ISSN (print) 1454-7406
ISSN (electronic) 2393-4603

“ION IONESCU DE LA BRAD” IASI UNIVERSITY OF LIFE SCIENCES (IULS)

SCIENTIFIC PAPERS VETERINARY MEDICINE

LUCRĂRI ȘTIINȚIFICE SERIA MEDICINĂ VETERINARĂ

VOLUME 65

NO. 3

PUBLISHING "ION IONESCU DE LA BRAD"

CONTENTS

ASSESSMENT OF THE TECHNOLOGICAL PARAMETERS OF BUTTER PRODUCTION AND THE INFLUENCE OF THE STORAGE CONDITIONS ON ITS FRESHNESS

Carmen Daniela PETCU[1], Dana TĂPĂLOAGĂ[1], Emilia CIOBOTARU-PÎRVU[1], Oana Diana MIHAI[1], Oana Mărgărita GHIMPEȚEANU[1], Otilia Cristina MURARIU[2]

e-mail: otiliamurariu@yahoo.ro

Abstract

Butter is the dairy product obtained by processing cream. It is a food rich in nutrients and is characterized by a very high digestibility. In the study carried out in a dairy products processing unit, located in the south of Romania, the technological flow of obtaining different types of butter, as well as their physico-chemical characteristics were followed. Laboratory determinations were carried out regarding the freshness of the butter (organoleptic examination, acidity determination, Kreiss reaction etc.). At the same time, a questionnaire was carried out in which 170 people participated, regarding the consumption of butter, a study performed through the Google forms platform. Following the determination of the acidity of the fresh, refrigerated and frozen butter samples, values below the maximum allowed limit were recorded, all samples being considered compliant, and the butter samples stored at temperatures higher than 4°C exceeded the maximum allowed value from the first days of the deviations temperature, being classified as non-compliant products. This aspect highlights the importance of respecting the storage conditions recommended by the manufacturers. Following the opinion poll on butter consumption, 71.2% confirmed the preference to consume butter in the morning. For most participants, the producing company is the determining factor in the choice of butter.

Key words: butter, fat, acidity, storage conditions.

Butter has been known since ancient times, from the Greeks, from where its preparation was taken over by the Romans, then gradually spreading to other nations (Dulce D., 2013). It has been considered a symbol of the "good life" throughout most of human civilization and is a staple food in many cultures. It is said that people ate butter and cheese instead of raw milk (Frey J., 2014).

Butter is the product obtained by processing pasteurized cream and sown with selected lactic starter cultures (Savu C., Petcu C.D., 2002; Floriștean V., Borș A., 2021).

This product is characterized by a very high digestibility, over 95%, due to the increased content of volatile fatty acids over 20% of their total (Usturoi M.G., 2007; Sala C., 2008).

Butter is obtained from sweet cream, but it can also be obtained from bacteriologically fermented cream. Since the 19th century, butter has been obtained from cream naturally separated from milk, floating to the surface of the milk, followed by placing the cream in a cylindrical wooden container, called churn (figure 1). Butter was churned manually, but this processing method also had disadvantages, namely, it favored contamination with microorganisms and the product's validity was limited (Savu C., Petcu C.D., 2002).

Currently, butter is obtained at an industrial level, and the obtaining technology is based on scientific progress and the experience in the field of bacterial acidification and thermal treatments (Savu C., Petcu C.D., 2002).

Moreover, particular attention must also be paid to food safety (Murariu F., 2019; Murariu O.C., 2016; Borș A. *et al*., 2018), the manufacture, packaging and storage of final dairy products (Petcu, 2006; Petcu, 2021).

Figure 1 **Cylindrical wooden container for butter churning (traditional churn)**

Figure 2 **Industrial churn**

The difference between the butter types all around the world

Depending on the country where it is obtained, the butter can differ due to different production methods, so each of these butters will be slightly different.

Therefore, when we talk about butter produced in Europe, we are actually talking about a style of butter produced throughout Europe. European-style butter refers to butter inoculated with selected dairy cultures that has been churned for a long time to obtain up to 82% fat. Traditionally, butter is left to ferment to achieve a slightly sour taste, but most often we will identify butter with the addition of selected lactic cultures. European-style butter is generally preferred for its rich flavor (this is a direct result of the higher milk fat content). More creaminess also means a softer texture, faster melting, and usually a saturated yellow hue. Due to its low moisture content but high fat content, it is often used in bakery, giving the dough an aromatic taste and a softer texture.

Butter made in the United States is monitored and overseen by the U.S. Department of Agriculture. For a dairy product to be considered butter, it must contain up to 80% fat. This butter, unlike butter from Europe, does not include any added lactic culture, resulting in a more neutral taste (Owen E., 2017; Ramdene H.B., 2019).

The benefits of butter consumption

As for consuming butter, it has been shown to have a number of benefits.

Butter is rich in vitamin A, which is easily absorbed and necessary for thyroid and adrenal glands health (Fallon S., Mary G., 2000; Weston P., 2020).

It contains lauric acid, which is important for treating fungal infections as well as those caused by *Candida albicans* (Arnfinnsson J. *et al.*, 2001).

It contains lecithin, which is essential for cholesterol metabolism (Fallon S., Mary G., 2000; Craciun O.C. *et. al.*, 2012).

In addition, it contains antioxidants, which can prevent free radical damage and dilate arteries (Brezeanu C. *et al*., 2020), thus helping to increase blood flow (Fallon S., Mary G., 2000).

It helps reduce the risk of type 2 diabetes (King I.B. *et al.*, 2018; Campos H., 2016).

It is an important source of vitamin E and K (Fallon S., Mary G., 2000; Weston P., 2020). It is a rich source of selenium (Fallon S., Mary G., 2000).

Saturated fats in butter may have antitumor and anticarcinogenic properties (Choi K., *et al*., 1986; Fallon S., Mary G., 2000;).

Butter contains conjugated linoleic acid, which is an effective anticarcinogenic agent, muscle and immune enhancer (Cho H.Y. *et al*., 2005).

Vitamin D in butter is essential for calcium absorption (Fallon S., Mary G., 2000; Weston P., 2020).

It is the only source of anti-stiffness factor (Vulsen Factor) and prevents joints calcification (Weston P., 2020).

It is a source of "Activator X" (vitamin K_2), which helps the body absorb minerals (Price, W.A., 2010). In addition, it is a highly absorbable source of iodine (Fallon S., Mary G., 2000).

It is a quick source of energy and is not stored in the adipose tissue of our body (Fallon S., Mary G., 2000).

Cholesterol contained in milk fat is essential for the development of the brain and nervous system of children (Fallon S., Mary G., 2000; Arenas E. *et al*., 2009).

It contains arachidonic acid (AA), which plays a role in brain function and is an important part of cell membranes (Weston P., 2020).

It can prevent gastrointestinal infections in children or the elderly (Weston P., 2020).

According to the National Institute of Statistics, butter production in February 2020 was 1039 tons, and in February 2021 it was 785 tons, thus showing a decrease in butter production by 254 tons (24.4%).

In the period January-February 2020, the butter production recorded in Romania was 1957 tons, and in the period January-February 2021 1759 tons. Butter production decreased by 198 tons (10.11%) (www.insse.ro).

MATERIAL AND METHOD

The study was conducted in a dairy unit located in southern Romania.

Milk and dairy products can be sold in a wide variety of packaging methods and are subject to a large number of analyzes before being put on the market (Visoescu D.I. *et al.,* 2015; Petcu C.D. *et al*., 2020). Thus, the aim is to know the chemical composition and assess the nutritional value, identify possible counterfeits etc. (Oprea O.D. *et al.*, 2020; Petcu C.D. *et al.,* 2021; Petcu C.D. *et al*., 2022).

The dairy processing unit under study is supplied with raw milk from sanitary-veterinary authorized farms and the frequency of supply depends on the performance of the animals and the amount of milk obtained from them (Oprea O.D. *et al.*, 2019). The milked milk is cooled in isothermal tanks and then is collected by the company's tankers and transported to the processing unit, where it is analyzed and subsequently processed. At the milk reception, a series of qualitative parameters are tested in the laboratory, by which it is assessed whether the properties of the milk have undergone changes due to non-compliance with the hygiene rules during milking, transport or if falsifications have been made (Verraes C. *et al.,* 2015).

In the study, the technology for obtaining the butter assortments, as well as their physico-chemical characteristics was followed. The technological diagram has been verified in the unit with the food safety team. Each technological stage was monitored until the finished product was obtained. Also, laboratory determinations were carried out regarding the freshness of the butter (organoleptic examination, acidity determination, Kreiss reaction). At the same time, a questionnaire was carried out on butter consumption among consumers.

The statistical study was carried out through the Google forms platform, a study in which 170 people participated. The participants expressed their opinion regarding the frequency and time of day they consume butter, up to opinions related to the price of butter on the Romanian market.

The freshness determination study involved the examination and analysis of 12 butter samples groups of 5 samples each group, stored under different temperature conditions: freezing (-18°C), refrigeration (4°C) and temperatures above 4°C, maintained for different time intervals namely: 0 days, 21 days, 30 days and 48 days. The duration of the study is March-July 2022.

Sampling was done by buying packages of butter with 82% fat content from a commercial unit.

Until the determinations were made, the butter samples were packed in non-vacuum polyethylene bags, after which they were labeled and stored in the three storage conditions previously mentioned.

Simultaneously with the study on the freshness degree of butter, the organoleptic properties were also assessed according to the storage conditions and time intervals, respectively, T_0=the moment of sample collection (day zero), day 21, day 30 and day 48 of storage. The following properties were appreciated: color, appearance, consistency, smell and taste.

RESULTS AND DISCUSSIONS

It has been shown that there is a direct correlation between the quality of the milk used in the production of dairy products and the quality of the finished products obtained from it.

Results and discussions on the technology of obtaining butter in the specialized unit

All raw milk comes from sanitary-veterinary authorized farms and is transported to the processing unit by isothermal tanks.

Results and discussion regarding the assessment of the freshness state by determining the acidity of butter

To determine acidity, 12 butter samples groups with a 82% fat content, stored in different environmental conditions, respectively, samples A0, A1, A2 and A3 were stored at refrigeration temperatures (4°C), samples B0, B1, B2 and B3 were stored at temperatures above 4°C, and samples C0, C1, C2 and C3 at freezing temperatures (-18°C).

The determinations were performed on each sample separately, and then the results obtained for each group of samples were averaged.

Following the acidity determination, it was found that 75% of the analyzed samples had acidity values below the maximum allowed for butter, namely 3.5°A being considered compliant products, and 25% recorded values above the maximum allowed limit (table 1, figure 3).

Table 1

Results regarding the determination of butter acidity

Sample	Storage period	Storage condition °C	Quantity taken for analysis	No ml NaOH use for titration	Acidity value	Result interpretation
A0	after manufacturing	4°C	5 g	0.2	0.4	compliant
A1	21 days	4°C	5 g	1.1	2.2	compliant
A2	30 days	4°C	5 g	1.4	2.8	compliant
A3	48 days	4°C	5 g	1.5	3.0	compliant
B0	after manufacturing	ambient temperature	5 g	0.2	0.4	compliant
B1	21 days	ambient temperature	5 g	2.4	4.8	non-compliant
B2	30 days	ambient temperature	5 g	2.6	5.2	non-compliant
B3	48 days	ambient temperature	5 g	2.7	5.4	non-compliant
C0	after manufacturing	-18°C	5 g	0.2	0.4	compliant
C1	21 days	-18°C	5 g	0.2	0.4	compliant
C2	30 days	-18°C	5 g	0.3	0.6	compliant
C3	48 days	-18°C	5 g	0.35	0.7	compliant

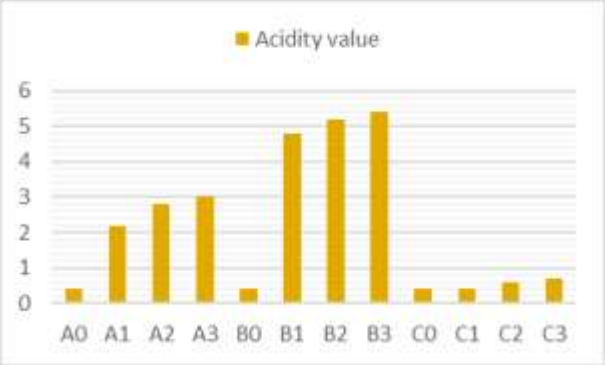


Figure 3 **The variation of butter acidity depending on the storage period and storage conditions**

With regard to the Kreiss reaction, all analyzed samples obtained negative results.

Results and discussion of organoleptic examination of butter according to storage conditions and different time intervals

The organoleptic examination of the butter indicated that:

- 91.7% of the total samples presented a clean appearance and 8.3% a partially clean appearance;
- 58.4% had a yellowish-white color; 33.3% had yellowish color and 8.3% had yellowish-blackish color;
- 66.7% had an unctuous consistency and 33.3% had an oily consistency;
- 50% kept their specific smell, 33.3% had a non-specific smell and 16.7% had a rancid smell;
- 66.7% had the specific taste, 8.3% neutral taste, 16.7% metallic/oily taste and 8.3% musty taste.

It is noteworthy that a percentage of 91.7% of the samples kept their clean appearance even when exposed to different environmental conditions and only 8.3% displayed a partially-clean appearance, most likely due to contamination with mold spores.

Results and discussion following the opinion poll on butter consumption

Following the opinion poll carried out by means of 170 participants, 9 essential questions related to the butter consumption were answered, questions such as the frequency and time of day in which it is consumed, the preferred category and fat content, but also the determining factor in choosing the butter assortment.

To the question "At what time of day do you generally consume butter?" 71.2% of participants consume butter in the morning.

48.2% of consumers preferred butter with 65% fat, 50% butter with 82% fat and 0.8% of participants preferred other types of butter.

Regarding the price of butter on the Romanian market, 51.2% of the participants mentioned that the purchase price is high.

CONCLUSIONS

The raw material milk used in the study unit comes from authorized farms and is subject to qualitative and quantitative examination.

In the study regarding the assessment of the freshness status of the butter, by determining the acidity over a predetermined period of time, using samples of butter stored in different temperature conditions, it was concluded that 75% of the samples had acidity values below the maximum allowed limit, being considered compliant samples, and 25% exceeded the maximum allowed value, being considered non-compliant. This aspect highlights the importance of complying with the storage conditions recommended by the manufacturers, as well as observing the thermal chain.

Following the opinion poll on butter consumption, attended by people of different ages and genders, it was concluded that 33.5% of the participants consume butter 4-5 times a month and only 1.8% do not consume butter. 71.2% of the questionnaire participants confirmed their preference for eating butter in the morning. The percentages were roughly equal in terms of consumers' preferred category of butter, with 49.4% preferring salted butter and 50.6% opting for sweet butter. 33.5% of 170 people chose the producing company as the determining factor in choosing butter.

REFERENCES

Arenas E., Gustafsson J.Å., Hall C., Hazenberg C., Hovatta O., Liste I., Parish C.L., Richter Ä.L., Sacchetti P., Sousa K.M., Steffenser K.R., Theofilopoulus S., 2009 - *Liver X Receptors and oxysterols promote ventral midbrain neurogenesis in vivo and in human embryonic stem cells*. Cell Stem Cell.

Arnfinnsson J., Bergsson G., Steingrimsson Ó., Thormar H., 2001 - *In vitro killing of Candida albicans by fatty acids and monoglycerides*. Antimicrob Agents Chemother. 45(11):3209-12. https://doi.org/10.1128/AAC.45.11.3209-3212.2001.

Borș A., Rusu O.R., Borș I., Floriștean V., 2018 - *Application of HACCP (hazard analysis critical control point) to cow telemea cheese production*. LSMV, 61:135-141. https://repository.uaiasi.ro/xmlui/handle/20.500.12811/1007.

Breazeanu C., Robu T., Trofin A., Brezeanu P., Murariu O.C., 2020 - *Research on the physico–chemical characterization of improved genotypes for Lycopersicon esculentem Mill. Obtained in the ecological system*, Lucrări Științifice, vol. 63 (1)/ 2020, seria Agronomie, pag. 95 - 100.

Campos H., Hu F.B., Mozaffarian D., Orav E.J., Rexrode K.M., Shi P., Willett W.C., Yakoob

M.Y., 2016 - *Circulating Biomarkers of Dairy Fat and Risk of Incident Diabetes Mellitus Among Men and Women in the United States in Two Large Prospective Cohorts*. Circulation. https://doi.org/10.1161/CIRCULATIONAHA.115.018410.

Cho H.Y., Kim Y.J., Lee H.J., Lee K.W., 2005 - *Role of the conjugated linoleic acid in the prevention of cancer*. Crit Rev Food Sci Nutr. 2005;45(2):135-44. https://doi.org/10.1080/10408690490911800

Choi K., Cohen L.A., Karmali R.A., Rose D.P., Thompson D.O., 1986 - *Dietary fat and mammary cancer, II: modulation of serum and tumor lipid composition and tumor prostaglandins by different dietary fats:association with tumor incidence patterns*. Journal of the National Cancer Institute,77:43-51. https://pubmed.ncbi.nlm.nih.gov/3459925/.

Crăciun O. C., Lazăr R., Boişteanu P.C., 2012 – *Research regarding the biological value of meat protein from sheep*, Scientific Papers Journal, Zootehnic Series, Iasi, ISSN 1454-7368, vol. 57: 56 – 60;

Dulce D., 2013 - *Operații și aparate în industria alimentară*, Proiect, Universitatea de Științe Agronomice și Medicină Veterinară Iași, Facultatea de Agricultură, specializarea: T.P.P.A, îndrumător științific Șef lucr. Dr.: Budui Costin.

Fallon S., Mary G., 2000 - *Why Butter Is Better*, The Weston A. Price Foundation. https://www.westonaprice.org/health-topics/know-your-fats/why-butter-is-better.

Florişţean V., Borş A., 2021 - *Igienă şi tehnologie alimentară. Laptele şi produsele lactate* – Manual practic, „Ed. Ion Ionescu de la Brad", Iaşi, ISBN 978-973-147-445-8.

Frey J., 2014 - *A brief history of butter*, The churncraft journal. https://churncraft.com/blogs/the-churncraft-journal/a-brief-history-of-butter.

Hackfort A., 2021 - *Your Complete Guide to the Different Types of Butter*, TasteofHome. https://www.tasteofhome.com/article/your-complete-guide-to-butter/.

King I.B., Lemaitre R.N., Marcia C.O.O., Siscovick D.S., Song X., 2018 - *Serial measures of circulating biomarkers of dairy fat and total and cause-specific mortality in older adults: the Cardiovascular Health Study*. The American Journal of Clinical Nutrition.

Murariu F., Voda A. D., Murariu O.C., 2019 - *Researches on food safety assessment - supporting a healthy lifestyle for the population from NE of Romania*. Journal of Biotechnology, vol.305, pp. s68-s68, Supplement.

Murariu O.C., Irimia L.M., Diaconu C.M., Murariu F., **2016** - *The right of t a healthy live provided by a healthy eating support*, The legal universe, Monthly Review, ISSN 2393 - 3445.

Oprea O.D., Petcu C.D., Ciobotaru-Pîrvu E., 2019 - *A study concerning quality assessment and processing particularities in certain dairy products.* Scientific Works. Series C. Veterinary Medicine. Vol. LXV (1), 121-126, ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295.

Oprea, O.D., Răduță, A., Călin (Nicolae), S., Andrei, C. 2020 - *Research on percentage variation concerning cow's milk protein, lactose and fat, depending on the season.* Scientific Papers. Series D. Animal Science, LXIII(1), 182-186.

Owen E., 2017 - *The Real Difference Between American and European Butter*, Travelandleisure. https://www.travelandleisure.com/food-drink/european-american-butter.

Petcu C., 2006 - *HACCP-Food safety guarantor*. Bucharest, RO: Idea Design Publishing House.

Petcu, C.D., Ciobotaru-Pîrvu, E., Oprea, O.D., Ghimpețeanu, O.M. 2020 - *Ecological dairy products: healthy or just a trend?* Scientific Works. Series C. Veterinary Medicine, LXVI (1), 87-95.

Petcu, C.D., Ghiță, M., Raba, D.N., Codreanu, I., Bălăceanu, R., Andrei, C., Mihai (Oprea), O.D. 2021 - *Research on the quantitative and qualitative evolution of sheep's milk during the lactation period.* Scientific Papers. Series D. Animal Science, LXIV (1), 462-467, ISSN 2285-5750; e-ISSN 2393-2260.

