pseudohermafroditism in a 1.5 year old tomcat, a metis, owned by a citizen of Bucharest.

We want to present in this paper some anatomical features encountered in the studied tomcat, particularities that have not been described in this species, in Romania so far.

Keywords: pseudohermafroditism, tomcat, anatomical features

Introduction

Pseudohermaphroditism in animals is characterized by testicles placed intra-abdominally and the female genital tract. The labia are poorly developed, reduced vulva, hypertrophied clitoris, hypoplastic gonads, without seminal line, but hypertrophied Leydig gland.

These types of hermaphrodites are highly masculinised. It is found mainly in pigs, rarely in cattle and horses. In the case of horses, it is accompanied by aggressive behaviour.

Material and methods

In 1997, a case was described in a paper by the Faculty of Veterinary Medicine in Oslo, Norway.

A 1.5-year-old tomcat, common breed, red tabby, owned by a citizen of Bucharest, was presented at C.M.V.I. Dr. Grigorescu Paul for orhidectomy.

Results and discussions

The owner requested the neutering as a result of the typical instinctive-hormonal-pheromone-territorial behaviour of the mature tomcat. The most disturbing being the miction on the walls of the house, which gives off a typical, persistent remnant door. The owner also denounced the tomcat's very noisy behaviour when he noticed the presence of cats in heat around.

The surprise came after examining the genital tract on the sedated tomcat (Figure 1-4). After removal of local hair, we found the testicles in separate bags, between them a slit was visible, which turned out to be a vulva. Initially, at the lower commissure of the vulva, it was thought to have a copulatory organ, but it was found to be a hypertrophied clitoris.

The excised testicles showed no anatomical changes, being normally conformed.



Figure 1. (orig.) *Hermaphroditism in tomcat*

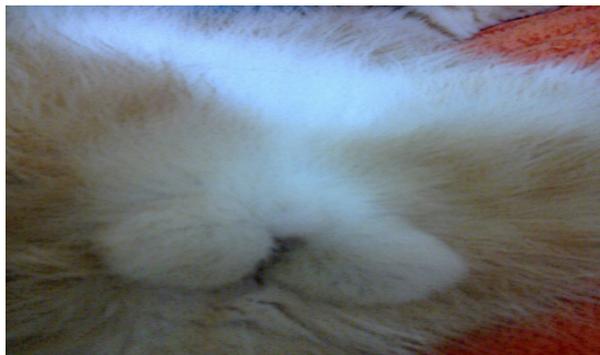


Figure 2. (orig.) *Hermaphroditism in tomcat (detail)*



Figure 3. (orig.) *Separate testicles bags, vulva and clitoris hypertrophy*



Figure 4. (orig.) *Separate testicles bags, vulva and clitoris Hypertrophy (detail)*

Conclusions

1. Pseudohermafroditism in tomcat is a congenital disorder, extremely rare.
2. So far, this morbid entity has not been reported in a tomcat until now, on the territory of our country.
3. The research should have been extended by exploring the genital tract in the abdominal-pelvic cavity. This implies the owner's approval.
4. The fact that this type of congenital condition is discovered for the first in the Romanian veterinary medicine, we felt the need to present the case in an academic environment.

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MORPHOTOPOMETRIC ANATOMICAL CONSIDERATIONS IN DOG'S MEDIASTINUM

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Abstract

Recent studies on mediastin include data on the microscopic and biochemical aspect, most often correlated with its pathology. In recent years, specialized journals have published many articles whose subjects are centred on the vegetative nervous system, in particular with studies of its pathology in pets such as dogs and cats.

For the study of organs, serous and visceral formations in the chest cavity, 20 (11 females and 9 males) were dissected. The working method was based on dissection to the limit of vision with the magnifying glass (15 D). In some cases, vascular formations have been injected with a mixture of food gelatine and food colouring substances. In all cases, the tissues were brushed with 10% acetic acid solution. For the topographic study, 10% formalin solution was injected on the aorta and directly into the cavity before opening the chest cavity. After the opening of the chest cavity, the intestinal mass was washed, taking care not to alter the normal topography of the viscera. For cutting the tissues, appropriate knives and scissors were used. When cutting the spine and other bone formations, an electric saw was used. The ready-made photos were placed on a flat surface (stainless steel) for good contrast.