Petcu C.D., Gâjâilă G., Mihai (Oprea) O.D., Călin(Nicolae) Simona, Ghimpețeanu O.M., Codreanu I., Ciobotaru-Pîrvu E., 2022 – *Research on the influence of lactation stage on goat's milk characteristics.* Scientific Papers. Series D. Animal Science. Vol. LXV (1), 536-541, ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750.

Price W.A., 2010 - *Nutrition and physical degeneration: A comparison of primitive and modern diets and their effects*, Milton Keynes.

Ramdene H.B., 2019 - *What's the Difference Between European and American Butter?,* Kitchn. https://www.thekitchn.com/whats-the-difference-between-european-and- american-butter-229135.

Sala C.C., 2008 - *Igiena, tehnologia şi controlul laptelui şi a produselor derivate*. Timişoara, România, Editura Eurobit.

Savu C., Petcu C.D., 2002 - *Hygiene and control of products of animal origin.* Bucharest, România, Semne Publishing House.

Usturoi M.G., 2007 - *Tehnologia laptelui și a produselor derivate*, Editura Alfa, Iași, România.

Verraes C., Vlaemynck G., Van Weyenberg S., De Zutter L., Daube G., Sindic M., Uyttendaele M., Herman L., 2015 - *A review of the microbiological hazard of dairy products made from raw milk*. International Dairy Journal, 50:32-44.

Visoescu D.I., Petcu C.D., Tapaloaga D., 2015 - *Researches regarding the influence of packaging on the quality of some dairy products.* Journal of Biotechnology, 208, S19-S19.

Weston P., 2020 - *Know Your Fats*, The Weston A. Price Foundation. https://www. westonaprice. org/know-your-fats/.

www.insse.ro – Institutul Național de Statistică, Comunicat de presă Nr. 90, / 12 aprilie 2021.

ANAESTHETIC MANAGEMENT OF CANINE PATIENTS WITH MITRAL VALVE REGURGITATION UNDERGOING SOFT TISSUE SURGERY

Ruxandra Georgiana PAVEL[1], Roxana Maria TURCU [1], Ruxandra COSTEA[1],

e-mail (first author): tudor_ruxandra89@yahoo.com

Abstract

The purpose of this study was to establish the anaesthetic management of canine patients with mitral valve regurgitation that underwent soft tissue surgery and, also to understand the physiopathology of this cardiac disease and how anaesthesia might influence the outcome of this patients.
The study was conducted on 30 canine patients aged between 9-14 years old belonging to different breeds represented by mixed breed, Basset Hound, French Bulldog, Bichon and Pekinese.

Key words: anesthesia, geriatric dog, mitral valve disease

Mitral valve regurgitation it is represented by a degeneration of the atrioventricular valve of the left side of the heart. It is defined by regurgitant blood flow travelling from the left ventricle back into the left atrium during ventricular contraction (*Mattin et al, 2015*). Being a degenerative disease, it is more prevalent in older dogs, with studies reporting that approximately 30% of dogs over 10 years of age are affected *(Fox PR, 2012).*

Anesthetizing a patient with cardiac disease relies on the proper selection and interpretation of the tests performed during preanesthetic evaluation represented by: medical history, physical examination with special attention to cardiac auscultation followed by electrocardiography (EKG), radiography and non-invasive blood pressure measurement *(Hopkins A., 2014).*

In cardiac patients with mitral valve disease, any substance that causes a significant decrease in heart rate should be avoided, which causes an increase in regurgitation *(Fox PR, 2012).*

Opioids such as methadone and butorphanol in combination with acepromazine cause adequate sedation and analgesia. Whenever possible, induction of anesthesia should be performed under close monitoring and pre-oxygenation. In severe cases, it is recommended to use etomidate with minimal effects on the cardiovascular system *(Gaya S. et al., 2018).*

For stable patients, low doses of ketamine are a good alternative with benzodiazepines or low doses of propofol. Negative inotropic substances, such as propofol and thiopental may increase the regurgitation fraction in patients with severe pathology and should be used with caution *(Miller C., 2019).*

In order to maintain anesthesia, inhalator anesthetics can be used at the lowest possible concentrations. Another possibility would be to use total or partial intravenous anesthesia using propofol, fentanyl or ketamine at subanesthetic doses. In case of hypotension or bradycardia, the use of anticholinergics should be considered *(Robinson R., et al., 2018).*

For this purpose, the heart rate should be within the preanesthetic or slightly low values. If hypotension is not accompanied by bradycardia and does not return to normal values after the decrease in anesthetic concentration, inotropic positive substances should be used such as dobutamine *(Gaya S. et al., 2018).*

MATERIAL AND METHODS

The present study was performed on a number of 30 canine patients, aged between 9-14 years old, belonging to different breeds represented by mixed breed (5 dogs), Basset Hound (2 dogs), French Bulldog (5 dogs), Bichon (10 dogs) and Pekinese (8 dogs).

[1] University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Patients were anesthetized for various soft tissue surgical procedures represented by: mammectomy, ovariohysterectomy, orchidectomy. Physical examination, complete blood exams, Electrocardiography and radiography were also taken into consideration (Figure 1).

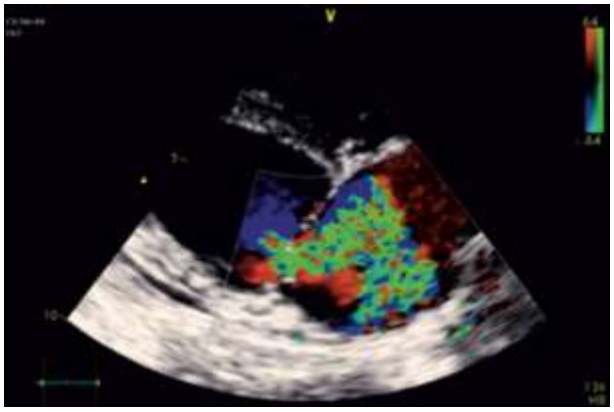


Figure 1: Colour Doppler examination in a dog with degenerative mitral valve disease - A right parasternal long axis view. The image shows the left atrium and ventricle - which are dilated

We included in the study patients who had a cardiac pathology represented by mitral valve disease in different stages of evolution and had an ASA (American Society of Anesthesiologists) score of III (Figure 2).

Figure 2: Bichon, 14 years old with mitral valve disease stage B2

Preanesthetic examination for all the patients was carried out in a quiet environment, to manipulate and stress out the patient as little as possible. In all cases, intramuscular administration of sedative substances was administered before approaching venous access in order to reduce patient stress along with preoxygenation.

Premedication was made with butorphanol 0.3 mg/kg and midazolam 0.2 mg/kg administered intramuscularly (IM). Anaesthesia was induced with Propofol (2 - 4 mg/kg IV). All patients were intubated and maintained with isoflurane. Crystalloid solutions were administered at 3-5 ml/kg/hour IV throughout anesthesia (*Costea, 2017).*

In all cases, patients were breathing spontaneous during anaesthesia which represents a low stress for the cardiovascular system compared to mechanical ventilation. (*(Gaya S. et al., 2018).*

Mechanical ventilation can lead to an increase in intrathoracic pressure followed by a compression of venous vessels and a decrease in venous return. Therefore, low pressure ventilation (approx. 12 cm H_2O) is recommended if we need to ventilate the patient.

Oxygen flow was initially delivered at 2 L/min with the vaporizer set to achieve a minimum alveolar concentration C% of 2.0% isoflurane within 10 minutes of induction (*Tudor R.G. et al., 2018*).

After the target concentration was achieved, oxygen flow was decreased to (500+10/kg) ml/min, and isoflurane was constantly maintained at 1.5 vol. % in all cases.

Electrocardiography, heart rate, end tidal concentration ($EtCO_2$), blood haemoglobin saturation (SpO_2) and esophageal temperature were monitored. Temperature was maintained between 38ºC-38.6ºC by using a warm electrical blanket (Figure 3).

Figure 3: Monitoring during anaesthesia

At the beginning of the surgery, during skin incision if the patient responded to surgical stimulation by a rapid increase of the heart rate, mean arterial pressure or had any signs of tachypnea additional analgesic and anaesthetic drugs boluses were given: Ketamine 1 mg/kg IV.

RESULTS AND DISCUSSIONS

In our study, from the total number of 30 canine patients (Figure 4), 13 had a mitral valve disease in stage B1 (43.3%) and 17 patients had a B2 stage of mitral valve disease (56.6%) (Figure 5,6).

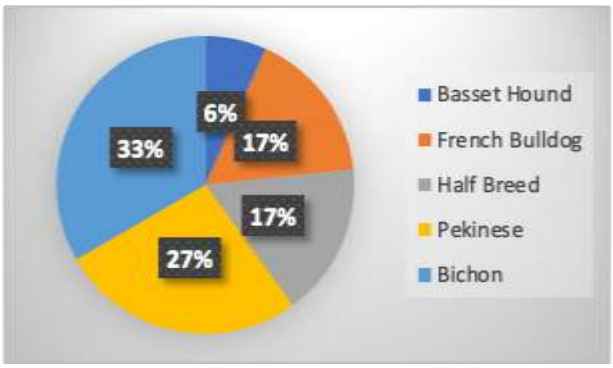


Figure 4: Patients included in the study

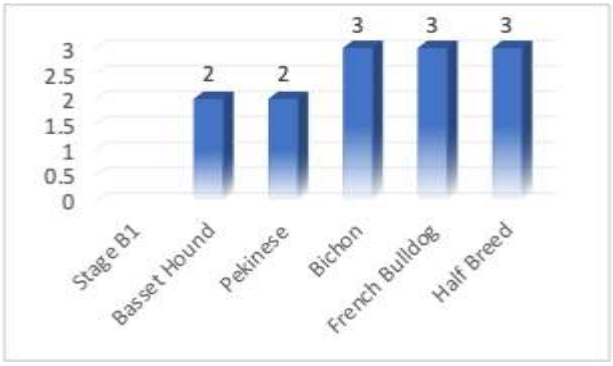


Figure 5: Patients with mitral valve disease in stage B1

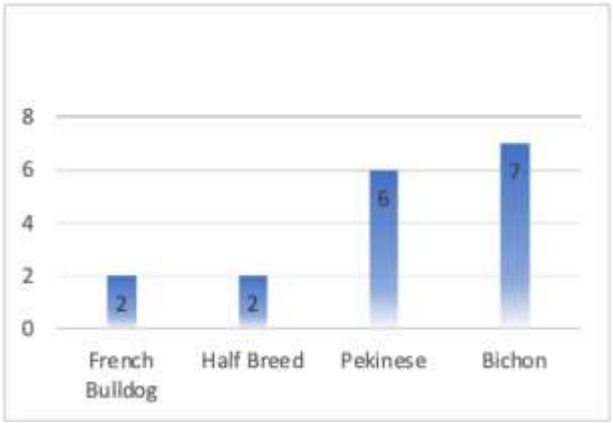


Figure 6: Patients with mitral valve disease in stage B2

Stage B1 refers to patients that have heart disease, which is characterized by a typical mitral regurgitation murmur, but they do not have an enlarged heart or clinical signs of the disease.

Stage B2 is referring to patients where evidence of cardiac enlargement is present, but we still do not have any clinical signs of the disease *(Ljungvall et al, 2014).*

In the induction phase, 5 minutes before propofol administration, all dogs were preoxygenated to reduce myocardial hypoxia and prevent apnea.

From the total of 30 patients included in the study, 2 dogs (6.7%) developed apnea in the induction phase at a propofol dose of 3 mg/kg and needed to be manually ventilated till they began to spontaneously breathe (approx. 5 minutes).

The purpose of the entire anaesthesia protocol was to maintain the cardiovascular system stable. Because of the cardiovascular depression achieved by most anaesthetic substances, changes in the cardiac function and pulse compared with an unanesthetized patient are inevitable but should be minimal as a result.

During surgery, 3 female dogs (10%) who had ovariohysterectomy required a bolus of ketamine of 1 mg/kg IV for analgesia supplement while reaching the ovary ligament due to a rapid increase in heart rate and blood pressure.

The postoperative recovery period is critical for all animals with approximately 47% of anaesthetic-related deaths occurring during this period (*Brodbelt et al, 2008*).

During recovery phase, all dogs received buprenorphine at a dose of 20 mcg/kg IV. They were closely monitored and received supplemental oxygen as well as continuously monitoring the heart rate, blood pressure and temperature (Figure 7) and recovered without any complications.

Figure 7: Oxygenation of the patient in the recovery period

CONCLUSION

Before carrying out anesthesia in a cardiac patient it is very important to diagnose the type of the condition and to start the medication in unstable cases.

The entire perianaesthetic period should be least stressful for the patient. Obtaining venous access, intubating the patient, and providing oxygen are indispensable measures for any cardiac patient to be anesthetized.

For most patients studied, premedication with an opioid and a benzodiazepine and induction of anesthesia with propofol at low doses is the right choice.

Anesthesia monitoring should include clinical monitoring, electrocardiography, blood pressure, capnography, and pulse oximetry.

REFERENCES

Ay T., (2002), Anesthesia of patients with cardiac disease, In: Veterinary anesthesia and pain management secrets,(Ed.) Hanley&Belfus, Philadelphia, USA, 157-164

Beardow A.W., Buchanan J.W., (1993), Chronic mitral valve disease in Cavalier King Charles Spaniels: 95 ca- ses (1987-1991). J Am Vet Med Assoc, 203:1023-1029

Borgarelli M, Buchanan JW (2012). Historical review, epidemiology and natural history of degenerative mitral valve disease. J Vet Cardiol. 2012;14(1):93 101.https://doi.org/10.1016/j.jvc.2012.01.011

Costea, R., Degan, A., & Tudor, R. (2017). Crystalloids/colloids ratio for fluid resuscitation during anesthesia. *Scientific Works. Series C. Veterinary Medicine*, *63*(1), 65-66.

Detweiler D.K., Patterson D.F., (1965), The prevalence and types of cardiovascular disease in dogs. Ann New York Acad Sci, 127:481-516

Evans A.T., (1992), Anesthesia for severe mitral and tricuspid regurgitation. Vet Clin North Am Small Anim Pract, 22:465-466

Fox PR. Pathology of myxomatous mitral valve disease in the dog. J Vet Cardiol. 2012;14(1):103–126. https://doi.org/10.1016/j.jvc.2012.02.001

Goya S, Wada T, Shimada K, Hirao D, Tanaka R. (2018) Dose-dependent effects of isoflurane and dobutamine on cardiovascular function in dogs with experimental mitral regurgitation. Vet Anaesth Analg. 2018;45(4):432–442. https://doi.org/10.1016/j. vaa.2018.03.010

Hopkins A, Giuffrida M, Larenza MP. (2014) Midazolam, as a co-induction agent, has propofol sparing effects but also decreases systolic blood pressure in healthy dogs. Vet Anaesth Analg. 2014;41(1):64–72. https://doi.org/10.1111/vaa.12088

Miller C, Hughes E, Gurney M. (2019) Co-induction of anaesthesia with alfaxalone and midazolam in dogs: a randomized, blinded clinical trial. Vet Anaesth Analg. 2019;46(5):613–619. https://doi.org/10.1016/j.vaa.2019.03.009

Muzi M., Berens R.A., Kampine J.P., Ebert T.J., (1992), Venodilation contributes to propofol-mediated hypo- tension in humans. Anesth Analg, 74:877-883

Robinson R, Borer-Weir K. (2013) A dose titration study into the effects of diazepam or midazolam on the propofol dose requirements for induction of general anaesthesia in client owned dogs, premedicated with methadone and acepromazine. Vet Anaesth Analg. 2013;40(5):455–463.https://doi.org/10.1111/vaa.12052

Tudor R.G., Degan A., Costea R., Predoi G., (2018), Post-operative analgesic management of geriatric dogs that underwent soft tissue surgery. Scientific Works. Series C. Veterinary Medicine, 64(2):82-87

CORN PROTEIN CONTENT – A COMPARISON BETWEEN METHODS, ANALYSORS AND FARMERS EXPECTATIONS

Bianca Cornelia LUNGU[1,2],Catalin IONESCU[1], Beatrice Ana-Maria TUDOR[2], Ovidiu Ionut GEORGESCU[2], Calin MIRCU[1,2*], Ioan HUTU [1,2]

* e-mail of corresponding author: calin.mircu@usab-tm.ro

Abstract

In a process of problem solving and diet formulation the analysis offeed ingredients' proteins have to be done accurately, rapidly, cheep and if is possible at the farm level. As a general rule the accurate methods last too long and real time methods are not so exactly. The study is doing the comparison between the results of three methods 1. Classical chemistry (*Kjeldahl*method*)*, 2. Near infrared spectroscopy analysis (NIR) and 3. By FT-NIR spectrometer used for analyzing the corn's protein content. The "reference sample" was considered the result obtained from classical chemistry - Kjeldahl method and the comparison was between classical, NIR and FT-NIR methods, for corn's grains and corn's flour. Each measurement was performed twice and no significant difference was found between repetitions ($p<0.00$). The average protein content in corn for 39 samples was 7.82±0.16% by Kjeldahl method, by NIR equipment, 7.47±0.17% in corn's grains and 7.57±0.15% in corn's flour and by FT-NIR equipment, 8.40±0.29% in corn's grains and 7.80±0.12% in corn's flour.Comparing the results of NIR and FT-NIR measurements with Kjeldahl method results significant differences between first measurement (F=3.625 at p =0.007), second measurement (F=3.255 at p =0.013) and average measurements (F=3.486 at p =0.009). The smaller differences between reference results was in case of FT-NIR in corn's flour (0.02%) but were no significant differences by Turkey test comparison for any of NIR, FT-NIR, grains or flour. In conclusion, irrelevant to the method or equipment used for measurements it appears more feasible to run the samples at farm level.

Key words: Kjeldahl, NIR, FT-NIR, protein, corn

.

The corn is the most used feed ingredient in swine and dairy industry even in pasture systems (Onan G., Huțu, I., 2016 Onan G., Huțu, I., Radunz, 2016). During several farm visits, in a process of problem solving the evaluation of diet and correction of diet weakness the evaluation and analysis of feed ingredients have to be done accurate, cheep and if is possible "in real time" at the farm level. On the practice of farm visit the extension service (Hutu I., 2004) have two variants: One is to take the sample, to send those to the Animal Nutrition Laboratory and to do the diet formulation in a high specialized software (*Brill*® family software) according with the result of laboratory analysis. Another one is to do the analysis in the frame of two hours during farm visit – in this case the ultra rapid analyses need it, regularly there are used near infrared spectroscopy analysis by portable NIR equipments.

All time between speedy and accuracy of results appears to be a contradictory relation. As a general rule the accurate methods take long time and speedy methods are not so exactly, and the time and all inexactitudes generate the additional costs for farm and decreases in gross margin for the production.

For all those reasons the extension service has to be sure that the result used is as close as possible with the reality of the farm (Matiuti, 2017). The study take into consideration the most expensive nutrient-the proteins form corn and the analysis of it. The study is performing the comparison between the results of three methods of protein content in corn: 1. classical (humid) chemistry – by *Kjeldahlmethod* and 2. by near infrared spectroscopy analysis with two types of equipments- one form *Foss InfraXact*® and the other form *Bruker Optics*®.

The specific objective of this report was to establish which method is feasible for "real time analysis" of protein content at the farm level – being the case of protein content of corn as a row material in two forms - grains and flour.

MATERIAL AND METHOD

Corn sample collection and preparation: 40 sample of 300 g corn grains (several variety such as Fundulea, KWS®, Pioneer ® and possible other varieties) was sampled from 85 partner farms of Extension unit were collected during farm visit of extension service during fall and winter season in West Romania. Each sample was divided in three: 1/3 for classical chemistry, 1/3 for near infrared

[1]*Horia Cernescu* Research Unit – USV Timisoara, RO.
[2] University of Life Science "*The King Michael I*" – Faculty of Veterinary Medicine, Timisoara, RO.

spectroscopy analysis (NIR) as a grain and other one 1/3 was milled for using for NIR as flour. In case of classical chemistry, before milling the grain was dryedin a drying stove at 105°C for 5 to 8 hours, depending on humidity of row samples. For NIR and FTNIR methods, in order to keep the humidity and the proportion of chemical components the milling was done in *Foss Cyclotec^{TM} 1093*grinding mill with same sieve.

Analysis of protein content was done for each sample two times for each three methods/equipments:

The first method used was classical *Kjeldahl* (humid chemistry), by fully automated *Kjeldahl^{TM}8400 Analyzer Unit (FOSS Hillerød 3400, DK)*from Animal Nutrition Laboratory – *Horia Cernescu Research Unit (figure 1).*

The second method was near infrared Near-infrared reflectance spectroscopy(NIR) with equipment *Foss InfraXact*® (Hillerød 3400, DK). The *Foss InfraXact* specters were developed for the accurate and fast prediction by equipment producers' calibrations *(figure 2)*. The equipment was on electrical power independent laboratory (Auto laboratory of Animal Production).

The third equipment was a FT-NIRspectrometer (*BrukerMatrix I ®,* Boston, MA, SUA) with INGOT® calibrations(multiple calibration dairy package, swine and pet food).

NIR spectroscopy is an outstanding tool due to its simple operation, short analysis time, small sample requirement, low-cost and non-destructive measurement (Haughey et al. 2008; Spragg, 2017; Yang et al. 2013).

Theoretically, the differences between equipments are given by differences between NIR and FTNIR (signal Fourier-transformed): IR spectroscopy gets absorption of a monochromatic IR light at a time and draws the spectrum, whereas, in FTIR, multi-chromatic takes a summarized absorption of light and distributes it to create a spectrum using FTIR.

By specialized noncommercial sources (Burns & Ciurczak, E.W. 2011, *Hussain et all, 2019*) there are some points of difference between IR and FTIR such as:

- NIR takes a single spectrum -FTIR takes a number of scans.
- NIR used monochromatic light whereas FTIR used polychromatic light.
- FTIR scans up to 50 times in a minute and giving better resolution.
- FTIR is a fast technique than IR.
- Sample preparation in the traditional IR is time-consuming, while nowadays in FTIR uses Attenuated Total Reflection (ATR). Therefore, there is no need to prepare the sample.

So, according to several suppliers the FT-NIR spectrometers are more modern, given rapid analysis, high quality spectrum, reproducibility, ease of use and low maintenance.

Figure 1. ***Kjeldahl^{TM}8400 Analyzer Unit,* Animal Nutrition Laboratory**

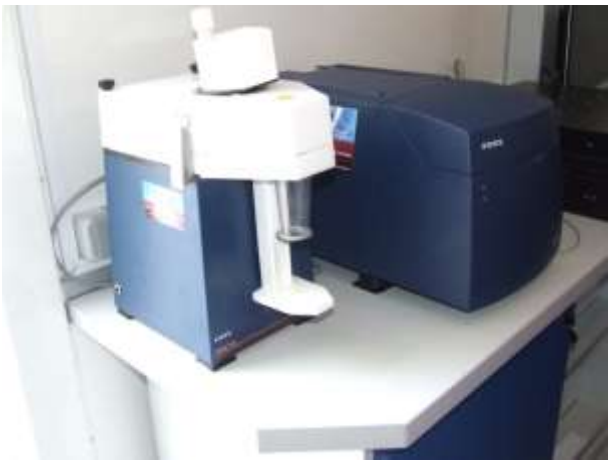


Figure 2. **NIR *Foss InfraXact*® in the Animal Production auto laboratory**

Statistical Analysis: Analysis of external results – protein values - were performed using one way *Analysis of Variance (ANOVA)*for the classical chemistry, NIR and FT-NIR equipments. For NIR and FT-NIR grain and also flour samples were used. The "reference sample" was considered the result obtained from classical chemistry - Kjeldahl method and the comparison was between classical and NIR and FT-NIR methods, for corn's grains and corn's flour. Also the comparison between first and second measurements was performing by *Student test* (paired samples).

RESULTS AND DISCUSSIONS

The measures were performed twice on each method – classical chemistry, NIR and FTNIR and a average between was also done.

The average protein content in corn for 39 samples was 7.82±0.16% by classical chemistry *Kjeldahl*, 7.47±0.17% by NIR in corn's grains, 7.57±0.15% by NIR in corn's flour, 8.40±0.29%

by FT-NIR in corn's grains and 7.80±0.12% by FT-NIR in corn's flour.

The results of the study show significant correlations between measurements but no significant difference between repetitions. All correlations between the first and the second measurements was significant (p<0,001): in generally, the correlations was higher in classical chemistry (r = +0.990, at p =0.00) and for milled samples (r = +0.990, at p =0.00 for NIR and r = +0.944, at p =0.00 for FTNIR method). The differences between the first and the second measurements was higher in corn grains, but not significant statistically (0.082±0.06% at p=0.205 for NIR and 0.127±0.11% at p=0.273 for FTNIR method).

As a first observation the variability of measurement is smaller in classical chemistry (in terms of standard deviation, SD = 0.147%) and milled sample (corn's flour) both NIR (SD=0.147%) and FTNIR methods (SD=0.264%).

Comparing the results of NIR and FT-NIR, for grain and flour measurements with Kjeldahl method results, the study found the significant differences between the first measure (F=3.625 at p =0.007), the second measure (F=3.255 at p =0.013) and the average of both measurements (F=3.486 at p =0.009).

Comparison of classical Kjeldahl with NIR and FTNIR by *Student* test, the differences between averages of measurements are significantly in case of NIR for corn's grains (*paired difference* = 0.35±0.10, t=3.526 at p =0.001) and corn's flour (*paired difference* =0.25±0.05, t=5.178 at p =0.000)and FTNIR for corn's grains (*paired difference* = -0.57±0.22, t=-2.555 at p =0.015). The study statistics underline the accuracy of FTNIR for corn's flour with classical results (*paired difference* =0.23±0.07, t=0.302 at p =0.764). In our study the tendency of NIR method was to overestimate the protein percent and underestimate the protein percent for FTNIR form corn's grain. The best estimation was obtained after milling the sample in FTNIR method.

Regarding the time, the most time consuming is classical chemistry: draying 5-8 hours, milling, weighting (1/4 hour), mineralization (6 hours), cooling, distillation and titration (1/2 hour) that means 2-3 working days in the lab. In case of using NIR and FTNIR equipment just 5 minutes, the time for spectrums collection, are needed. If the flour is use as a material the time for milling increases with maximum 10 minutes.

Conventional methods of analysis for quality control involve time consumption, a lot of labour, expensive procedures and chemical reagents (Cozzolino, 2012, Karoui et al., 2010, Weeranantanaphan et al., 2011).

In conclusion, by farmer expectations, time consumption, preparation of sample (grains or flour) and speed of obtained results, the use of any instruments (NIR and FTNIR) at the farm level is better than taken sample for analysis in laboratory.

By the accuracy of results the milled samples and FTNIR equipment is better but NIR equipment is robust and easy to work in the farm conditions. Even the differences between estimations of corns' flour are significant (average NIR estimation = 7.57±0.16%, average FTNIR estimation = 7.80±0.12, *paired difference* = -0.22±0.06, t=-3.682 at p =0.001) practically, the value of analysis can be applicable at farm level. Like in other study, the results indicated that corn nutritive values could be fast and accurately predicted by NIRS (Yang et al., 2011). In conclusion, in extension service, any portable NIR or FTIR instruments with adequate calibrations are preferable for analyzing quality and safety of cereals (Hussain *et al.* 2019) to time consuming classical chemistry.

CONCLUSIONS

The results for protein content in corn have a limited variability given by type of raw material (corn's grain or corn's flour) and type of the used method (Kjeldahl, NIR or FT-NIR).

By theoretically point of view the differences are significant but by practical point of view in extension services, the time to obtain the results is more important than accuracy of the results.

In extension service conducted in animal farms, any portable NIR or FTIR equipment with adequate calibrations is preferable to time consuming classical chemistry.

ACKNOWLEGMENTS

The research was financed by Extension Unit, ONG, in Research Contract no 6584 from 19.10.2015 – *Const analysis and evaluation of needs in Horia Cernescu Reseach*. The authors acknowledge to the Faculty of Veterinary Medicine students (Aurel Nies, Adelina Proteasa and Madalina Serb) for their volunteer activities, to the Animal production Lab and to Animal nutrition lab personnelfor work and collaboration during trial period.

REFERENCES

Burns, D.A. Ciurczak, E.W. *Handbook of Near-infrared Analysis*(2008), 10.1021/ja015320c

Cozzolino D.,*Recent trends on the use of infrared spectroscopy to trace and authenticate natural and agricultural food products***,** Applied Spectroscopy Reviews, 47 (2012), pp. 518-530.

Haughey S.A., Graham S.F., Cancouët E., Elliott C.T.,*The application of Near-Infrared Reflectance Spectroscopy (NIRS) to detect melamine adulteration of soya bean meal*, Food Chemistry, 2011, 136:1557-1561

Hussain N., Sun, D.W., Pu., H.B.,*Classical and emerging non-destructive technologies for safety and quality evaluation of cereals: A review of recent applications*, Trends in Food Science & Technology, 2019, 91:598-608.

Karoui R., Downey, G., Blecker Ch., *Mid-infrared spectroscopy coupled with chemometrics: A tool for the analysis of intact food systems and the exploration of their molecular structure-quality relationships — A review***,** Chemical Reviews, 110 (2010), pp. 6144-6168.

Matiuti M., Hutu, I., Diaconescu D., Sonea C., *Rural Pole for Competitivity: A Pilot Project for Circular Bioeconomy*, Journal of Environmental Protection and Ecology, 2017, 18(2):802–808

Onan G., Huțu, I.,*Grass-fed beef,* Volume: : Lucrări ştiinţifice medicină veterinară, 2016, 49(1): 142-153, Agroprint Timisoara, Romania. DOI: 10.13140/RG.2.2.14605.90083

Onan G., Huțu, I., Radunz, A.,*Residual feed intake as a measure of feed efficiency,* Volume: Lucrări ştiinţifice medicină veterinară, 2016, 49(1): 154-162, Agroprint Timisoara, Romania. DOI: 10.13140/RG.2.2.28027.67365

Spragg, R.A. *Encyclopedia of spectroscopy and spectrometry***,** (2017), 10.1016/B978-0-12-803224-4.00088-1

Weeranantanaphan J., Downey G., Allen P., Sun D.W., *A review of near infrared spectroscopy in muscle food analysis: 2005–2010.* **J** Near Infrared Spectroscopy, 19 (2011), pp. 61-104.

Yang X.S., Wang L.L., Zhou X.R., Shuang S.M., Zhu Z.H., Li, N. *et al., Determination of protein, fat, starch, and amino acids in Foxtail Millet[Setariaitalica (L.) Beauv.]* by Fourier transform near-infrared reflectance spectroscopy, Food Science &Biotechnolgy, 2013, 22:1495-1500.

Yang, F., Xie C., He, D.,"*Analysis and Estimate of Corn Quality by Near Infrared Reflectance (NIR) Spectroscopy*," 2011 Symposium on Photonics and Optoelectronics (SOPO), 2011, pp. 1-4, doi: 10.1109/SOPO.2011.5780611.

.

OBSERVATIONS ON SENSITIVITY AND *AMR* OF *E.coli* IN PIGS AND HUMANS

Bianca Cornelia LUNGU[1,2], Beatrice Ana-Maria TUDOR[2], Ovidiu Ionut GEORGESCU[2], Irina SPATARU[2], Iuliu TORDA[1,2], Călin MIRCU[1,2], Ioan HUȚU[1,2]*

e-mail: calin.mircu@fmvt.ro

Abstract

Antimicrobial resistance of pathogenic *E. coli* impacts the swine industry due to the limited treatment options and growing public health concerns caused by potential transfer of antimicrobial resistance genes into the food chain. The study was carried out throughout the whole year; the samples were collected from the small intestine following necropsies performed in young pigs. From a laboratory database, 78 samples were positive for *E. coli* and were sampled and processed in order to describe the AMR following a classical microbiological exam. Each sample was cultured on selective media (Mac Conkey Agar) and antibiograms were performed using MicroScan Walk Away System. The antibiogram examinations were performed for 20 antibiotics and the results were presented in the form of the following categories: resistant, intermediate, susceptible. In 4 of the 20 antibiotics tested, the bacterial agent showed a sensitivity of over 85% and in 6 of the 20 antibiotics, it showed resistance of over 85%. On average, 47.8±37% of *E. coli* showed sensitivity to the 20 antibiotics tested and 48.5±38% resistance; 3.2±4% were classified as "intermediate". Similarities were noted in terms of sensitivity and resistance between the antibiograms from animal *E.coli* versus human *E.coli*. The same sensitivity was observed in 5 of the 20 antibiotics, and in 3 of the 20 antibiotics we noticed common resistance, but a future molecular biology analysis will be performed in order to identify the genes associated with AMR.

Key words: *antibiograms, E. coli, pig industry, AMR*

Introduction

Escherichia coli produces a series of diseases among pigs, generating serious losses both at the farm level and through possible transmission to humans. Swine colibacillosis is a multifactorial syndrome caused by *Escherichia coli* which exhibits three main disease conditions, namely, oedema disease, neonatal diarrhoea and post-weaning diarrhoea. Each of them can be differentiated through their pathogenesis, age-range of the affected animals, and the involved pathotype: Shiga toxin–producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), and atypical enteropathogenic *E. coli* (aEPEC). *(García-Meniñoet et a, 2020; Abubakar R. H. et al, 2017; Fairbrother J. M. et al, 2012, Moga et al. 2001)*

Antimicrobial resistance of pathogenic *E. coli* impacts the swine industry because of limited treatment options and growing public health concerns due to the potential transfer of antimicrobial resistance genes into the food chain. *(Fairbrother J. M. et al, 2012; MISUMI W. et al, 2021; Borow et al, 2022)*

The aim of the research was to describe in a transversal survey the sensitivity and resistance of *E. coli* to 20 types of antibiotics. The similarity between human and animal *E. coli* cases was also observed

Materials and methods

Animals sampling: The farm – a unit specialised in pig farming with a closed circuit. By clinical anamnesis of young pigs (aged between 4 and 11 weeks), the cadavers were selected for necropsy. Only cases with morphopathological signs were selected for microbiological exam. The samples were taken from the small intestine after the necropsy examination.

One sample was taken from an employee of the farm (who had direct contact with animals). The urine sample was taken because of their very frequent urinary infections, and then processed at

[1] University of Life Science "*The King Michael I*" – Faculty of Veterinary Medicine, Timisoara, RO
[2] *Horia Cernescu* Research Unit – USV Timisoara, RO.

an accredited laboratory. The result of the analysis was the infection with *E. coli*, which is the reason for some differences observed in the results of antibiograms (see table 1 - 77 vs. 78 results).

Microbiology analysis by C*OPAN's ESwab™* system was conducted for all collected samples. The samples were sent and processed in the laboratory 12 hours after sampling. The germs were isolated by classical microbiological exam. Each sample was cultured on selective media (*Mac Conkey Agar - figure 1*), and after, placed in a thermostat for 24 hours for incubation. From the resulting culture, after identifying and confirming the existence of the bacteria by macroscopic examination, antibiograms were performed. The types of germs and antimicrobial sensitivity and resistance were analysed by *Walk Away System (figure 2)* using *MicroScan® Dried Panels – type NBC-42. (Cubin et al, 2022, Lungu, 2020)*

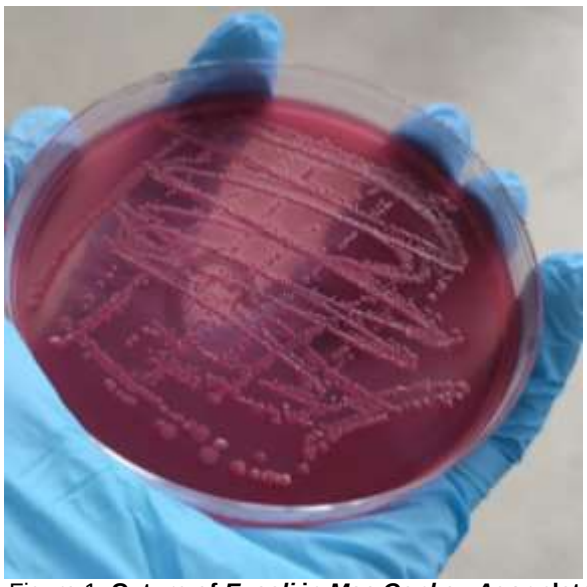


Figure 1 **Cuture of *E. coli* in *Mac Conkey Agar* plate**

Figure 2 **Entering data into the system and issuing the barcode for each sample**

Panels for human use were used because the transmission of the AMR phenomenon from animals to humans was assessed. Part of the bacterial culture obtained was stored in the freezer for subsequent genetic determinations. The information from the antibiograms was printed, recorded and processed. The antibiogram analyses were performed for 30 antibiotics, however only the results for 20 antibiotics were used in the present manuscript.

Statistical analysis - From a laboratory database, 78 samples positive to *E.coli* were chosen and processed in order to describe the AMR following a classical microbiological exam, and the microbiological cut-off of 20 from 30 antibiotics in following categories: resistant (R), intermediate (I), susceptible (S).

Results and discussion

The output was printed and analysed. After centralising the data obtained from *Walk Away System* (antibiograms) there results are shown in the table 1:

On average, 47.8±37% of *E. coli* showed sensitivity to the 20 antibiotics tested and 48.5±38% resistance; 3.2±4% was classified as "intermediate". Practically, for half of the antibiotics, resistance can be discussed and for the other half, sensitivity, with both classes having a rather large variability of 37-38%.

Considering the results obtained in the antibiograms (table 1), we can observe the following:

- the bacteria show sensitivity in 78/78 (100%) antibiograms to Amikacin and Meropenem, in 69/78 (88,5%) antibiograms to Pip/Tazo and in 74/77 (96,1%) antibiograms to Tigecycline.
- *E. coli* shows resistance in 76/78 (97,4%) antibiograms to Ampicillin, in 73/78 (93,5%) antibiograms to Ciprofloxacin, in 72/78 (92,3%) antibiograms to Levofloxacin, in 75/77 (97,4%) antibiograms to Mezlocillin, in 77/77 (100%) antibiograms to Moxifloxacin, in 67/78 (85,9%) antibiograms to Trimeth/Sulfa, in 58/78 (74,35%) antibiograms to Piperacillin and in 52/77 (67,5%) antibiograms to Tetracycline.

Table 1

Pharmacological and Microbiological cut-off of *E.coli* for 20 antibiotics

Antibiotic and pharmacological cut-off(*mg/l*)	Microbiological classes		
	Susceptible	**Intermediate**	**Resistant**
Amikacin (S≤16)	78	0	0
Amox/K Clav(S<=8/4;I 16/8; R>16/8)	53	14	11
Ampicillin(I 16; R>16)	0	2	76
Cefuroxime(S<=4; I 16; R>16)	33	2	43
Cefazolin(S<=8; I 16; R>16)	29	8	40
Cefepime(S<=8; R>16)	52	0	25
Cefoxitin(S<=8; R>8)	61	0	16
Ciprofloxacin(S<=2; I 2; R>2)	1	4	73
Fosfomycin(S<=32; R>32)	75	0	3
Gentamicin(S<=4; I 8; R>8)	43	4	31
Levofloxacin(S<=2; I 4; R>4)	3	3	72
Meropenem(S<=1)	78	0	0
Mezlocillin(I 64; R>64)	0	2	75
Moxifloxacin(R>1)	0	0	77
Pip/Tazo(S<=16; I 64; R>64)	69	5	4
Piperacillin(S<=16; I 64; R>64)	18	2	58
Tetracycline(S<=4; R>8)	25	0	52
Tigecycline(S<=1; I 2)	74	3	0
Tobramycin(S<=4; I 8; R>8)	42	1	34
Trimeth/Sulfa(S<=2/38; R>2/38)	11	0	67
Averages(n/78)	**47,8%**	**3,2%**	**48,5%**
Standard Deviation	**37%**	**4%**	**38%**

Similarities were noted in terms of sensitivity and resistance between the antibiograms from animal *E. coli* versus human *E. coli*. The same sensitivity was observed for Amikacin, Amox/K Clav, Meropenem, Pip/Tazo. There was also noted a common resistance to Ampicillin, Piperacillin and Tetracycline.

Antibiotics such as Florfenicol, Marbofloxacin, Enrofloxacin, Amoxicillin, Penicillin, Lincomycin and Tylosin are largely used in the pig industry. They are used both in individual and collective treatments, both *per os* and administered parenterally.