Based on the results of his 20-piece research, the following conclusions can be drawn regarding the morphology, vascularisation and intimacy of the mediastinum:

The former mediastinum abounds in vascular nerve and lymph formations in dog. The volume of the previous mediastinal cavity is small in size (from 3 cm to 6 cm), which is why the base of the large vessels together with all the brachiocephalic artery collateral advances in this mediastinal compartment.

Median mediastinum is occupied in a proportion of 60% of the cord that is suspended in the fibrous pericardium. By means of the fibrous pericardium, due to the special cord curvature in the dog, there is also the freno-prickard ligament. Because of the small size of the caudal mediastinal compartment, the caudal cavern vein occupies a fairly important space in the middle mediastinal compartment

crossing the mediastinum under the right accessory lobe and on the right side of the lung ligament.

The size of the heart is closely related to the size of the animal, falling into three categories of size: small, medium, large.

Keywords: *dog, mediastinum, morphotopometry*

Introduction

The anatomical study of the mediastinum in the dog is a challenge for an anatomist, since clinically it presents one of the most recently approached areas, being at the same time the area with the highest variability from individual to individual. Evolution in surgical interventions makes it however difficult to clarify the morphological aspects of the anatomical components of the mediastinum in order to improve their medical and surgical approach and to develop fundamental experimental models for other areas of research.

The author also combines new research methods with a special presentation style, resulting in a rich work in detail, useful to both veterinarians and researchers in all branches of biology.

The mediastinum is systematized for embryological, topographic and physiological considerations in several segments or portions, distinct from the anatomical and physiological point of view, as follows:

- anterior or precardiac mediastinum;
- median or cardiac mediastinum;
- posterior or postcardiac mediastinum.

The term “mediastin” refers to the part of the thoracic cavity that is contained in the sagittal septum of the endothoracic fascia between the two pleures. As all formations in the chest cavity, excluding the lungs, live in this compartment, the area is of particular interest for clinical activity. The paper aims to present an update of the anatomical veterinary

Recent studies on mediastin include data on the microscopic and biochemical aspect, mostly in association with its pathology. In recent years, specialized journals have published articles whose subjects are centred on mediastin formations, especially in relation to studies of its pathology in pet animals such as dogs.

An example of what can be conclusive is the articles appearing in some medical journals, such as the work of Dr. Florin Eugen Grosu – “Radiological diagnosis – pneumothorax versus pneumomediastin” published in *Veterinarul* journal. The increased interest of veterinarians and animal lovers towards pet animals has led to the emergence of real veterinary clinics equipped with proprietary research laboratories, justifying concerns about the anatomy of macroscopy and microscopy of the thoracic cavity, focusing on decipherment of some physiological phenomena that have as substrate the anatomic particularities of vascular-nerve formations.

The research aimed at presenting the morphotopography of vasculo-nerve formations from mediastin to carnivores, with particular reference to topography of the heart and adjacent formations.

A complex study of these formations required preliminary investigation of the morphological support of the region, the detailed description of the thoracic cavity, the morphology of the viscera in these cavities, and the pericardic architectonics.

The paper aims to present an update of the anatomical veterinary terminology regarding the morphotopography of the anatomical formations contained in the mediastin. The literature particularly abounds in physiological and pharmacological data related to the vegetative nervous system and less in morphology data, all the more so as the practice of veterinary medicine is confronted with these problems, especially in carnivores.

Material and methods

For the study of organs, serous and visceral formations in the thoracic cavity, 20 (11 females and 9 males) were dissected with different waist. The working method was based on dissection to the limit of visibility with the magnifying glass (15 D). In some cases, the vascular formations were injected with a mixture of food gelatine and dye substances. In all cases, the tissues were brushed with 10% acetic acid solution. For the topographic study, 10% formalin solution was injected into the thoracic aorta and directly into the cavity before the chest cavity was opened. After opening the thoracic cavity, the washing of the anatomical formations was performed, with care not to alter the normal topography of the viscera. For cutting the tissues, appropriate knives and scissors were used. When cutting the spine and other bone formations an electric saw was used. Tracks ready to be photographed were placed on a black (flat) wooden surface to produce good contrast.

The cadavers came from the private offices and presented a diversified casuistry. Most (80%) were euthanasia animals. The anatomical formations were dissected to the limit of vision with the magnifying glass and using the stereomicroscope (SMZ 2B NIKON).