In 2021, Wang et al. studied the mechanisms of Tigecycline resistance in *Enterobacteriaceae* from a pig farm in China. The results of the study show that although tigecycline has not been approved in animals, tigecycline resistance mediated by the *tet*(A) variant and *tet*(X) via plasmid or ICE was observed on this pig farm. The use of tetracyclines such as doxycycline, one of the most used antimicrobial agents in food-producing animals in China, may be the reason for the emergence and transmission of tigecycline resistance. Appropriate measures should be taken to limit tigecycline resistance in animals. (Wang J. et al, 2021)

In 2021, Gruel et al. have tried to understand antimicrobial use in pigs, beef cattle, and poultry on farms on Guadeloupe, French West Indies, and to acquire data on AMR in *Escherichia coli* in these food-producing animals. This study provided the first baseline information on levels of antimicrobial use, on the dynamics of phenotypic and genotypic resistance to tetracyclines, and on ESBL- *E. coli* in small-scale pig, beef cattle, and poultry production on Guadeloupe. Despite rational use of antimicrobials, *E. coli* resistance to third-generation cephalosporins was found on these farms. Mechanisms other than selective pressure of these antimicrobials in the emergence of AMR remain to be elucidated. (Gruel G. et al, 2021)

In 2018, Lugsomyaet al. studied antimicrobial resistance (AMR) profiles in commensal *Escherichia coli* derived from healthy fattening pigs in Thai farms that used prophylactic antimicrobials (in-feed tiamulin fumarate and amoxicillin) [PAs], therapeutic antimicrobials (injectable enrofloxacin or gentamicin) [TAs], or no antimicrobials [NAs].The conclusions were that although *E. coli* isolates from all farms contained a

core set of resistance to b-lactams and tetracyclines, the routine use of PA increased resistance rates to other important antimicrobials. (Lugsomya K. et al, 2018)

In 2019, Pirolo et al. studied the unidirectional animal-to-human transmission of methicillin-resistant *Staphylococcus aureus* ST398 in pig farming. Given the potential exchange of MRSA between animals and humans, and the infection risk associated with human colonisation by MDR LA-MRSA, the study was conducted to investigate: (i) the prevalence, genetic characteristics and antimicrobial resistance profile of MRSA isolated from swine farm workers in southern Italy, and (ii) the genome-based relatedness of human and animal MRSA isolates, for a better understanding of MRSA transmission to professionally-exposed farm workers. It was observed that workers employed in farms with an intensive type of breeding had higher MRSA carriage rates compared with workers employed in farms that adopted a non-intensive breeding system (27.1% vs. 0%). (Pirolo M. et al, 2019)

In 2022,Torres et al. found high similarity of macro-restriction patterns for isolates of *Yersinia enterocolitica* 4/O:3 obtained at a pork production chain from Minas Gerais, Brazil. They proposed to determine the clonality and the antibiotic resistance profiles of a subset of these isolates (n = 23) and human clinical isolates (n = 3). The close genetic relationship amongst *Y. enterocolitica* obtained from a pork production chain and human clinical isolates in Brazil was confirmed, and the role of swine in the potential transmission of an antibiotic-resistant clones of a pathogenic bio-serotype to humans, or the transmission of these resistant bacteria from people to animals, was highlighted. (Torres Furtado Martins B. et al, 2022)

CONCLUSIONS

- *E. coli* had sensitivity in 78/78 (100%) antibiograms to Amikacin, Meropenem, 69/78 (88.5%) antibiograms to Pip/Tazo and in 74/77 (96.1%) antibiograms to Tigecycline.
- *E. coli* shows resistance in 76/78 (97.4%) antibiograms to Ampicillin, 73/78 (93.5%) antibiograms to Ciprofloxacin, 72/78 (92.3%) antibiograms to Levofloxacin, 75/77 (97.4%) antibiograms to Mezlocillin, 77/77 (100%) antibiograms to Moxifloxacin, 67/78 (85.9%) antibiograms to Trimeth/Sulfa, 58/78 (74.35%) antibiograms to Piperacillin and 52/77 (67.5%) antibiograms to Tetracycline.
- Studied *E. coli* had similarities in terms of sensitivity and resistance to tested antibiotics, probably as a result of the transmission of these resistant *E. coli* from pigs to humans.

Acknowledgement

The research was carried in the Animal Production Laboratory, in the frame of the project: *A bio-economical approach of the antimicrobial agents – use and resistance*, contract PCCDI 7/2018, code: PN-III P1-1.2-FPRD- 2017.

REFERENCES

1. **Abubakar R. H., Madoroba E., Adenubi O., Morar-Leather D., Fasina F.** O.2017. *Bacterial pathogens of pigs with particular reference to* Escherichia coli*: a systematic review and meta-analysis*. *J. Vet. Med. Anim. Health* 9: 159–185.;
2. **Barrow, P., Bianca Lungu, Hortensja Brzoska, C. Mircu, I. Hutu**, *Antibiotic Resistance in Enteric Infections – Exploring a New Approach*, Rev Rom Med Vet (2022) 32(1): 83-88.
3. **Cubin S., Tripon R.M., Brzóska H., Gaşpar C.M., Tulcan C., Lăzărescu C.F., Ţibru I., Huţu I.** *The Effect of Temperature on in vitro Biofilm formation in Multidrug Resistant Escherichia Coli and Staphylococcus Aureus,* Lucrări ştiinţifice medicină veterinară, 2022, vol. LV(1):31-37, Timişoara
4. **Fairbrother J. M., Gyles C. L.** 2012. *Colibacillosis*, pp. 723–749. In: *Diseases of Swine*, 10th ed. (Zimmerman, J. J., Karriker, L. A., Ramirez, A., Schwartz, K. J. and Stevenson, G. W. eds.), Wiley-Blackwell, Hoboken.
5. **García-Meniño, I., García, V., Alonso, M. P., Blanco, J. E., Blanco, J., & Mora, A**. (2020). *Clones of enterotoxigenic and Shiga toxin-producing Escherichia coli implicated in swine enteric colibacillosis in Spain and rates of antibiotic resistance*. Veterinary Microbiology, 108924. doi:10.1016/j.vetmic.2020.108924 ;
6. **Gruel G., Sellin A., Riveiro H., Pot M., Breurec S., Guyomard-Rabenirina S., Talarmin A, Ferdinand S.,** 2021. *Antimicrobial use and resistance in Escherichia coli from healthy food-producing animals in Guadeloupe*. BMC Vet Res. 2021 Mar 8;17(1)
7. **Lugsomya K., Chatsuwan T., Niyomtham W., Tummaruk P., Hampson D. J., Prapasarakul N.,** 2018. *Routine Prophylactic Antimicrobial Use Is Associated with Increased Phenotypic and*

Genotypic Resistance in Commensal Escherichia coli Isolates Recovered from Healthy Fattening Pigs on Farms in Thailand. Microb Drug Resist. 2018, 24(2):213-223

8. **Lungu B.,** 2020 – *Studiu asupra fenomenului rezistenței la preparate antimicrobiene în mamitele stafilococice, FMV Timisoara, Lucrare de licenta.*
9. **Misumi W., FunamoriT., Hamada K., Iwamoto J., Fujisono S.,Chitose K. ,KusomotoM**., 2021, *Association between antimicrobial treatment and resistance of pathogenic Escherichia coli isolated from diseased swine in Kagoshima Prefecture*, Japan. J Vet Med Sci. 2021 Mar; 83(3): 358–369.
10. **Pirolo, M., Visaggio, D., Gioffrè, A., Artuso, I., Gherardi, M., Pavia, G., Visca, P**. (2019). *Unidirectional animal-to-human transmission of methicillin-resistant Staphylococcus aureus ST398 in pig farming; evidence from a surveillance study in southern Italy.* Antimicrobial Resistance & Infection Control, 8(1). doi:10.1186/s13756-019-0650-z
11. **Radu Moga Mânzat, Cătană N., Ervin E., Herman V., Perianu T., Popovici V., Răducănescu H., Secașiu V., Știrbu C., Țogoe I., Verdeș N.** *Boliinfecțioase ale animlelor-bacterioze*. Timișoara. Brumar, 2001. ISBN 973-8057-60-4
12. **Torres Furtado Martins B.,de Meirelles J. L., Pine Omori W., de Oliveira R. R., Seiti Yamatogi R., Call D.R., Nero L. A.**, *Comparative genomics and antibiotic resistance of Yersinia enterocolitica obtained from a pork production chain and human clinical cases in Brazil*. Food Research International, Volume 152, February 2022, 110917.
12. **Wang J., Wu H., Mei C-Y., Wang Y., Wang Z-Y., Lu M-J., Pan Z-M., and Jiao X**., 2021. *Multiple Mechanisms of Tigecycline Resistance in Enterobacteriaceae from a Pig Farm*, China. MicrobiolSpectr. 2021 Sep-Oct; 9(2)

EPIDEMIOLOGICAL STUDY OF GASTROINTESTINAL PARASITISM IN HORSES FROM MEHEDINTI COUNTY

Ioan Cristian DREGHICIU[1], Sorin MORARIU[1], Ion OPRESCU[1], Mirela IMRE[1], Gabriel ORGHICI[1], Gheorghe DĂRĂBUȘ[1], Marius Stelian ILIE[1]*

*) Corresponding author: marius.ilie@fmvt.ro

Abstract

The presence of digestive parasites can alter behaviour, fertility, body condition, juvenile development, decreased resistance to other pathogens, or the performance for which animals are bred. The aim of this study was to monitor gastrointestinal parasite infestation in equines in Mehedinți County. The horses, sport and working, various breeds and half-breeds, came from different localities from Mehedinți County. Samples were collected in plastic bags and examined by flotation, sedimentation, and Baermann methods. Horses were divided into several categories: sex, age, breed, and service. *Strongylidae* and *Parascaris* spp. represented parasitism in recreational and working horses studied in Mehedinți county, no other parasitic elements were identified. The overall prevalence was 43.5% for *Strongylidae* and 21.17% for *Parascaris* spp. The simultaneous presence of at least two parasite species was observed in 17.64% of the horses examined.

Key words: horses, gastrointestinal parasites prevalence, *Strongylidae, Parascaris*

INTRODUCTION

Horses can be affected by more than 150 species of internal parasites. A horse may be infested with one or more parasites at a time, but the nature and severity of the damage varies with the type and degree of infestation [Güiris A.D., 2010].

The presence of digestive parasites can alter behaviour, fertility, body condition, juvenile development, decreased resistance to other pathogens or the performance for which animals are bred [Cernea M.; 2015, Cernea M., 2008].

The etiology and evolution of digestive parasitism and serous cavities in equines in our country have been little systematically studied, usually, in small areas, thus imposing the need to complete the data through detailed analyses [Morariu S., 2016, Güiris A.D., 2010].

The aim of this study was to monitor gastrointestinal parasite infestation in horses in Mehedinți County.

Worldwide, research and analysis of the digestive tract and serous cavities of equine animals have led to the diagnosis of the following parasitoses: eimeriosis, giardiosis, fasciolosis, dicroceliosis, cestodosis, habronemosis, parascaridosis, strongyloidosis, strongylidosis, cyathostominoses, oxyuroses, and gastrophilosis.

MATERIALS AND METHODS

For the purpose of this work, 85 faecal samples were collected from equine animals from March 2018 to May 2020 in Mehedinți County (*Figure 1*). The horses came from localities within the area of the CSVs Malovăț, Șimian, Hinova, Pătulele, and the samples were examined at the Clinic of Parasitology and Parasitic Diseases of the Faculty of Veterinary Medicine, Timișoara, Romania.

Breeds of horses of the riding centre taken in the study and from the households of the population: Hungarian sport horse, Croatian sport horse, Romanian sport horse, Semi-German horse, German sport horse, Arabian Shagya,

- Friesian,
- Lipitan,
- common breed horse.

Faecal samples were collected in plastic bags, labelled and refrigerated until examination.

The examination was carried out by Willis, successive washes and Baerman coproscopic methods.

[1]University of Life Sciences "King Mihai I" Timisoara, Faculty of Veterinary Medicine Timișoara, Romania

The data obtained were statistically analyzed using GraphPad Software Inc.® (QuickCalcs, 2018) and differences were considered significant when p≤0.05.

RESULTS AND DISCUSSIONS

The horses came from a variety of backgrounds, including both sports and working horses. Where possible, age, sex, and service data were obtained for each animal, based on which horses were divided into several categories. This, according to sex, 2 groups (53 males and 32 females) were obtained, according to age, 3 categories (young animals: 0-5 years-11 mares, adults: 6-15 years-46 mares, elderly: >16-28 mares), according to breed, 20 purebred horses, and 65 half-breeds, and according to service, 2 groups (sport, 30, and work, 55) (*Table 1*).

Strongylidae infestations were the most common parasitic infestations found in the samples examined in this study (43.5%).

Other infestations detected in the samples were with *Parascaris* spp. (21.17%).

Polyparasitism was observed in 15 animals (17.64%).

A total of 40 horses were found to be infested with at least one species of parasite, giving an overall prevalence of 47.05%.

The prevalence of infestation with strongyles and ascarids in relation to age groups was 45.5 and 72.7 % for the under-five age group, 43.5 and 15.2 % for the six to 15 age group and 42.9 and 10.7 % for the 16-age group.

In terms of sex-specific prevalence, parasitism with strongyles and ascarids was found to be 56.3 and 37.5% in females and 49.1 and 11.3% in males.

Reported to the breed of horses, it was observed that 35 % of purebred horses and 46.2 % and 20 % of half-breeds respectively were positive in the parasitological examination for strongyles and ascarids.

Table 1

Summary of positive samples from the epidemiological investigation of gastrointestinal parasite infestation in 85 study horses

Epidemiological data	*Strongylidae*		*Parascaris* spp.	
	Willis test			
age	**n=**	**%**	**n=**	**%**
≤ 5 years (*n=11*)	5	45.5	8	72.7
> 6 - 15 years (*n=46*)	20	43.5	7	15.2
≥ 16 years (n= 28)	12	42.9	3	10.7
sex				
females (*n=32*)	18	56.3	12	37.5
males (*n=53*)	26	49.1	6	11.3
race				
pure (*n=20*)	7	35.0	5	25.0
half-breeds (*n=65*)	30	46.2	13	20.0
Service				
leisure (*n=30*)	10	33.3	7	23.3
Labour (*n=55*)	27	49.1	11	20.0
Total	**37 /85**	**43,5**	**18/85**	**21,17**

n = positive horses; % - prevalence obtained.

Regarding the lifestyle or service of the horses, it was observed that in recreational horses, the prevalence was 33.3% and 23.3 % respectively, and in working horses, it was 49.1% and 20 % for strongyles and ascarids respectively (*Figure 5*).

Statistical analysis revealed that in the case of strongyles parasitism there were no statistically significant differences between prevalence values reported by age, breed, sex, and horse service.

In the case of ascarid parasitism, however, there were statistically significant differences

between the prevalence values reported by age (between the under-5 group compared to the other two, but not between the 6-15 and +16 groups) and by race. No statistically significant differences were recorded for the other 2 groups (sex, service).

Among the studies carried out in Romania [Buzatu M.C., et al., 2013, 2014, 2016; Cernea M., et al., 2003, 2008, 2013; Covaşă C.T., Miron L.D., 2011; Morariu S., et al., 2012], is found and those carried out by Morariu S. et al., 2016, focused on the identification of small strongyle species. Forty-seven working horses from Romania have been examined post-mortem for evidence of infestation with small strongyles (*Cyathostominae*). All horses were found to be infected. Twenty-four species were identified. *Cyathostomum catinatum, Cylicocyclus insigne* and *C. Nassatus* had a prevalence of 100%. More than 50% of the horses were infected with *Coronocyclus coronatus, Cylicostephanus calicatus, C. goldi and C. longibursatus*. Other predominant species (34%-45%) were *Cyathostomum tetracanthum, Cylicostephanus minutus* and *Gyalocephalus capitatus*. *Coronocyclus labiatus, Parapoteriostomum mettami, Poteriostomum imparidentatum* and *P. ratzii* had the lowest prevalence [Morariu S., 2016].

A study carried out in Suceava, Botoșani, Iași, Neamț, Bacău counties and, in addition to the Moldavian region, Bistrița-Năsăud County identified *Parascaris equorum* infestation by coproscopy and necropsy, establishing a prevalence of 40.62%. Cases of increased infestation in this study were rare, and the number of parasites observed in necropsied animals ranged from 3 to 67. Among the large strongyles, two species were identified: *Strongylus vulgaris* and *Strongylus edentatus*. Together with the large strongyles, the nematode infestation of the subfam. *Cyathostominae* had a prevalence of 100%, thus 25 species belonging to the following 7 genera were detected: *Cyathostomum, Coronocyclus, Cylicocyclus, Cylicostephanus, Skrjabinodentus, Tridentoinfundibulum* and *Petrovinema* [Cernea M., 2015].

A study in Finland tested young horses for *Parascaris* spp. and strongyles. In this study, the 112 horses evaluated, aged between one and three years, were housed in training stables. The prevalence of *Parascaris* spp. was 21% and that of strongyles was 48% [Aromaa M., 2018].

In the US, the prevalence of internal parasites was determined by examining eggs in faeces from thoroughbred foals in central Kentucky in 2003. Faecal samples were examined from 733 foals from 14 farms. The age of the foals in the study ranged from 10 to 223 days old. Prevalence (mean %) was determined for eggs of *Strongyloides westeri* (1.5%), *Parascaris equorum* (22.4%) and strongyles (27.6%) as well as *Eimeria leuckarti* oocysts (41.6%) identified in foal faeces. Foals had infestations with *S. westeri* on six farms (42.9%), with *P. equorum* on 12 farms (86%) and with strongyles and *E. leuckarti* on all 14 farms (100%) [Lyons E.T., 2003].

Avcıoğlu H., et al, 2016, in Turkey conducted a study on faecal samples, which were collected from 76 horses of different ages, sexes and breeds in the Erzurum region. Individual faecal samples were collected and then examined by flotation and sedimentation methods. The following species were detected: strongyles eggs (57.89%), *Parascaris equorum* (10.52%), *Dicrocoelium dendriticum* (2.63%), *Fasciola* spp. (2.63%) and *Eimeria* spp. (5.26%) [Avcıoğlu H., 2016].

A total of 512 horses (320 mares, 170 geldings and 22 stallions) were examined in Poland. In the group of 185 horses from individual farms, 119 animals (64.3%) were infected with gastrointestinal parasites. Of the 372 horses from agritourism farms, 169 (51.7%) were infected with parasites. Most of the animals expelled *Cyathostominae* eggs. In some individuals eggs of *Strongylus* spp., *Parascaris equorum*, *Strongyloides westeri* and *Anoplocephala* spp. were present. The number of infected horses on agritourism farms was lower than on individual farms, probably due to more regular deworming (usually twice a year) and more care of pastures [Sokół R., 2015].

A study carried out to estimate the prevalence of helminths in the horse population in the state of Brandenburg, Germany established the following values at the horse breeding level: *Cyathostominae* (98.4%), roundworms (16.7%), tapeworms (14.3%), pinworms (8.7%) and Strongyloides (4.0%). *Strongylus vulgaris* was identified in one farm. No liver flukes and lungworms were found [Hinney B., 2011].

CONCLUSIONS

Parasitism in recreational and working horses studied in Mehedinți County was represented by *Strongylidae* and *Parascaris* spp., no other parasite elements were identified.

The overall prevalence of identified species was 43.5% for *Strongylidae* and 21.17% for *Parascaris* spp.

The concomitant presence of at least two species of parasites was observed in 17.64% of the horses examined.

REFERENCES

1. **Aromaa M., Hautala K., Oksanen A., Sukura A., Näreaho A., 2018 -** *Parasite infections and their risk factors in foals and young horses in Finland*. Veterinary Parasitology: Regional Studies and Reports, 12:35-38.
2. **Avcıoğlu H., Güven E., Balkaya İ., et al. 2016 -** *Parasites Determined by Fecal Examination in Horses in Erzurum*. Türkiye Parazitoloji Dergisi, 40(3):147-151.
3. **Buzatu M.C., Ionita M., Mitrea I.L., 2013.** - *Coprological prevalence of intestinal parasites and strongyle EPG profiles of working horses from North-Eastern and South-Eastern Romania*. Scientific Works. Series C. Veterinary Medicine, Vol. LIX (3), 62-67.
4. **Buzatu M.C., Mitrea I.L., Lyons E., Ionita M. 2016** - *Epidemiological study on parasite infections in horses from different types of equine establishments, romania*. AgroLife Scientific Journal, 5(1):31-35.
5. **Buzatu M.C., Mitrea I.L., Miron L., Ioniță M., 2014 -** *Coprological investigations on strongyle EPGprofiles in working horses and horses residing in stud farms in Romania*. Journal of Biotechnology, 185, S42.
6. **Cernea M., 2008** - *Anthelmintic control strategies for equines strongylidosis*, Veterinary Medicine Vol II, No. 01/ISSN:1843-9527
7. **Cernea M., Cozma V., Cernea C., Gall A., 2003.** *The strongilidosis in horses from Mures district: epidemiology and diagnosis*. Bulletin USAMV Cluj Napoca, 60:196-201.
8. **Cernea M., Cristina R.T., Stefanut L.C., Madeira de Carvalho L.M., Taulescu M.A., Cozma V., 2015 -** *Screening for anthelmintic resistance in equid strongyles (Nematoda) in Romania*. Folia Parasitol (Praha), 62:2015.023.
9. **Cernea M., Madeira de Carvalho L.M., Cozma V., Cernea L.C., Răileanu Ș., Silberg R., Gut A., 2008 -** *Atlas of diagnosis of Equine Strongylidosis*, AcademicPres, Cluj-Napoca, 75-110.
10. **Covașă C.T., Miron L.D., 2011** - *Prevalence study of digestive and the serous cavities endoparasitosis in horses from Iassy city area*. Lucr. St. USAMV Iasi, Seria Medicina Veterinara, 54 (13),302-306.
11. **Güiris A.D., Rojas H.N., Berovides A.V., Sosa P.J, Pérez E.M., Cruz A.E., Chávez H.C., Moguel A.J., Jimenez-Coello M., Ortega-Pacheco A., 2010 -** *Biodiversity and distribution of helminths and protozoa in naturally infected horses from the biosphere reserve La Sierra Madre de Chiapas", Mexico*. Veterinary Parasitology, 170(3-4):268-277.
12. **Hinney B., Wirtherle N.C., Kyule M., Miethe N., Zessin K.H., Clausen P.H., 2011 -** *Prevalence of helminths in horses in the state of Brandenburg, Germany*, Parasitology Research, 108(5):1083-1091.
13. **Lyons E.T., Tolliver S.C., 2003 -** *Prevalence of parasite eggs (Strongyloides westeri, Parascaris equorum, and strongyles) and oocysts (Emeria leuckarti) in the feces of Thoroughbred foals on 14 farms in central Kentucky in*. Parasitology Research, 92(5):400-404.
14. **Morariu S., Mederle N., Badea C., Dărăbuș G., Ferrari N., Genchi C., 2016 -** *The prevalence, abundance and distribution of cyathostomins (small stongyles) in horses from Western Romania*, Veterinary Parasitology, 223:205-209.
15. **Morariu S., Oprescu I., Mederle N., Ilie M., Dărăbuș G., 2012 -** *Helminth Parasites in horses from five locations of Arad County*. Scientific Papers: Animal Science and Biotehnologies, 45(2): p. 184-187.
16. **Sokół R., Raś-Noryńska M., Michalczyk M., Raś A, Rapacz-Leonard A., Koziatek S., 2015 –** *E.stimation of infection of internal parasites in horses from different type of farms*. The Annals of Parasitology, 61(3):189-192.

CLINICAL AND PARACLINICAL ASPECTS IN THE ACUTE DIARRHEAL SYNDROME IN DOGS

Vasile BOGHIAN[1]

* E-mail correspondence: vboghian@yahoo.com

Abstract

The aim of this paper was to identify the clinical and paraclinical diagnostic elements of acute diarrheal syndrome in dogs, useful in streamling of treatment. The clinical diagnosis in haemorrhagical acute diarrheal was easy to establish given the profoundly altered general condition with cortical inhibition and melena; sometimes it has been clinically evolve as dysentery (frequent bloody defecation). In catarrhal acute diarrheal, it can sometimes be profuse (exhausting), accompanied by alteration of the patient's general condition and drowsiness. The ultrasonographical examination revealed inflammation of the intestinal wall with a hyperechoic appearance and a halo exterior hypoechogenic corresponding to parietal congestion. The haematological examination revealed hypochromic, normocytic anaemia and a systemic inflammatory syndrome (increased WBC=17.2±0.3x10³/mm³, decreased RBC=5.3±0.4x10⁶/mm³, HGB=11.8±0.4 g/dl and CHEM=31.0±0.2 g/dl). The blood biochemical examination showed subclinical liver failure without impairment of renal and exocrine pancreas functions. The sero-haemorrhagical acute diarrhea had clinically manifested by cyclic episodes of diarrhea. The radiological examination revealed inflammation of the intestinal mucosa and the presence of superficial ulcers, and the coproparasitological examination confirmed cryptosporidiosis. On the other hands, the sero-haemorrhagical acute diarrhea was the consequence of traumatic gastroenteritis, confirmed by radiological exam (dense, radiolucent contents in the gastrointestinal mass). In this situation, the haematological examination revealed a systemic inflammatory process (increased WBC=18.0±0.3x10³/mm³) and hypochromic, normocytic anemia (decreased CHEM=31.8±0.3 g/dl). The blood biochemical examination revealed subclinical liver failure (increased ALT=88.2±0.3 IU/L and ALP=120.3±0.4 IU/L, values only).

Key words: acute diarrhea, dogs, clinical, paraclinical

Introduction

The functional diarrhea is a typical clinical sign in enteritis, and in the dogs, the enteritis also evolves with clinical signs of gastritis due to the particularities of the digestive tract (Willard M.D. *et al.* 2014; Mortier F. *et al.* 2015).

Clinically, acute diarrheal syndrome in dogs have frequently a haemorrhagic, catarrhal or sero-haemorrhagic appearance, to which have add the common clinical signs of gastroenteritis (Berset-Istratescu C.M. *et al.* 2014; Hall E.J. *et al.* 2010; Davenport D.J. *et al.* 2010). On the other hand, the acute diarrheal syndrome in the dogs have a varied etiology that doesn`t allow it to be addressed by a unitary therapeutic protocol (Hubbard K. *et al.* 2007; Unterer S. *et al.* 2015; Hagiu N. *et al.* 1997). In order to establish the etiological diagnosis and to adopt a curative-prophylactic approach, it is often also necessary to carry out paraclinical diagnostic elements such as laboratory tests (haemolucogram, some parameters of the blood biochemical profile) and imaging investigations (ultrasound, radiography) (Singleton D.A. *et al.* 2019; Minamoto Y. *et al.* 2015).

Thus, the clarification of clinical and paraclinical diagnostic elements in the acute diarrheal syndrome frequently encountered in dogs, facilitates the clinical diagnosis and streamlines the treatment.

Materials and Methods

Research, has been done on dogs of different ages and breeds, showing clinical signs of acute diarrheal syndrome. The clinical diagnostic elements were the consistency and appearance of faeces, the frequency of defecation and the general condition of patient. From this point of view, the acute diarrheal syndrome was haemorrhagic, catarrhal or sero-haemorrhagic.

Subsequently, depending on the clinical diagnosis were performed paraclinical examinations in order to specify the primary disease and to recommend the appropriate

[1] University of Life Sciences, Iasi, Romania; e-mail: boghian_v@uaiasi.ro

treatment. For this purpose, were determined the haemoleukogram, some blood biochemical parameters of liver profile – aspartate-aminotransferase (AST), alanine-aminotransferaze (ALT), alkaline phosphatase (ALP), renal profile – creatinine (CRTN), blood-ureea-nitrogen (BUN) (Focak M. *et al*. 2017) and amylase (AMY), the ultrasound, radiological and coproparasitological examination.

For the haematological examination, blood was collected on anticoagulant (EDTA) and were determined RBC (red blood cells), HCT (hematocrit), HGB (haemoglobin), VEM (mean erythrocyte volume), HEM (mean erythrocyte hemoglobin), CHEM (mean erythrocyte hemoglobin concentration), WBC (white blood cells) and PLT (platelets). For these determinations was used the automatic hematology analyzer – *Abaxis Vet Scan HM5*.

For the biochemical examination, blood was collected in test tubes without anticoagulant for the serum expression and was used the automatic biochemical analyzer – *Abaxis Vet Scan VS2*.

The ultrasound examination was performe using the *Acuson NX3 Elite* ultrasound and the probe 5-8 MHz.

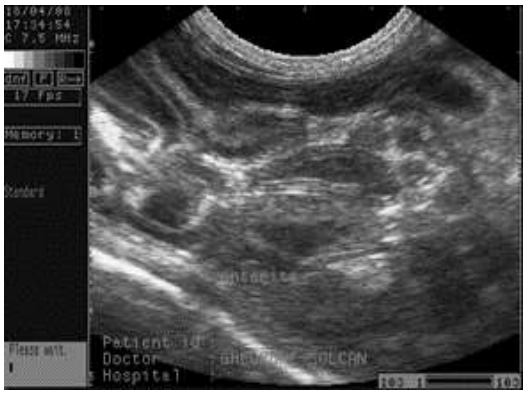
Results

The clinical signs in the haemorrhagic acute diarrhea started with inappetence and retching associated with vomiting and diarrhea with hemolyzed blood (melena). The patient's general condition was profoundly alter, with cortical inhibition (deviation) and pulse with diminished qualities (increased frequency, decreased amplitude and tension). The dehydration was severe (skin fold persisting for more than 60 sec.), the gaze was expressionless, and during examination the posterior train was observed soiled with soft, bloody and foul-smelling faeces (melena).

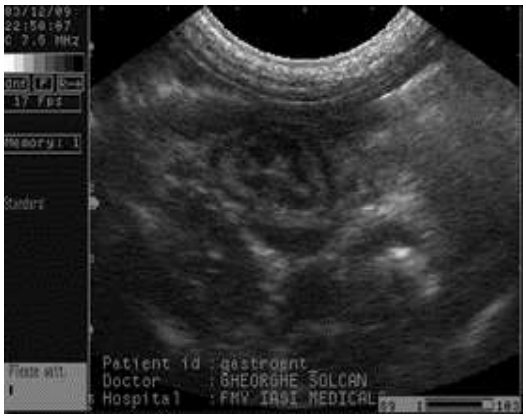
In some patients, the haemorrhagic acute diarrhea had appearance of dysentery (frequent bloody defecation and profound impairment of general condition). Clinical examination also revealed a thready pulse, inert attitudes, cyanosis and discoloration of the buccal mucosa, hypothermia and traces of soft, bloody faeces remaining on the thermometer.

The clinical signs in the catarrhal acute diarrhea started with inappetence, vomiting, discrete haematuria and catarrhal diarrhea. Later, clinical signs of cortical inhibition (deviation), moderate dehydration (skin fold returning in 50 seconds), tachycardia, cold extremities, elevated rectal temperature and soft faeces mixed with mucus remained after thermometry on thermometer. In some patients, the catarrhal diarrhea was profuse (exhausting), the patient's general condition deteriorated abruptly with repeated vomiting, followed by severe dehydration (skin fold persists over 60 seconds) and profound cortical inhibition (drowsiness).

On the ultrasonographic examination in catarrhal acute diarrhea was observe in which the intestinal loops have a hypoechoic fluid content, inflamed intestinal wall with hyperechogenic appearance and a halo hypoechogenic outside corresponding to parietal congestion. The pyloric mucosa was also thicken with obvious hyperechogenic folds and a hypoechogenic inflammatory halo (catarrhal gastroenteritis) (Figure 1, a, b).



a



b

Figure 1 – Catarrhal gastroenteritis
a - intestinal loops with hypoechoic content;
b -thickened pyloric mucosa

The haematological examination showed increased WBC ($17.2\pm0.3x10^3/mm^3$), decreased RBC ($5.3\pm0.4x10^6/mm^3$) and HGB (11.8±0.4 g/dl) and unchanged HCT and PLT values (Table 1).

The blood biochemical examination showed elevated values of AST (60.8±0.3 IU/L), ALT (178.0±0.4 IU/L), ALP (147.0±0.3 IU/L). At the same time CRTN, BUN and AMY values were within the average reference values (Table 2).

Table 1

Results of the haematological tests in dogs with catarrhal gastroenteritis

Blood parameter	WBC	PLT	RBC	HCT	HGB	VEM	HEM	CHEM
Unit of measuring	mii/µl	mii/µl	mil./µl	%	g/dl	µ³	pg	g/dl
Values of reference (Susan E. *et al.* 1998)	6-17	160-430	5.5-8.5	37-55	12-18	64-74	22-27	34-36
Determined values	17.2±0.3	378	5.3±0.4	38.0±03	11.8±0.4	71.6±03	22.2±0.3	31.0±0.2

Notes: WBC-white blood cells; PLT-Platelets; RBC-red blood cells; HCT-hematocrit; HGB-haemoglobin; VEM-mean erythrocyte volume; HEM-mean erythrocyte haemoglobin; CHEM-mean erythrocyte haemoglobin concentration

Table 2

Results of the blood biochemical tests in dogs with catarrhal gastroenteritis

Blood parameter	AST	ALT	ALP	CRTN	BUN	AMY
Unit of measuring	IU/L	IU/L	IU/L	mg/dl	mg/dl	IU/L
Values of reference (Susan E. *et al.* 1998)	8.9-48.5	8.2-57.3	10.6-100.7	0.5-1.6	8.8-25.9	269.5-1462.4
Determined values	60.8±0.3	178.0±0.4	147.0±0.3	0.9±0.2	12.4±0.2	752.0±0.5

Notes: AST-aspartate-aminotransferase; ALT-alanine-aminotransferase; ALP-alkaline phosphatase; CRTN-creatinine; BUN-blood urea nitrogen; AMY-amylase

Clinical signs in the sero-haemorrhagic acute diarrhea are the appetite present and the general condition slightly altered (listlessness). At long intervals of about 1-2 months, episodes of sero-haemorrhagicaly diarrhea were observe, sometimes even with streaks of non-haemolysed blood.

The radiological examination with contrast medium (barium sulphate) revealed inflammation of the intestinal mucosa and the presence of superficial ulcers. Coproparasitological examination indicated cryptosporidiosis (Figure 2).

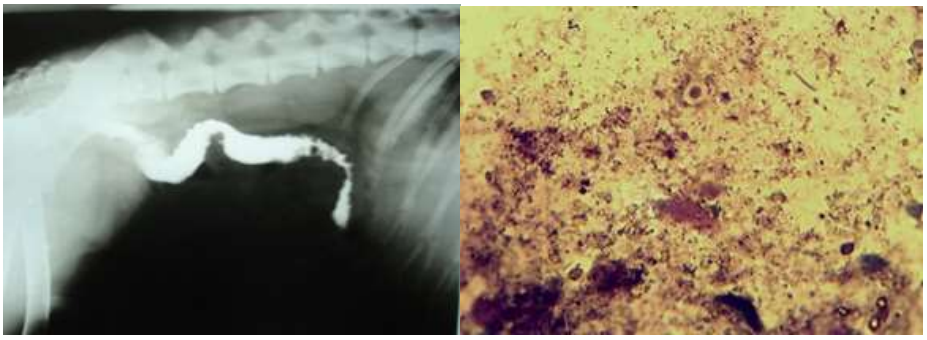


Figure 2 – Superficial intestinal ulcers, Cryptosporidiosis

In some patients, the sero-haemorrhagic diarrhea was the consequence of traumatic gastroenteritis. The clinical examination revealed inappetence, vomiting, alternating periods of watery diarrhea and constipation, abdominal contraction, tachypnea and tachycardia.

The haematological examination showed only an increased WBC (18.0±0.3x10³/mm³), the other haematological parameters determined had values within the physiological limits of the mean reference values. The calculated haematological parameters had values within physiological limits, except for CHEM (31.8±0.3 g/dl) (Table 3).

Table 3

Results of the haematological tests in dogs with traumatic gastroenteritis

Blood parameter	WBC	PLT	RBC	HCT	HGB	VEM	HEM	CHEM
Unit of measuring	mii/µl	mii/µl	mil./µl	%	g/dl	µ³	pg	g/dl
Values of reference (Susan E. *et al.* 1998)	6-17	160-430	5.5-8,5	37-55	12-18	64-74	22-27	34-36
Determined values	18.0±0.3	362	6.2±0.4	44.0±0.3	14.0±0.3	70.9±0.4	22.5±0.4	31.8±0.3

Notes: WBC-white blood cells; Plt-Platelets; RBC-red blood cells; HCT-hematocrit; HGB-haemoglobin; VEM-mean erythrocyte volume; HEM-mean erythrocyte haemoglobin; CHEM-mean erythrocyte haemoglobin concentration

Table 4

Results of the blood biochemical tests in dogs with traumatic gastroenteritis

Blood parameter	AST	ALT	ALP	CRTN	BUN	AMY
Unit of measuring	IU/L	IU/L	IU/L	mg/dl	mg/dl	IU/L
Values of reference (Susan E. *et al.* 1998)	8.9-48.5	8.2-57.3	10.6-100.7	0.5-1.6	8.8-25.9	269.5-1462.4
Determined values	37.8±0.3	88.2±0.3	120.3±0.4	1.0±0.2	18.2±0.2	640.0±0.5

Notes: AST-aspartate-aminotransferase; ALT-alanine-aminotransferase; ALP-alkaline phosphatase; CRTN-creatinine; BUN-blood urea nitrogen; AMY-amylase

On the blood biochemical examination only elevated ALT (88.2±0.3 IU/L) and ALP (120.3±0.4 IU/L) values were obtained. These data indicate a subclinical liver failure without impairment of renal and exocrine pancreas functions (Table 4).

On radiological examination, a dense, radiolucent content was observed in the gastrointestinal mass (Fig.3).

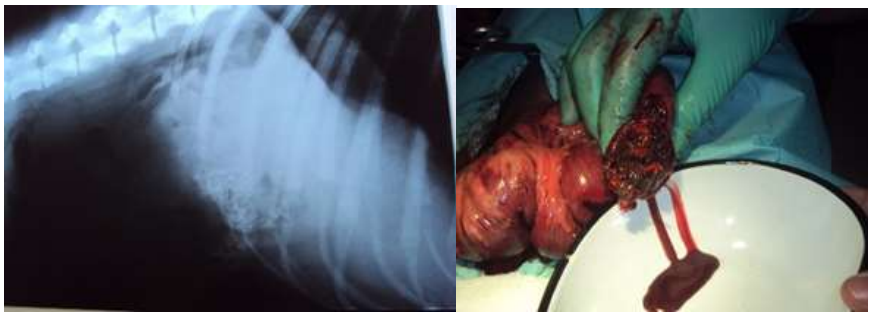


Figure 3 – Traumatic gastroenteritis
(through foreign bodies)

Discussion

In the haemorrhagic acute diarrheal no other paraclinical examinations were necessary to establish the clinical diagnosis and recommend treatment. The data obtained on clinical examination (bloody diarrhea) and anamnesis (lack of prophylactic vaccination) were sufficient to specify the diagnosis of haemorrhagic gastroenteritis, most likely viral in nature.

On the other hands, in the catarrhal gastroenteritis the clinical examination and history which revealed diarrhea, vomiting and the appearance of feces that were soft and mucusy, to which was added the inflammatory syndrome were the data that indicated the clinical diagnosis. At the same time the values of the calculated haematological parameters, namely MEV and MEH were within the limits of the mean reference values and CHEM (31.0±0.2 g/dl) was below the limit of the physiological mean values. These data indicate a hypochromic, normocytic anaemia and an inflammatory syndrome that often accompanies catarrhal gastroenteritis, while the blood biochemical examination indicate subclinical liver failure without impairment of renal and exocrine pancreas functions. This shows the serious evolution of this type of diarrhea.

In the sero-haemorrhagic diarrhea the etiological diagnosis of the primary disease is necessary for the appropriate treatment. Paraclinical examination indicate a systemic inflammatory process, a hypochromic, normocytic anaemia, subclinical liver or a traumatic gastroenteritis confirmed by a radiological examination; in this situation it is recommended surgical treatment.

Conclusions

In the haemorrhagic acute diarrhea, the clinical examination (bloody diarrhea) and the history (patient's age and lack of prophylactic vaccination) are often sufficient to establish the diagnosis and recommend treatment, without further paraclinical examinations. Sometimes, it can evolve as dysentery (frequent bloody defecation) with profound impairment of the general condition.

In the catarrhal acute diarrhea, in addition to the clinical examination, other paraclinical investigations are necessary to clarify the diagnosis, such as ultrasound, haematological or blood biochemical examination, in which case found an inflammatory syndrome or a pathophysiological syndrome of hepatocytolysis (increased serum transaminases). At other times, it may evolve clinically with the appearance of profuse (exhausting) diarrhea and profound cortical inhibition (drowsiness).

In the sero-haemorrhagic diarrhea, the paraclinical diagnostic elements (radiological, coproparasitological) are essential for the aetiological diagnosis (primary disease) and recommendation of appropriate treatment.

References

Willard M.D. Digestive System Disorders. In: Nelson R.W., Couto C.G., editors. *Small Animal Internal Medicine. 5-th ed*. Elsevier; St. Louis, MO, USA, 2014, 376-380.

Mortier F., Strohmeyer K., Hartmann K., Unterer S. Acute haemorrhagic diarrhoea syndrome in dogs: 108 cases. *Vet. Rec.* 2015, 176, 627. DOI: 10.1136/vr.103090.