For the differentiation of vegetative structures, in all cases the abdominal aorta was injected with a dye + duco + dye substance. The preparation of this substance consisted of 20% mixing of a colourless plastic "AGO" with acetone. A dye mixed with the initial mixture (DUCO red dye for arteries and vein blue) was incorporated into the obtained fluid. Also vegetative formations were treated with 10% acetic acid. Following the dissection, sketches were made in all cases, vegetative plexuses were photographed in 40 cases and vegetative structures were confirmed, vegetative lymph nodes were harvested and histologically processed. For microscopic evidence of vegetative cells, tricromatic staining, Gomori and Van Gieson staining were.

Results and discussions

The results obtained complete, check or correct existing data on topography, biometrics, the structure of some formations, and especially those around the heart. The delimitation of mediastin organs has an important applicability especially in clinical and scientific fields. As there is no way to demonstrate the existence of

nerve pathways or the vegetative plexus thickness of the vegetative lymph nodes by microscopic investigation, histological harvesting and histological processing of the lymph nodes was carried out, as the case may be.

For the presentation of the anatomical formations were used diagrams and photographs made after parts, photos after histological preparations.

The thoracic cavity is bounded by the chest cage and by the muscular and fibrous formations that impregnate this chest cage. In the thoracic cavity are located the two lungs, both right and left, housed in pleural sacs and separated from each other by the mediastinal septum. The thoracic cage is represented by the trunk bone walls: the thoracic backbone, the lateral, and the sternum, disposed ventral (Figure 1, 2).

The chest cavity is bounded by the chest cage that is taped by the endothoracic fascia and supplemented by the muscular formations of the costal wall. Inside the torsion cage the mediastinal septum made by the medial extension of the endothoracic fascia separates the two hemodials in which the plamons are housed in the pleural sacs. The medial septum of the fibrous nature fixes between the two lungs all the formations that traverse or live permanently in the chest cavity.

The caudal chest cavity is delimited by the abdominal cavity through the diaphragm (Figure 1). Mediastinum represents the median cavity of the thoracic cavity, defined by the fibrous cavity, also called the endothoracic fascia.

– Vegetative in 40 cases and for confirmation of vegetative structures, vegetative lymph nodes were harvested and subjected to histological processing. For microscopic evidence of vegetative cells, tricromatic staining, Gomori and Van Gieson staining were used.

– Physiology and pharmacology, linked to the vegetative nervous system and less in morphology data, all the more so as the practice of veterinary medicine is confronted with these problems, especially in carnivores.

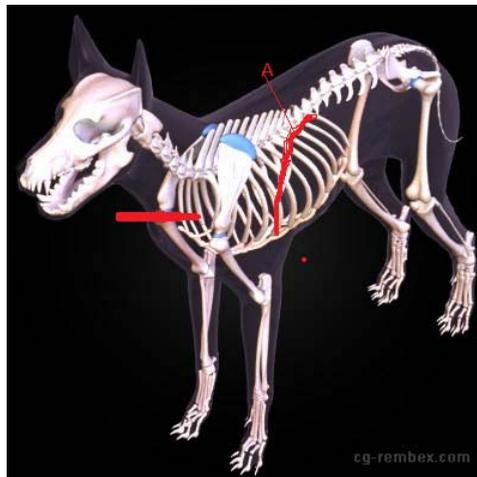


Figure 1. *Dog skeleton with thoracic aperture presentation circumscribed by the first thoracic vertebra, the first ribs and the ventral sternum (A – diaphragm)*



Figure 2. *Dog skeleton with skeleton presentation defining chest cage*
(A – thoracic vertebral column, B – ribs, C – stern)