Berset-Istratescu C.M., Glardon O.J., Magouras I., Frey C.F., Gobeli S., Burgener I. Follow-up of 100 dogs with acute diarrhoea in a primary care practice. *Vet. J.*, 2014, 199, 188-190. DOI: 10.1016/j.tvjl.2013.10.014.

Hall E.J., German A.E. (2010). Diseases of the small intestine. In: Ettinger S.J., Feldman E.C., editors. *Textbook of Veterinary Internal Medicine. 7th ed*. Elsevier Saunders; St. Louis, MO, USA, 2010, 1544-1546.

Davenport D.J., Remillard R.L. Acute Gastroenteritis and Enteritis. In: Hand M.S., Thatcher C.D., Remillard R.L., editors. *Small Animal Clinical Nutrition. 5th ed*. Mark Morris Ins.; Topeka, KS, USA, 2010, 1053-1064.

Hubbard K., Skelly B.J., McKelvie J., Wood J.L.N. Risk of vomiting and diarrhoea in dogs. *Veter. Rec.* 2007, 161, 755-757. DOI: 10.1136/vr.161.22.755.

Unterer S., Lechner E., Mueller R.S., Wolf G., Straubinger R., Schulz B.S., Hartmann K. Prospective study of bacteraemia in acute haemorrhagic diarrhoea syndrome in dogs. *Vet. Rec.* 2015, 176, 309. DOI: 10.1136/vr.102521.

Hagiu N., Solcan G., Hriţcu L.D., Beşchea Chiriac S.I. Tulburări digestive la câine și pisică provocate de deglutirea accidentală a corpilor străini. *Lucr. St. UAMV Iași*, 1997, 40, 103-107.

Singleton D.A., Noble P.J.M., Sánchez-Vizcaíno F., Dawson S., Pinchbeck G.L., Williams N.J., Radford A.D., Jones P.H. Pharmaceutical Prescription in Canine Acute Diarrhoea: A Longitudinal Electronic Health Record Analysis of First Opinion Veterinary Practices. *Front. Vet. Sci.* 2019, 6, 218. DOI: 10.3389/fvets.2019.00218.

Minamoto Y., Otoni C.C., Steelman S.M., Büyükleblebici O., Steiner J.M., Jergens A.E., Suchodolski J.S. Alteration of the fecal microbiota and serum metabolite profiles in dogs with idiopathic inflammatory bowel disease. *Gut Microbes.* 2015, 6, 33-47. DOI: 10.1080/19490976.2014.997612.

Focak M., Haskovic E., Suljevic D., Zahirovic A. Comparative evaluation of biochemical parameters during urinary infection in Maltese and Belgian shepherd dogs. *Cercetări Agronomice în Moldova*. 2017, 50, 3(171), 107-114. DOI: 10.1515/cerce-2017-0030.

Susan E., Aiello B.S. The Merck Veterinary Manual eighth edition, *Merck & Co. Inc*. 1998, 2192-2194.

EPIDEMIOLOGICAL IMPACT OF SWINE ZOONOTIC DIGESTIVE VIRUSES – REVIEW

Adriana ANIȚĂ[1], Dragoș ANIȚĂ[1],

[1]Faculty of Veterinary Medicine, University of life Sciences "Ion Ionescu De La Brad" Iași, Romania
e-mail:danita@uaiasi.ro

Abstract

Infectious diarrhea of farm pigs causes some of the most significant financial losses for producers working in the pork industry through the loss of efficiency, as well as the loss of overall profitability of production. Highly virulent diseases, such as porcine epidemic diarrhoea, result in very high piglet mortality, causing producers enormous financial difficulties. Recurrent and less endemic diseases however, have greater long-term overall effects on health and productivity. The prevalence of swine diarrhea can vary from country to country, from one farming system to another and even from one farm to another.

The increasing pressure of pig production, the wide network of imports-exports, the constant evolution of pathogens that allow them to develop new adaptation and diversification mechanisms, and climate change, are some of the challenges faced by the global pork industry. Thinking about viruses being transmitted across species implies taking into consideration all issues arising from these pathogens. They not only reveal the vulnerabilities in human societies, whose functioning is disrupted by these outbreaks, but also the fragilities of the environments in which they appear.

Key words: swine, astrovirus, rotavirus, norovirus, hepatitis E virus

Etiologic investigations of infectious diarrhea in swine were long limited to bacteria and protozoa. The advent of electron microscopy and molecular biology showed that diarrhea could also be caused by viruses, both in humans and in other animals. Several pathogens have been intensively studied, while others have yet to be investigated even though they have been reported in pigs all around the world. Recent events have shown the importance of the zoonotic potential of viruses, for example directly but also via potentially infected or contaminated food. The emergence of pathogens through inter-species passage corresponds to rare events, but a very high mutation rate increases their probability occurrence as evidenced by the over-representation of single-stranded RNA viruses among the known examples of species jumping.

Although the molecular mechanisms necessary to cross the species barrier are very diverse, the presence of compatible cell receptors represents a crucial step and several examples of crossing the species barrier have shown the determining role of mutations allowing viruses to recognize their receptor on cells of the new species target. Under these conditions, the risk of inter-species transmission will be higher that the receptors are phylogenetically conserved. This review evaluates the impact of porcine astroviruses, rotavirus, norovirus and hepatitis E virus in pig production network.

Porcine astrovirus (PAstV) has been poorly studied and has been associated mainly with gastroenteritis. Astroviruses are naked single-stranded RNA viruses, small in size (28 to 30 nm) belonging to the *Astroviridae* family. Astroviruses have a genome composed of 3 ORFs designated ORF1a, ORF1b and ORF2. ORF1a and ORF1b encode for non-structural polyproteins, including a protease and an RdRp. ORF2 encodes for the capsid structural protein transcribed by a subgenomic mRNA. The great diversity of *Astrovirus* species has led to multiple efforts to classify these different genera and species. This great genetic diversity makes it possible to find astroviruses in several animals in addition to humans, including cats, dogs, cattle, mice, deer, pigs, sheep, mink, bats, cheetahs, rats, rabbits and

[1]ROVETEMERG Research Center, Faculty of Veterinary Medicine, University of Life Sciences "Ion Ionescu de la Brad" Iași, Romania

other marine mammals, as well as in chickens, turkeys, ducks, pigeons and guinea fowl. Based on genetic analysis of the complete capsid region at the amino acid level, mammalian astroviruses will be divided into two main genogroups: genogroup I and genogroup II. Each genogroup includes astroviruses infecting different host species, and can be further subdivided based on both genetic and host species criteria (Rawal G, Linhares DC, 2022)

In pigs, five different groups of porcine astroviruses have been identified (porcine astrovirus groups 1-5), these differences being due to their large genomic disparities. However, only porcine astrovirus "1" is part of *Mamastrovirus*.

Genotypes A and B are able to infect humans (Carter, 2005). Among the viral agents known to cause enteric disease, importance of astroviruses as a cause of foodborne disease is perhaps the less well characterized (Percival et al., 2004). In infected people, the disease seems similar to that caused by rotaviruses, although it is much less severe (diarrhea of 2 3 days that does not cause significant dehydration). Other symptoms include headache, malaise, nausea, vomiting, and mild fever (Percival et al., 2004; Méndez and Arias, 2007).

Astroviruses can be transmitted through food, water, contaminated materials and person-to-person contact (Bosch et al., 2014). Astroviruses are remarkably resilient in the environments. They resist several treatments including chlorinated water (7-9), low pH, lipid solvents and ionic detergents and non-ionic (Maclachlan et al, 2016). Molecular analysis has revealed the close relationship of PAstV with astroviruses of humans (HAstV) and cats (FAstV).

Astroviruses appear to be ubiquitous in young animals and often associated with self-limiting gastroenteritis. Extra-intestinal infections have also been observed, causing encephalitis in cattle (Li et al., 2013), pigs (Arruda et al, 2017; Matias et al, 2019; Rawal, 2019) and mink (Blomström et al, 2010). Porcine astroviruses were detected almost everywhere around the world, including Greece (Stamelou et al, 2022), Hungary (Boros et al, 2017), Czech Republic (Indik et al, 2006), US (Rawal, Matias et al, 2019), Canada (Laurin et al, 2011; Luo et al, 2011) (Table 1).

Table 1.

Epidemiology of swine astrovirus based on molecular detection studies

Sample type	Overall detection	Country	Authors
Faecal samples	80%	Canada	Luo et al, 2011
Faecal samples	23,8%	Colombia	Ullua et Gutierrez, 2010
Faecal samples	61,33%	US	Mor et al, 2012
Faecal samples	34,25%	Czech Republic	Dufkova et al, 2013
Faecal samples	89,6%	Croatia	Brnic´ et al, 2014
Faecal samples	38%	Ireland	O'Shea et al, 2016
Brain stem and spinal cord	60%	Hungary	Boros et al, 2017
Brain stem and spinal cord	100%	US	Matias et al, 2019
Faecal samples	83%	US	Rawal, Matias, Macedo, et al, 2019
Faecal samples	95.4%	Greece	Stamelou et al, 2022

Swine rotavirus is endemic in pig populations, and all swine herds certainly have a history of RV infection and circulation. Rotaviruses belong to the order *Reovirales*, family *Sedoreoviridae* which contains the genus *Rotavirus* divided into 10 species designated Rotavirus A to J. Rotaviruses are non-enveloped, icosahedral viruses 75 nm in diameter and composed of a triple layer capsid. The genome consists in 11 linear dsRNA segments and is formed in a unique morphogenic pathway, which involves acquisition of a transient lipid envelope during budding of immature particles into the endoplasmic reticulum (ER) (www.ictv.global).

Each of the rotavirus species infects a wide variety of animal species. Humans are infected with rotaviruses belonging to Rotavirus A, B, and C species, while pigs are infected with Rotavirus A, B, C, E, and H species (Norman et al, 2014; Kwot et al, 2020; Vlasova et al, 2017). In addition to humans, Rotavirus A, the most common Rotavirus species, can be found in pigs, monkeys, cattle, sheep, horses, dogs, cats, mice, rabbits and birds. Rotavirus B species also infects cattle, sheep, and rats, while Rotavirus C species also infects dogs, cattle, and cats (Martela et al, 2010). Rotavirus E is poorly documented, not making part of the species ratified by the ICTV and Rotavirus H, although prevalent in a few studies (Molinari et al, 2015), is only very rarely found and reported (Marthaler et al, 2014).

Rotavirus A (RVA) and C (RVC) are the most common among all RV species reported in swine. RVA was considered most prevalent and pathogenic in swine; however, RVC has been emerging as a significant cause of enteritis in newborn piglets. Since no intra-uterine passage of immunoglobulins occur in swine during gestation, newborn piglets are highly susceptible to RV infection at birth. Moreover, rotaviruses are constantly evolving, either by point mutation or as a result of genetic reassortment and many unusual human and/or animal strains have emerged in many countries (Santos N & Hoshino Y, 2005) and the list of human rotavirus strains that can recognize being a genomic segment of animal origin is far from complete.

The structural proteins of the inner capsid VP7 and the outer capsid VP4 correspond to the G (Glycoprotein) and P (protease-sensitive) antigens respectively inducing the production of neutralizing antibodies. Thus, these antigens have made it possible to define the types G and P of the viral strains and thus to better understand on the one hand the predominant strains in human diarrhea and on the other hand to compare them with the strains isolated in animals with a view to identify a possible zoonotic risk. The comparison of the genetic sequences made it possible to show the existence of a close relationship between certain animal and human rotaviruses or to discover new human genotypes which could recognize an animal origin, demonstrating that the specificity of host could be broken easily. Since the new virus classification system defined by Matthunssens et al (2008), there is a better understanding of the complex interactions between human and animal rotaviruses. Currently there are at least 23 G (VP7) and 32 P (VP4) genotypes identified.

As early as 1996, it was shown experimentally that the Wa strain of human rotavirus G1P (Matthijnssens et al, 2008) was pathogenic in gnotobiotic pigs (Ward et al, 1996). In addition, only pathogenic human strains cause diarrhea in pigs while attenuated human strains do not (Azevedo et al, 2005), allowing the porcine model to be used in assays to verify the efficacy human rotavirus vaccines. In Europe, human strains of rotavirus resembling porcine strains of group P (VP4) have often been identified (Yuan et al, 2006) and other serotypes of porcine origin have been noted in infantile diarrhea observed in non-European countries (Brazil, Argentina, Paraguay, India, Thailand) (Martella et al, 2010). The presence of porcine rotavirus group C was also proved detected in a Brazilian child with diarrhea (Gabbay et al, 2008). It was highlighted the striking contrast between the low genetic diversity of the human strains isolated in group C (responsible for cases of diarrhea with hospitalization in less than 5% of children) and the great variability of the porcine strains of this group.

Swine Calicivirus belongs to a large viral family, *Caliciviridae*, of the order *Picornavirales* infecting a wide variety of animals. This family includes a total of 11 different genera, including the *Norovirus* and *Sapovirus* genera, both of which infect humans and pigs and cause gastroenteritis. The genera *Norovirus* and *Sapovirus*, have only one species, respectively Norwalk virus and Sapporo virus (www.ictv.global). Another recently discovered genus, *Valovirus*, including Saint Valerian virus can also be found in the gastrointestinal tract of pigs (Desselberg, 2019).

Calicivirus virions are 27–40nm in diameter, non-enveloped with a single-stranded, positive-sense genomic RNA of 6.4–8.5kb organized into either two or three major ORFs. Generally, caliciviruses are stable in the environment and enteric caliciviruses are acid-stable. Genotyping based on the complete capsid region (ORF2 and ORF3) was until recently the gold standard (Vinje et al, 2004) for noroviruses, but RNA recombinations make the task more difficult (Bull et al, 2008). The latest classification has 10 genogroups and p-groups (GI to GX), 49 genotypes (9 GI, 27 GII, 3 GIII, 2 GIV, 2 GV, 2 GVI and 1 each of GVII, GVIII, GIX and GX) and 60 p-types (14 GI, 37 GII, 2 GIII, 1 GIV, 2 GV, 2 GVI, 1 GVII and 1 GX).

A universal nomenclature has been proposed to use when depositing new norovirus sequences: Organism/Host/2-letter country code/Year of sample collection/Genotype[p-type]/Sample name.

Genogroups GI, GII and GIV infect humans, and only genotypes GII.11, GII.18 and GII.19 have been detected in pigs (Chhabra et al, 2019).

The detection of *swine norovirus* dates back to 1997 when Sugieda et al. reported for the first time the presence of norovirus particles in swine faeces collected in Japan. Noroviruses of animal origin could infect humans, these are genogroup II noroviruses. Some have been detected in asymptomatic pigs in the Netherlands (Van der Poel et, 2000) and Japan (Sigieda et al, 1998). The detection of this virus in the faeces of sick domestic animals shows that it could be a zoonosis without knowing exactly how these viruses circulate from animals to humans and vice versa or whether the animals represent a reservoir of these pathogens for humans (Table 2).

Table 2.

Epidemiology of swine norovirus in European countries

Genotype	Overall detection	Country	Authors
GII.11 and GII.18	1,2%	Slovenia	Mijovski et al, 2010
-	12.2%	Spain	Halaihel et al, 2010
GII	5.8%	Hungary	Reuter et al, 2007
GII.19	4.6%	Belgium	Mauroy et al, 2008
GII.11	0.5%	Italy	Di Bartolo et al, 2014
GII.18	14.2%	Germany	Machnowska et al, 2014
GII.P11	2.53%	Italy	Laconi et al., 2020,
GII.P11 and GII.P18	11.4%	Italy	Cavicchio et al., 2020

In 2020, human noroviruses and other caliciviruses were the fourth most frequently reported causative agents of food and waterborne outbreaks in the European Union, associated with 130 outbreaks in thirteen member states (i.e., Belgium, Czechia, Denmark, Finland, France, Germany, Italy, Latvia, Malta, the Netherlands, Poland, Spain, and Sweden).

Sapoviruses is classified in 19 genogroups, subdivided into a total of 52 genotypes, based on the nucleotide sequence of VP1. Human sapoviruses are divided into 7 genotypes from each of the first 2 genogroups (GI.1 to GI.7 and GII.1 to GII.7), GIV and 2 genotypes of GV (GV.1 and GV.2) (Oka et al, 2015). Sapoviruses from pigs and wild boars are divided in 8 genogroups and 21 genotypes: GIII, GV.3 and 5, GVI.1 to 3, GVII.1 to 6, GVIII.1 and 2, GIX.1 to 3, GX 1 and 2 and GXI 1 to 3 (Sunaga et al, 2019).

The first particles of sapovirus were found in human faeces by electron microscopy during manifestations of clinical signs of diarrhea in 1976 in the United Kingdom, then recognized as pathogens causing gastroenteritis. Epidemics of sapovirus gastroenteritis are fewer than those of norovirus in humans.

Sapovirus RNA has frequently been detected in pig faeces, being found worldwide. The prevalence of porcine sapovirus vary greatly between countries, 30.4% in Brazil (Barry et al, 2008), in 75% of farms in Canada (L'Homme et al, 2009), 28.6% of farms in China (250), and also in

different farms from Denmark, Finland, Hungary, Italy, Slovenia and Spain (Reuter et al., 2010). The age group with the highest prevalence of sapovirus in pigs is 2 to 8 weeks.

Although there have been no cases of human infection from either porcine norovirus or sapovirus, there are still several factors that cause these viruses to be categorized as viral agents. potentially zoonotic. These two viruses have diverse genetic material, divided into multiple genogroups, but still have genogroups common to pigs and humans such as norovirus GII and sapovirus GV. Recombination events can occur in RdRp and VP1 regions within these genogroups (Wand et al, 2005). In addition, replication of a human GII norovirus has been demonstrated in gnotobiotic piglets (Cheetham et al, 2006) and antibodies against human noroviruses have been found in pigs in Venezuela (Farkas et al, 2005). The emergence of new strains with genetic material recombinant would be most likely to occur in countries where high livestock densities are close to human populations and where farming practices create close contact between humans and pigs.

Hepatitis E virus is classified into the *Hepeviridae* family. The family *Hepeviridae* includes enterically transmitted quasi-enveloped (blood or tissue culture) or non-enveloped (faeces) positive-sense single-stranded RNA viruses infecting mammals and birds (subfamily *Orthohepevirinae*) or fish (*Parahepevirinae*). Members of the subfamily *Orthohepevirinae* infect humans (genera *Paslahepevirus*) and domestic and wild mammals (genera *Rocahepevirus*) (Purdy et al, 2022). Although hepatitis E virus (HEV) is shed through faeces as a non-enveloped virus there is evidence that, HEV can hijack host membranes on assembly and exit. Possession of a host-derived envelope may allow the virus to circulate in a patient's blood escaping detection by neutralizing antibodies (Yin et al, 2016).

Hepatitis E virus infections are found worldwide in human and swine population. Different genotypes infect humans and can be divided into two different groups: genotypes 1 and 2, found in endemic areas, in developing countries where sanitary conditions are poor and genotypes 3 and 4 in sporadic cases, in industrialized countries. Genotype 7 has only very rarely been found in humans.

Over the past 2 decades, evidence has accumulated implicating pigs and other animals in the zoonotic transmission of G3 HEV to humans. In swine, hepatitis E virus infections are attributed to genotypes 3, 4 and 5, in the same regions where the virus is sporadic in humans. Genotype 3 infections in pigs have also been found in areas where the hepatitis E virus is endemic in humans (Pischke et al, 2013).

Table 3.

Hepatitis E virus molecular detection in pig food products in Europe

Country	Food product	ARN prevalence	Authors
UK	pig liver	3%	Berto et al, 2012
	sausages	10%	
France	pig liver sausages	30%	Pavio et al, 2014
	dry salted liver	3%	
	liver quenelles	25%	
Germany	liver sausages	22%	Szabo et al, 2015
	raw salami	20%	
Netherlands	cervelat	10.8%	Boxman et al, 2020
	salami	18.5%	
	metworst	26.1%	
	snijworst	16.3%	
Switzerland	raw liver sausages	11.8%	Giannini et al, 2017
Switzerland	meat products	11.1%	Moor et al, 2018
	liver sausages	18,9%	
	raw meat sausages	5.7%	
France	porc liver	2.8%	Feurer et al, 2018
Italy	traditional pork meat products	0%	Montone et al, 2019
	wild boar homemade meat and liver sausages	6.3%	

Result of the studies developed in UK and Switzerland (Dalton et al, 2007; Fraga et al, 2017) identified that indigenous cases of HEV were occurring in economically developed countries (Table 3). It is now widely accepted that pigs are a zoonotic source of HEV transmission, though increased detection and awareness of HEV may also play a small role in the observed increase in cases. There is a risk of contracting HEV from undercooked pig products, and there is also potential for other livestock species to be unidentified hosts for the virus. Generally, foodstuffs containing raw pork meat is more likely to cause a foodborne infection than cooked foods due to no thermal inactivation of the virus.

Some strains included in genotypes 3, 4 and 7 of the hepatitis E virus have been identified as zoonotic strains of the hepatitis E virus. Several cases of transmission to humans of viral strains infecting pigs and vice versa have been listed, as well as the ingestion of camel meat contaminated with genotype 7 of the hepatitis E virus (Lee et al, 2016). The pig would therefore be the primary reservoir of the hepatitis E virus in sporadic, non-endemic areas due to the autochthonous nature of the infections.

Consumption of raw or undercooked meat, game meat (Kamar et al, 2012), figatelli (a fresh sausage made from pork liver, smoked or dried) (Pavio et al, 2014), liver pâtés (Diez-Valcare et al, 2012) are known sources of zoonotic hepatitis E virus infection.

In conclusion, efforts devoted to the implementation of biosecurity measures are necessary, because swine viral infections are contagious and may cause harmful clinical manifestations that can cause great economic losses either by growth retardation or by the death of the animals. Viral infections in swine can cause several different pathologies, including polysystemic diseases, respiratory, urinary and reproductive system, skin and intestinal system, whose dysfunctions causing diarrhea are common problems in pig farms. These diarrheas can be caused by different pathogens, including many viral infections with zoonotic character. The control of zoonotic viral diarrhea requires a holistic view, including several factors such as washes and disinfection, immunity, pathogen treatments, gut microbiota, nutrition, stress reduction, and effective management practices. Pigs can act as intermediate and amplifying hosts for the viruses with zoonotic potential, thus the One Health approach should be applied in order to prevent the complex represented by public and animal health issues.

References

1. **Arruda B., Arruda P., Hensch M., Chen Q. I., Zheng Y., Yang C., Gatto I. R. H., Ferreyra F. M., Gauger P., Schwartz K., Bradner L., Harmon K., Hause B., Li, G**., 2017 - Porcine Astrovirus type 3 in central nervous system of Swine with Polioencephalomyelitis. Emerging Infectious Diseases, 23(12), 2097–2100.
2. **Azevedo M.S., Yuan L., Jeong K.I**. et al., 2005 - Viremia and nasal and rectal shedding of rotavirus in gnotobiotic pigs inoculated with Wa human rotavirus. J. Virol., 79, 5428-5436.
3. **Barry AF, Alfieri AF, Alfieri AA**., 2008 - High genetic diversity in RdRp gene of Brazilian porcine sapovirus strains. Vet Microbiol. 131(1-2):185-91.
4. **Berto A, Martelli F, Grierson S, Banks M**., 2012 - Hepatitis E virus in pork food chain, United Kingdom, 2009–2010. Emerging Infectious Diseases.18(8):1358
5. **Boros Á., Albert M., Pankovics P., Bíró H., Pesavento P.A., Phan T.G., Delwart E., Reuter G**., 2017 - Outbreaks of neuroinvasive Astrovirus associated with encephalomyelitis, weakness, and paralysis among weaned pigs, Hungary. Emerging Infectious Diseases, 23(12), 1999–2010.
6. **Bosch A., Pintó R.M., Guix S**., 2014 - Human astroviruses. Clin. Microbiol. Rev., 27(4): 1048–1074.
7. **Boxman I.L.A., Jansen C.C.C., Zwartkruis-Nahuis A.J.T., Hägele G., Sosef N.P., Dirks R.A.M**. 2020 - Detection and quantification of hepatitis E virus RNA in ready to eat raw pork sausages in the Netherlands. Int J Food Microbiol. 16;333:108791.
8. **Brnić D., Jemeršić L., Keros T., Prpić J**. 2014 - High prevalence and genetic heterogeneity of porcine astroviruses in domestic pigs. The Veterinary Journal, 202(2), 390–392.
9. **Bull R.A., Tanaka M.M., White P.A**. 2007 - Norovirus recombination. The Journal of general virology.88(Pt 12):3347-59.
10. **Carter M.J**., 2005 - Enterically infecting viruses: Pathogenicity, transmission and significance for food and waterborne infection. J. Appl. Microbiol., 98(6): 1354–1380.
11. **Cheetham S, Souza M, Meulia T, Grimes S, Han MG, Saif LJ., 2006 -** Pathogenesis of a genogroup II human norovirus in gnotobiotic pigs. Journal of virology.80(21):10372-81.

12. **Chhabra P, de Graaf M, Parra GI, Chan MC, Green K, Martella V, et al**. 2019 - Updated classification of norovirus genogroups and genotypes. The Journal of general virology.100(10):1393-406.
13. **Dalton H, Thurairajah P, Fellows H, Hussaini H, Mitchell J, Bendall R, Banks M, Ijaz S, Teo CG, Levine D**. 2007 - Autochthonous hepatitis E in southwest England. Journal of Viral Hepatitis.14(5):304–309
14. **Desselberger U**. 2019 - Caliciviridae Other Than Noroviruses. Viruses. 11(3).
15. **Di Bartolo I, Tofani S, Angeloni G, Ponterio E, Ostanello F, Ruggeri FM**. 2014 - Detection and characterization of porcine caliciviruses in Italy. Arch Virol. 159(9):2479-84.
16. **Diez-Valcarce M, Kokkinos P, Soderberg K, Bouwknegt M, Willems K, de RodaHusman AM, et al**. 2012 - Occurrence of human enteric viruses in commercial mussels at retail level in three European countries. Food Environ Virol. 4(2):73-80.
17. **Dufkova L., Sčigalková I., Moutelíková R., Malenovská H., Prodelaova J.** 2013 - Genetic diversity of porcine sapoviruses, kobuviruses, and astroviruses in asymptomatic pigs: An emerging new sapovirus GIII genotype. Archives of Virology, 158(3), 549–558.
18. **Farkas T, Nakajima S, Sugieda M, Deng X, Zhong W, Jiang X**. 2005 - Seroprevalence of noroviruses in swine. J Clin Microbiol. 43(2):657-61.
19. **Feurer C, Le Roux A, Rossel R, Barnaud E, Dumarest M, Garry P, Pavio N**. 2018 - High load of hepatitis E viral RNA in pork livers but absence in pork muscle at French slaughterhouses. International Journal of Food Microbiology. 264:25–30.
20. **Fraga M., Doerig C., Moulin H., Bihl F., Brunner F, Müllhaupt B., Ripellino P., Semela D, Stickel F, Terziroli Beretta-Piccoli B**. 2017 - Hepatitis E virus as a cause of acute hepatitis acquired in Switzerland. Liver International. 38(4):619–626.
21. **Gabbay Y.B., Borges A.A., Oliveira D.S., et al**. 2008 - Evidence for zoonotic transmission of group C rotaviruses among children in Belém. Braz. J. Med. Virol, 80, 1666-1674.
22. **Giannini P., Jermini M., Leggeri L., Nüesch-Inderbinen M., Stephan R.** 2017 - Detection of hepatitis E virus RNA in raw cured sausages and raw cured sausages containing pig liver at retail stores in Switzerland. Journal of Food Protection. 81(1):43–45.
23. **Halaihel N., Masía R.M., Fernández-Jiménez M., Ribes J.M., Montava R., De Blas I., Gironés O., Alonso J.L., Buesa J**. 2010 - Enteric calicivirus and rotavirus infections in domestic pigs. Epidemiol. Infect. 138:542–548.
24. **Indik S., Valicek L., Smid B., Dvorakova H., Rodak L.** 2006 - Isolation and partial characterization of a novel porcine astrovirus. Vet Microbiol. 117(2-4):276-83.
25. International Committee on Taxonomy of Viruses. Virus Taxonomy. Available from https://ictv.global/taxonomy.
26. **Kamar N., Bendall R., Legrand-Abravanel F., Xia N.S., Ijaz S., Izopet J., et al**. 2012 - Hepatitis E. Lancet. 379(9835):2477-88
27. **Kwok K.T., Nieuwenhuijse D.F., Phan M.V.T., Koopmans M.P.G**., 2020 - Virus Metagenomics in Farm Animals: A Systematic Review. Viruses. 12(1).
28. **Laconi A., Cavicchio L., Tassoni L., Cunial G., Milani A., Ustulin M., Di Martino G., Forzan M., Campalto M., Monne I.**, et al. 2020 - Identification of two divergent swine Noroviruses detected at the slaughterhouse in North East Italy. Porc. Health Manag. 6:9.
29. **Laurin M.A., Dastor M., L'Homme Y**. 2011 - Detection and genetic characterization of a novel pig astrovirus: relationship to other astroviruses. Archives of virology. 156(11):2095-9. 30.
30. **Lee G.H., Tan B.H., Teo E.C., Lim S.G., Dan Y.Y., Wee A.**, et al. 2016 - Chronic Infection with Camelid Hepatitis E Virus in a Liver Transplant Recipient Who Regularly Consumes Camel Meat and Milk. Gastroenterology. 150(2):355-7 e
31. **Li L., Diab S., McGraw S., Barr B., Traslavina R., Higgins R., Talbot T., Blanchard P., Rimoldi G., Fahsbender E., Page B., Phan T.G., Wang C., Deng X., Pesavento P., Delwart E.** 2013 - Divergent astrovivrus associated with neurologic disease in cattle. Emerging Infectious Diseases, 19, 1385–1392.
32. **L'Homme Y., Sansregret R Plante-Fortier E., Lamontagne A.M., Lacroix G., Ouardani M.,** et al. 2009 - Genetic diversity of porcine Norovirus and Sapovirus: Canada, 2005-2007. Archives of virology.154(4):581-93.
33. **Luo Z., Roi S., Dastor M., Gallice E., Laurin M.A., L'Homme Y.**. 2011 - Multiple novel and prevalent astroviruses in pigs. Vet Microbiol.149(3-4):316-23.
34. **Machnowska P., Ellerbroek L., Johne R. 2014 -** Detection and characterization of potentially zoonotic viruses in faeces of pigs at slaughter in Germany. Vet Microbiol. 10;168(1):60-8.
35. **Maclachlan N.J, Dubovi E.J**. Fenner's veterinary virology. Fifth ed: London, United Kingdom: Academic Press is an imprint of Elsevier; 2016.
36. **Martella V., Banyai V., Jmatthijnssens J**. et al. 2010 - Zoonotic aspects of rotaviruses. Vet Microbiol , 140, 246-255.
37. **Matias Ferreyra F.S., Bradner L.K., Burrough E.R., Cooper V.L., Derscheid R.J., Gauger P.C., Harmon, K.M., Madson D., Piñeyro P.E., Schwartz K.J., Stevenson, G.W., Zeller M.A., Arruda B.L**. 2019 - Polioencephalomyelitis in domestic swine associated with Porcine Astrovirus Type 3. Veterinary Pathology, 57(1), 82–89.
38. **Matthijnssens J., Ciarlet M., Rahman M.** et al. 2008 - Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch Virol, 153, 1621-1629.
39. **Matthijnssens J., Ciarlet M., Heiman E.** et al. 2008 - Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1—like and bovine rotavirus strains. J Virol , 82 , 3204-3219.
40. **Martella V., Banyai K., Matthijnssens J., Buonavoglia C., Ciarlet M.**. 2010 - Zoonotic aspects of rotaviruses. Vet Microbiol. 40(3-4):246-55.
41. **Marthaler D, Rossow K, Culhane M, Goyal S, Collins J, Matthijnssens J,** et al. 2014 - Widespread rotavirus H in commercially raised pigs, United States. Emerg Infect Dis. 20(7):1195-8.
42. **Mauroy A., Scipioni A., Mathijs E., Miry C., Ziant D., Thys C., Thiry E.** 2008 **-** Noroviruses and sapoviruses in pigs in Belgium. Arch. Virol.153:1927–1931.
43. **Méndez E., Arias C.F.** 2007 - Astroviruses. Dans: Fields Virology. Knipe, D.M. and Howley, P.M. (eds.). Fifth edition. Lippincott Williams & Wilkins, Philadelphia, PA.
44. **Mijovski J.Z., Poljšak-Prijatelj M., Steyer A., Barlič-Maganja D., Koren S.** 2010 - Detection and molecular characterisation of noroviruses and sapoviruses in asymptomatic swine and cattle in Slovenian farms. Infect. Genet. Evol.; 10:413–420.
45. **Mladenova Z., Papp H., Lengyel G., Kisfali P., Steyer A., Steyer A.F., Esona M.D., Iturriza-Gomara M.,**

Banyai K. 2012 **-** Detection of rare reassortant G5P[6] rotavirus, Bulgaria. Infect. Genet. Evol. 12:1676–1684.
46. **Molinari BL, Alfieri AF, Alfieri AA.** 2015 - Genetic variability of VP6, VP7, VP4, and NSP4 genes of porcine rotavirus group H detected in Brazil. Virus Res. 197:48-53.
47. **Montone A.M.I., De Sabato L., Suffredini E., Alise M., Zaccherini A., Volzone P., Di Maro O., Neola B., Capuano,F., Di Bartolo I.,** 2019 - Occurrence of HEV-RNA in Italian regional pork and wild boar food products. Food Environ. Virol. 11, 420–426.
48. **Moor D., Liniger M., Baumgartner A., Felleisen R**., 2018 - Screening of ready-to-eat meat products for hepatitis E virus in Switzerland. Food Environ. Virol. 10, 263–271.
49. **Mor S. K., Chander Y., Marthaler D., Patnayak D. P., Goyal S. M.** 2012 - Detection and molecular characterization of Porcine astrovirus strains associated with swine diarrhea. Journal of Veterinary Diagnostic Investigation, 24(6), 1064–1067.
50. **Norman J.M., Handley S.A., Virgin H.W**. 2014 - Kingdom-agnostic metagenomics and the importance of complete characterization of enteric microbial communities. Gastroenterology. 146(6):1459-69.
51. **Oka T., Wang Q., Katayama K., Saif L.J**. 2015 - Comprehensive review of human sapoviruses. Clin Microbiol Rev. 28:32–53.
52. **O'Shea E., Collins P. J., Gunn L., Matthijnssens J., Zeller M., Heylen E., Conceicao-Neto N., McKillen J., Morgan J., Staines A., O'Shea H**. 2016 - Isolation and analysis of porcine enteric viruses in Ireland: Near complete genome sequences of porcine astrovirus and porcine teschovirus. IPVS 2016-1378.
53. **Pavio N, Merbah T, Thebault A**. 2014 - Frequent hepatitis E virus contamination in food containing raw pork liver, France. Emerg Infect Dis. 20(11):1925-7.
54. **Percival S.L., Chalmers R.L., Embrey M., Hunter P.R., Sellwood J., Wyn-Jones P.** 2004 - Microbiology of waterborne diseases. Elsevier Academic Press, San Diego, CA.
55. **Pischke S., Wedemeyer H.** 2013 - Hepatitis E virus infection: multiple faces of an underestimated problem. J Hepatol. 58(5):1045-6
56. **Purdy M.A, Drexler J.F, Meng X.J, Norder H., Okamoto H., Van der Poel W.H.M, Reuter G., de Souza W.M, Ulrich R.G, Smith D.B**. 2022 - ICTV Virus Taxonomy Profile: Hepeviridae 2022. J Gen Virol.103(9).
57. **Rawal G., Linhares D.C.L**. 2022 - Scoping review on the epidemiology, diagnostics and clinical significance of porcine astroviruses. Transbound Emerg Dis. 69(3):974-985.
58. **Rawal G., Ferreyra F.M., Macedo N.R., Bradner L.K., Harmon K.M., Allison G., Linhares D.C.L., Arruda B. L.** 2020 - Ecology of Porcine Astrovirus Type 3 on a Swine farm with associated neurologic disease. Viruses, 12(9), 992.
59. **Rawal G., Matias F.F., Macedo N.R., Bradner L.K., Harmon K.M., Mueller A., Allison G., Linhares D.C. L., Arruda B.L**. 2019 - Detection and cellular tropism of porcine astrovirus type 3 on breeding farms. Viruses, 11(11), 1051.
60. **Reuter G., Bíró H., Szücs G**. 2007 - Enteric caliciviruses in domestic pigs in Hungary. Arch. Virol. 152:611–614.
61. **Reuter G., Zimsek-Mijovski J., Poljsak-Prijatelj M., Di Bartolo I., Ruggeri F.M., Kantala T.**, et al. 2010 - Incidence, diversity, and molecular epidemiology of sapoviruses in swine across Europe. J Clin Microbiol. 48(2):363-8
62. **Santos N., Hoshino Y.** 2005 - Global distribution of rotavirus serotypes/genotypes and its implication for the development and implémentation of an effective rotavirus vaccine. Rev. Med. Virol. 15, 29-56.
63. **Sigieda M., Nagaoka Y., Ohshita T**. et al.1998 - Detection of Norwalk-like virus genes in the caecum contents of pigs. Arch. Virol., 143 , 1215-1221
64. **Stamelou E., Giantsis I.A., Papageorgiou K.V., Petridou E., Davidson I., Polizopoulou Z.S., Papa A., Kritas S.K. 2022 -** Epidemiology of Astrovirus, Norovirus and Sapovirus in Greek pig farms indicates high prevalence of Mamastrovirus suggesting the potential need for systematic surveillance. Porcine Health Manag. ;8(1):5.
65. **Sugieda M., Nakajima K**. 1996 - Outbreaks of Norwalk-like virus-associated gastroenteritis traced to shellfish: Coexistence of two genotypes in one specimen. Epidemiol. Infect. 116:339–346.
66. **Sunaga F., Masuda T., Aoki H., Ito M., Sano K., Naoi Y., Katayama Y., Omatsu T., Oba M., Furuya T., Shirai J., Mizutani T., Oka T., Nagai M.** 2019 **-** Complete genome sequencing and genetic characterization of porcine sapovirus genogroup (G) X and GXI: GVI, GVII, GX, and GXI sapoviruses share common genomic features and form a unique porcine SaV clade. Infect Genet Evol. 75:103959.
67. **Szabo K., Trojnar E., Anheyer-Behmenburg H., Binder A., Schotte U., Ellerbroek L., Klein G., Johne R.,** 2015 - Detection of hepatitis E virus RNA in raw sausages and liver sausages from retail in Germany using an optimized method. Int. J. Food Microbiol. 215, 149–156
68. **Truong T.C., Nguyen T.H., Kim W.** 2022 **-** Multiple reassortment and interspecies transmission events contribute to the diversity of porcine-like human rotavirus C strains detected in South Korea. Arch Virol. 167(11):2163-2171.
69. **Ulloa J.C., Gutiérrez M.F.** 2010 - Genomic analysis of two ORF2 segments of new porcine astrovirus isolates and their close relationship with human astroviruses. Canadian Journal of Microbiology. 2010, 56(7): 569-577.
70. **Yin X., Ambardekar C., Lu Y., Feng Z.** 2016 - Distinct entry mechanisms for nonenveloped and quasi-enveloped hepatitis E viruses. J Virol, 90:4232–4242.
71. **Van Der Poel W.H.M., Vinje J., Van Der Heide R.** et al. 2000 - Norwalk-like calicivirus genes in farm animals. Emerg. Infect. Dis., 6 , 36-41.
72. **Vinje J., Hamidjaja R.A., Sobsey M.D**. 2004 - Development and application of a capsid VP1 (region D) based reverse transcription PCR assay for genotyping of genogroup I and II noroviruses. J Virol Methods. 116(2):109-17.
73. **Vlasova A.N., Amimo J.O., Saif L.J.** 2017 **-** Porcine Rotaviruses: Epidemiology, Immune Responses and Control Strategies. Viruses. 2017;9(3).
74. **Wang Q.H., Han M.G., Cheetham S., Souza M., Funk J.A., Saif L.J**. 2005 - Porcine noroviruses related to human noroviruses. Emerg Infect Dis.11(12):1874-81.
75. **Ward L.A., Yuan L., Rosen I.** et al., 1996 - Development of mucosal and systemic lymphoproliferative responses and protective immunity to human group A rotaviruses in a gnotobiotic pig model. Clin Diagn Lab Immunol, 3, 342-350.
76. **Yuan L., Stevenson G., Saif L.** 2006 - Rotavirus and reovirus. In Diseases of Swine, 9e ed., Ed Strauw B. et al, Blackwell Publ. Ames, 2006, p. 435-454.