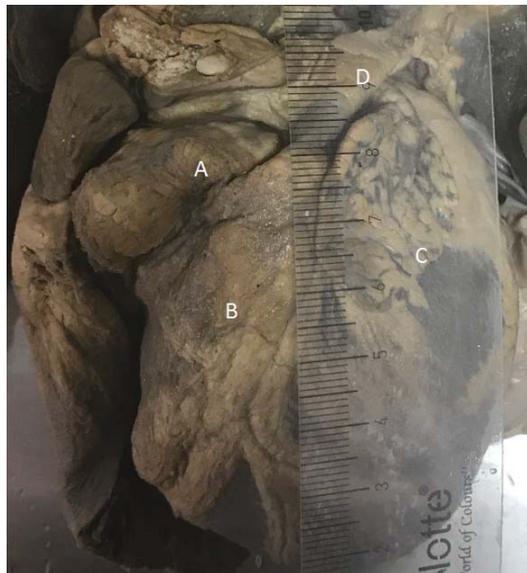


Figure 3. *Dog heart*
(A – right atria; B – right ventricle; C – left ventricle; D – pulmonary artery)

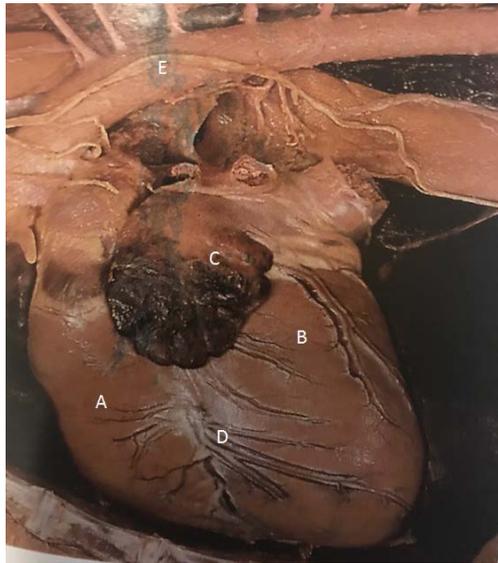


Figure 4. *Cardia plexis*
(A – right ventricle; B – left ventricle; C – left auriculus;
D – paraconal groove; E – n.vag)



Figure 5. *Small sized dog heart*
(A – right ventricle; B – left ventricle)



Figure 6. *Dog heart – bog sized*

(A – right ventricle; B – left ventricle; C – left atria; D – paraconal groove; E – vortex)

The innervation of organs in the mediastinum is ensured by the vegetative nervous system with its two components, the sympathetic system and the parasympathetic system. The intraneuraxial portion of the vegetative nervous system is disposed in the spinal cord in the intermediolateral corners of the cervical marrow segment in the thoracic cervical segment, as well as in the spinal bulb in the vagal cardio-pneumo-enteric nucleus. The extravascular centres of the vegetative nervous system are located in the sympathetic paravertebral nodes and in the prevertebral lymph nodes for the parasympathetic component. These lymph nodes are connected by post-ganglion fibres to the mediastinum organs. These connections can be made directly or through secondary plexuses. The parasympathetic nodes are disposed near the organs they innervate, being contained in the nerve tissue of the mixed plexus. Cranial, middle and caudal heart nodules that come from the sympathetic ganglion chain and the thread of the depressor nerve of the vagus make many anastomoses and converge to the large vessels at the base of the heart, forming around them a complex nervous complex called cardiac plexus. Several microscopic vegetative lymph nodes are dissected along the fibres of this plexus. In principle, sympathetic and parasympathetic cardiac nerves on the left side cross the left slope of the descending aorta, forming the superficial cardiac plexus, and the right-hand cardiac nerves cross the right aorta and the brachiocephalic trunk to the right of space between the two main bronchi forming the deep aortic-pulmonary plexuses (figure 4).

The nerves that distribute to the heart come from the middle cervix, the cervical-thoracic ganglion, the subclavicular plexus and the vagus nerve.

Dissected cases were grouped into three study groups according to the size of the animals: small, medium and large.

- o Lot 1 (Table 1): 6 small animals with a length of up to 30 cm (figure 5, 7, 8)

- Lot 2 (Table 2): 7 medium sized animals up to 60 cm in length (figure 9)
- Lot 3 (Table 3): 6 large size animals up to 80 cm in size (figure 6, 10, 11)

Table 1

Lot no. 1 – small sized

Nr.	Case dissected	Dog size	L/l thoracic cave	Heart length	Heart rate
1	York Toy	25 cm	10/5 cm	6 cm	3,5 cm
2	Teckel	30 cm	12/6 cm	6,4 cm	3,9 cm
3	Bichon Toy	32 cm	12,8/6,2 cm	6,7 cm	4 cm
4	Pincher	23cm	9/5 cm	5,8cm	3,4 cm
5	Cavalier King Charles Spaniel	30 cm	12,2/7 cm	6,5 cm	3,5 cm
6	Common breed	30 cm	13/7 cm	6,5 cm	3,5 cm

Table 2

Lot no. 2 – medium sized

Nr.	Case dissected	Dog size	L/l thoracic cave	Heart length	Heart rate
1	Common breed	60 cm	22/12 cm	8,2 cm	5,3 cm
2	Shiba Inu	37 cm	18/10 cm	7,5	4,2
3	Whippet	48 cm	20/11 cm	7,6	4,5
4	Border Collie	51 cm	21/11,5 cm	7,8	4,7
5	Irish Terrier	46 cm	19/11cm	7,2	4,6
6	French Bulldog	35 cm	17,5/9 cm	6,6	4,2
7	Spaniel Cocker	39 cm	17,8/8	6,7	4,3

Table 3

Lot no. 3 – big sized

Nr.	Cases	Size dog	L/l thoracic cave	Heart length	Hert rate
1	Common breed	80 cm	35/20cm	12 cm	5,6 cm
2	Argentinean Dog	70 cm	30/18	10 cm	5 cm
3	Rottweiler	69 cm	29/17	9,8 cm	4.9 cm
4	German Dog	81 cm	36/21	12 cm	5,7cm
5	Mastiff	76 cm	35/20	11 cm	5,2cm
6	Common breed	70 cm	31/18,5	10 cm	5,2cm



Figure 7. *Lot no. 1 – small sized*



Figure 8. *Lot no. 1 – small sized*



Figure 9. *Lot no. 2 – medium sized*

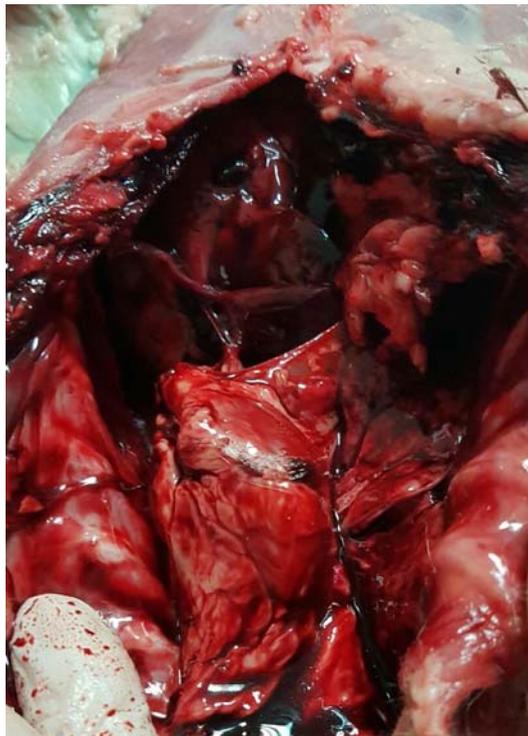


Figure 10. *Lot no. 3 – big sized*



Figure 11. *Lot no. 3 – big sized*

Conclusions

1. Cardiac mediastinum occupies a rather large space within the thoracic mediastinum.
2. In the former mediastinum there are also some of the large vessels (pulmonary artery and brachiocephalic artery).
3. The caudal vein can be projected on a long side of the middle mediastinum.
4. The study resulted in a number of three groups of animals systemized by waist size.
5. Small animals have a heart size between 8 cm and 5 cm.
6. Medium sized animals have a heart size between 9 cm and 6.6 cm.
7. Large animals have a heart size of 12 cm to 9.8 cm.
8. The vegetative nervous system comprises a great variety of branches belonging to the cervico-thoracic ganglion and the vagus nerve.
9. The former mediastinum includes: the origin of the large vessels, the cranial vein, the brachiocephalic trunk, the thymus, the oesophagus, the trachea, the vagal nerves, the thoracic cervical plexus, the thoracic duct, the cranial mediastinal lymph nodes.
10. Medium mediastinum includes: the medulla in the middle compartment of the mediastinum, the endothoracic fascia forming at this level the pericardial fibrous sac that deflects the sternum where it forms the sternopericardial ligament.

11. The posterior mediastinum includes: thoracic aorta, caudal vein, esophagus, nn. frenici, nn. vage, thoracic channel.

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