MONIEZIAIS OF RUMINANT IN SERBIA – PRELIMINARY OBSERVATIONS

Ivan PAVLOVIĆ[1], Nemanja ZDRAVKOVIĆ[1], Oliver RADANOVIĆ[1], Jovan BOJKOVSKI[2], Milan P.PETROVIĆ[3], Violeta Caro-PETROVIĆ[3], Igor STOJANOV[4], Stanko MINIĆ[5], Ferenc CSORDÁS[6], Ivan DOBROSAVLJEVIĆ[7], Bisa RADOVIĆ[8], Valentina MILANOVIĆ[8], Vladica MLADENOVIĆ[9], Slobodan STANOJEVIĆ[1], Aleksandra TASIĆ[1], Renata RELIĆ[10]

e-mail (first author): dripavlovic58@gmail.com

Abstract

Monieziais is a global parasite disease of ruminants. It is caused by *Moniezia expans* and *Moniezia benedini*. As all tapeworms, *Moniezia* spp has an indirect life cycle with ruminants as final hosts, and oribatid mites (also called "moss mites" and "beetle mites") as intermediate hosts. The oribatid mites ingest the eggs, which hatch in their gut and develop to cysticercoids in the body cavity of the mites. They are infective for the final hosts. These thugs inhabit the small intestine of the host and clinical presentation is most common in young animals. There are disorders of profuse diarrhea, intestinal convulsions and obstruction, bloated abdomen, cachexia and death. Research on the prevalence of monieziais in ruminants has not been done in Serbia for more than fifty years. In our work, we presented the results of a preliminary examination of the prevalence of monieziais in certain regions of Serbia in large and small ruminants in the last ten years. *Moniezia benedeni* is a common tapeworm of cattle in Serbia and depending on the region, the prevalence is 3 to 5%. *Moniezia expansa* is more frequent in sheep and goats and occured in 11 to 23% of examined animals. Diagnosis is based on fecal examination for the presence of gravid segments (proglottids) or of eggs with a characteristic morphology. In the treatment of the disease, the most commonly used preparations are bezamidazole, niclosamide, and combination of praziquantel and levamisole.

Key words: sheep, goat, cattle, *Moniezia expansa*, *Moniezia benedini*

The parasitic fauna of ruminants is rich and diverse. Among them, the largest percentage is made up of nematodes (gastrointestinal and lung parasites), while planthelminths make up only 20% of the total number. In addition, tapeworms are represented in adult form only by one genus from the family Anoplocephalidae, the family Moniezia and the species *Moniezia expansa*, *M.benedini, M. autumnalis* and *M. Baeri* (Soulsby E.J.L,1977, Roberts R.L.. *et al*.2005, Mehlhorn H., 2008)

[1] Scientific Institute of Veterinary Medicine of Serbia, Belgrade, Serbia
[2]Faculty of Veterinary Medicine,University of Belgrade, Serbia
[3]Institue for Animal Husbandry,Belgrade-Zemun, Serbia
[4] Scientific Veterinary Institute Novi Sad, Novi Sad, Serbia
[5] Veterinary Station "Minić", Starčevo, Serbia
[6] Veterinary Ambulance FeriTom, Zrenjanin, Serbia
[7] Veterinary Institute Požarevac, Požarevac, Serbia
[8] Faculty of Agriculture, Lesak, University of Pristina, Kosovska Mitrovica, Serbia
[9] Veterinary Farmacy Vethem plus, Velika Plana, Serbia
[10] Faculty of Agriculture, University in Belgrade, Serbia

Moniezia species occur in ruminants in most parts of the world, *M. benedeni* primarily in cattle and *M. expansa* in cattle and sheep (Denegri G., *et al.*1998, Guo A., 2017)

As all tapeworms, *Moniezia* spp has an indirect life cycle, where ruminants is final hosts, and oribatid mites as intermediate hosts (Denegri G.,1993). In transmissio of Moniezia occurs during the summer, and parasite burdens increase into the late summer and fall, when parts of adult parasites may be shed in the feces (Ardeleanu D. *et al*.2017). The young are more likely to have heavy infection than are older animals (Guo A.,2017).

The grazing semi-intensive breeding allows ruminats to constant contact with tintermedial hosts (oribatids, mollusks, etc.) and eggs and larval forms of parasites, so that there is numerous animals that is infected with at least one parasitic species (Kenyon F. *et al.*2007, Bersissa et al.2011, Pavlovic I. and Ivanović S.,2019).

Research on the prevalence of endoparasites, especially tapeworms, in ruminants has not been done in Serbia for more than fifty years (Babić B.P.,1965,Aleksić S.D.,1986,Marušić S.,1988). Then they were partially continued at the beginning of the nineties of the last century, but due to all the events in these areas, they were interrupted (Pavlović I. et al.1991, 1995, 2003, 2007).

Finally, in the last ten years (from 2011 to 2020), continuous research on the ruminant parasite fauna in Serbia has started again, and here we present the results of thet research with emphases on the prevalence of monieziais in large and small ruminants.

MATERIAL AND METHOD

Our research was systematically carried out in the all regions of Serbian in the period from 2011 to 2020. Examination were performed in all areas where small and large ruminants are raised in semi-intensive breeding. Biodiversity of parasites, their prevalence and seasonal distribution, as well as economic indicators of the harmful effects of parasites, were monitored (Pavlović I., et al.2009).

In this period, more than 350 herds of sheep (approximately 5,500 animals), 270 herds of goats (approximately 3,100 animals) and 90 herds of cattle (approximately 800 animals) were examined. In this period, approximately 9000 samples of sheep, goat and cattle feces were examined. Coprological examinations were performed in all herds using standard coprological methods (Euzebyn J.,1981, Pavlović I., Rogožarski D.2017). Examinations we performed with Carl Zeiss AxioLab A1 microscope with the Axiocam 105 Color microscope camera and Zen Lite software.

At same time more than 750 animals were examined postmortem (either on the slaughter line or after death).

Determination of eggs and adult parasites we performed by morphological characteristic by key given by Euzeby J. (1981) and Anderson R.C.(2000).

RESULTS AND DISCUSSION

During our examination *Moniezia benedeni* is a common tapeworm of cattle in Serbia and depending on the region, the prevalence is 3 to 5%. Most prevalente are in northern part of Serbia (Vojvodina) where the prevalence ranges from 3-19%, while in the south of Serbia the prevalence ranges from 1 to 3 percent. The most endangered are lowland pastures, where the largest number of transitional hosts - oribatids - are found.

Moniezia expansa is more frequent in sheep and goats and occured in 11 to 23% of examined animals. It is mostly found in central Serbia, where the population of sheep and goats is present in the largest number (Petrović P.M.,. *et al*.2021, Pavlović I.,Ivanović S.,2022)... *M.benedeni* were occured in les than 1% (Pavlović I.,Kulišić Z., 2007).

Adult *Moniezia* belong to the largest parasitic tapeworms of livestock (SoulsbyE.J.L., 1977). They can reach up to 10 m in length. *Moniezia expansa* can be up to 1.5 cm wide, *M.benedeni* up to 2,5 cm (Mehlhorn H., 2008) The head (scolex) measures about 0.8 cm and has 4 prominent suckers but no hooks. The main body (or *strobila*) has hundreds and up to thousands of segments (called *proglottids*). The segments are much broader than long. As in all tapeworms, each segment has its own reproductive organs of both sexes (i.e. they are hermaphroditic) and excretory cells known as flame cells (*protonephridia*). The reproductive organs in each segment have a common opening called the genital pore. In young segments all these organs are still rudimentary. They develop progressively, which increases the size of the segment as it is pushed towards the tail (Soulsby E.J.L.1977).

Mature gravid segments are full of eggs (several thousands) and detach from the strobila (i.e. the chain of segments) to be shed outside the host with its feces. Otherwise, as other tapeworms, they have neither a digestive tube, nor a circulatory or respiratory systems. They don't need them because each segment absorbs what it needs directly through its tegument. Individual gravid segments in the feces are visible by the naked eye (Pavlović I.,and Rogožarski D.,2017)

Gravid segments containing the eggs are shed out and release the eggs only outside the host. The eggs are sticky and adhere to the vegetation or soil particles. Depending on the species and the region they can survive for months in the environment and some may survive cold winters, but they are very sensitive to dessication. The oribatid mites ingest the eggs, which hatch in their gut and develop to cysticercoids in the body cavity of the mites (Denegri G.,1993)They are infective for the final hosts. Cysticercoids can survive for months inside the mites, which on their turn have a live span of up to 18 months (Akrami M.A.,*et al*.,2007)

The final host becomes infected after ingesting contaminated mites while grazing. The mites are digested and release the cysticercoids that attach to the iner surface of the small intestine using a strong muscular sucers on their head and develop to adult tapeworms within a several weeks, depending on the worm species and the final host. The adult worms live for up to 18 months inside their final host. *Moniezia* infections are rather benign for adult livestock and usually do not cause clinical signs.

The pathogenic effect of parasite is manifested by mechanical and toxic action

directly affecting the resorptive capacity of the intestine.

Due to the presence of parasites, partial intestinal obturation occurs, followed by intussusception, volvulus and sometimes intestinal rupture. Low-intensity infection is asymptomatic. High-intensity infections (especially in lambs and kids) are accompanied by digestive tract dysfunction, profuse diarrhea, intestinal convulsions and obstructions, distended abdomen, cachexia and death. In cattle, camels and wild ruminants, the infection proceeds with less pronounced symptoms (Pavlović I., *et al.* 2012, 2013, 2015,2017).

Based on our experience, we recommend preimaginal deworming therapy in the spring (April, early May) - prevention of clinical manifestations of the disease and contamination of pastures with a large number of eggs. In case of new infections, deworming is repeated 30-40 days after the first one. Measures for the reduction of transitional hosts in the pasture - practically impossible (Truong P.N., Bake, D., 1998, Skipp R.A., *et al.,* 2000) In the treatment of diseases, preparations based on bezamidazole, niclosamide and combinations of levamisole and preziquantel are most often used (Southworth J., *et al.*,1996, Ivanović S.,Pavlović I.,2015, Petrović P.M., *et al.*2021,PavlovićI.,Ivanović S., 2022)

CONCLUSIONS

Prevalence of monieziais in Serbia in large and small ruminants in the last ten years shown that *Moniezia benedeni* is a common tapeworm of cattle in Serbia and depending on the region, the prevalence is 3 to 5%. *Moniezia expansa* is more frequent in sheep and goats and occured in 11 to 23% of examined animals. The pathogenic effect of parasites is manifested by mechanical and toxic action directly affecting the resorptive capacity of the intestine.

Given that these are only preliminary studies, we are of the opinion that more attention should be paid to the study of the prevalence of monieziais in small and large ruminants in Serbia, as well as the development of control measures.

ACKNOWLEGMENTS

The study was funded by the Serbian Ministry of Education, Science and Technological Development (Contract No 451- 03-68/2022- 14/200030).

REFERENCES

Aleksić S. D., 1986 - Helmintoze goveda i ovaca Timočke Krajine s posebnim osvrtom na fasciolozu i dicroceliozu. BSc thesis, Faculty of Veterinary Medicine,University of Belgrade.

Akrami M.A., Saboori A., Eslami A. 2007 - Observations on oribatid mites (Acari: Oribatida) serving as intermediate hosts of *Moniezia expansa* (Cestoda: Anoplocephalidae) in Iran. International Journal of Acarology, 33, 4:365-369.

Ardeleanu D., Pivodă C., Neacşu M., Ida A., 2017 - Bio-ecolgical phenomenon of poly-parasitism –actual major problem in breeding of sheep and goats. Lucrari Stiintifica Zootechnica. şi Biotehchologia, 40(2):309-317.

Anderson R.C., 2000 - Nematode parasites of vertebrates: their develpment and transmision. 2nd ed. Wellingford,UK; NewYork USA.

Babić B. P., 1965 - Helminti digestivnih organa goveda Srbije sa naročitim osvrtom na njihovo pojavljivanje i nedovoljno opisane vrste. PhD disertation, Faculty of Veterinary Medicine,University of Belgrade.

Bersissa K, Tigist T., Teshale S., Reta D., Beru H., 2011 - Helminths of Sheep and Goats in Central Oromia (Ethiopia) During the Dry Season, Journal of Animal and Veterinary Advances, 10: 1845-1849.

Denegri G. 1993 - Review of oribatid mites as intermediate hosts of tapeworms of the Anoplocephalidae. Experimental and Apply Acarology, 17: 567–580.

Denegriđ G; Bernadina W., Perez-Serrano, J., Rodriguez-Caabeiro F, 1998 - Anoplocephalid cestodes of veterinary and medical significance: a review. Folia Parasitologica, 45 (1): 1–8

Denev I., Kostov R.,1984 - Seasonal variations in the principal helminth infections on a sheep-grazing complex. Veterinarno-meditsinska Nauki, 21:81-88.

Euzeby J., 1981 - Diagnostic experimental de helminthoses animals", ITVC, Paris, France.

Guo A., 2017 - Moniezia benedeni and Moniezia expansa are distinct cestode species based on complete mitochondrial genomes Journal Acta Tropica 166:287-292.

Ivanović S., Pavlović I., 2015 - Meso koza - bezbedna namirnica.NIVS, Beograd.

Kenyona F., Sargisonb N.D., Skucea P.J., Jacksona F., 2009 - Sheep helminth parasitic disease in south eastern Scotland arising as a possible consequence of climate change. Veterinary Parasitology 163, 4:293–297.

Marušić S., 1988 - Endoparazitoze goveda u području rada veterinarske stanice u Smederevskoj Palanci i razrada mera za njihovo suzbijanje. BSc thesis, Faculty of Veterinary Medicine,University of Belgrade

Mehlhorn H., 2008 - Encyclopedia of parasitology, Volume 1 (3rd edn). Springer.

Pavlović I,, Kulišić Z., 2007 - Moniezioza ovaca na području Beograda. Zbornik radova i kratkih sadržaja 19.savetovanja veterinara Vrnjačka Banja, Vrnjačka Banja, 45-46

Pavlović I., Rogožarski D., 2017 - Parazitske bolesti domaćih životinja sa osnovima parazitologije i dijagnostike parazitskih bolesti. Naučna KMD, Beograd.

Pavlović I., Ivanović S.. 2019 - Gastrointestinal helminths of goats breed in central part of Serbia. Proceedings of International symposium on animal science (ISAS), Herceg Novi, Montenegro, 143-148,

Pavlović I., Ivanović S. (2022) Goats from pastures to table. LAP Lambert Academic Publishing.

Pavlović I., Nešić D., Ilić G., Jovanović D., Vlater D., 1991 - Parasitoses of sheep in Timok district during 1990. Proceeding of 1th International Summer Conference for Advancement of Sheep and Goat Production, Ohrid, SFRJ, 387-389.

Pavlović I., Kulišić Z., Nešić D., Romanić S., 1995 - Endoparasites of sheep and goats in Prizren district. Proceedeing of 3rd International Conference of Sheep and Goats Production, Ohrid, Macedonia, 106-110.

Pavlović I,, Ivanović S,, Žujović M., 2003 - Moniezioza ovaca na području Dimitrovgrada. Zbornik radova i kratkih sadržaja 5. Epizootiološki dani, Subotica, 140-141.

.

Pavlović I., Savić B., Ivetić V., Radanović O., Žutić M., Jakić-Dimić D., Bojkovski J ., 2009 - The effect of parasitic infections to production results of sheep. Proceeding of IV Balkan Conference of Animal Science BALNIMALCON 2009, Challanges of the Balkan Animal industry and the Role of science and Cooperation,.Stara Zagora, Bulgaria, 389-391.

Pavlović I., Ivanovic S., Žujović M., Tomić Z.,Memiši N., 2012 - Studies on the endoparasites of goats in spread Belgrade area in period 2009-2010. Archiva Zootechnica 15(4): 27-31

Pavlović I., Ivanović S, Stokić-Nikolić S, Bojkovski J, Šekler M, Savić B, Žutić M., 2013 - Season distribution of gastroitestinal helminths of goats in south-east Serbia. Lucrări Ştiinţifice Medicină Veterinară XLVI (3), Timişoara: 138-143.

Pavlović I., Ivanović S., Savić M., Ćirković D., Jovčevski S., Jovcevski St., Savić B., Bečkei Ž., Marčić D., 2015 - Gastointestinal helmints of goats breeding at Stara plana area (Serbia). Lucrări Ştiinţifice Medicină Veterinară Timisoara XLVIII (3): 159-166

Pavlović I., Becskei Z., Ivanović S., Petrović P.M., Savić M., Caro Petrović V., Bojkovski J., 2017 - Biodiversity of helminths of sheep breed in Vojvojvodina (Northern Serbia). Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca 74 (2):162-166.

Petrović P.M., Ilić Z., Caro Petrović V., Pavlović I., 2021 - Uspešno i isplativo ovčarstvo. Ruske akademije prirodnih nauka, Balkanski naučni centar, Beograd.

Roberts R.L., Janovy J.Jr., McGraw H., 2005 - Foundations of Parasitology. Higher Education, Boston

Skipp R.A., Hay F.S., Leathwick D.M., Popay I., 2000 - Biological control of gastrointestinal nematodes of livestock in faeces and in pastures. Meat New Zeland Reporton Project 96PR36/1.

Soulsby E.J.L.,1977 - Helminths, Artgropods and Protozoa of Domesticated Animals. Bailler,Tinadall and Cassell, London, UK.

Southworth J., Harvey C., Larson S.,1996 - Use of praziquantel for the control of *Moniezia expansa* in lambs. New Zeland Veterinary Journal, *44: 112–115.*

Truong P.N., Bake, D.,1998 - Vetiver grass system for environmental protection. Technical Bulletin N0. 1998/1. Pacific Rim Vetiver Network. Royal Development Projects Board, Bangkok, Thailand.

APPLICABILITY OF THORACIC NON-CARDIAC ULTRASONOGRAPHY IN THE DIAGNOSTIC PROTOCOL OF ACUTE DYSPNOEA IN FELINE PATIENTS

Andreea Cătălina TURCU[1], Radu Andrei BAISAN[1], Eusebiu Ionuț CONDURACHI[1], Vasile VULPE[1]

Corresponding author: Andreea Cătălina Turcu catalina.andreea.turcu@gmail.com

Abstract

Lung ultrasonography (LUS) is a non-invasive and rapid method used for the diagnostic of respiratory diseases in all mammals. The changes in clinically observed respiratory patterns can be correlated with the modifications observed during transthoracic ultrasonography, thus the clinician can select an appropriate protocol for managing the animals with respiratory distress. The aim of this study is to emphasize the clinical utility of lung ultrasonography when dealing with cats in an emergency setting. Cats presented with respiratory distress were retrospectively reviewed and classified based on the aetiology of the disease. Lung ultrasonography was performed using the thoracic fast protocol (T-FAST). Vet BLUE ultrasonography and chest radiography were performed only when the clinical status of the patient was suitable for these diagnostic methods. Thirty-nine cats met the inclusion criteria. The presence of free fluid, B-lines, shred sign, nodule sign, organ sign and barcode sign have been assessed for each patient. Based on the findings, a differential diagnostic and a management protocol had been proposed. Lung ultrasonography is a non-invasive, stress-free and fast examination with high sensibility and specificity for diagnosing cats with breathing difficulty.

Key words: lung ultrasonography; dyspnoea; acute respiratory distress; T-fast; B-lines;

INTRODUCTION

Shortness of breath is one the most common causes of emergency presentation. Dyspnoea is an unspecific clinical sign associated with increased breathing rate and changes in the respiratory pattern. It is often a marker of significant underlying disease, therefore requires prompt treatment. Investigations performed to discover the underlying aetiology must be balanced with the status of the animal, as stress can be detrimental in these patients (Tseng L. W., 2000).

Ultrasonographic examination of the lung has been described in both human and veterinary medicine, with targeted point-of-care protocols for obtaining a fast diagnostic. Two point-of-care protocols are designed for the veterinary patients: the Thoracic Focus Assessment with Sonography for Trauma protocol (T-FAST) and the Veterinary Brief Lung Ultrasonography Examination protocol (Vet BLUE). Both protocols were designed with two precise, different purposes. T-FAST protocol monitors the presence of free fluid (pleural effusion, pericardial effusion and cardiac tamponade) and air (pneumothorax), whereas the Vet BLUE protocol evaluates the regional lung parenchyma (recommends an assessment system for the presence of B and A lines, as well as other changes located on the periphery of the lung lobes) (Lisciandro G. R., 2011; Lisciandro G. R., 2017).

The T-FAST technique evaluates the changes occurring in four main points at the level of both sides of the chest, guided by the principle that "air rises and liquid descends". The right and left chest tube sites (CTS) are assessed for the presence of gliding sign. The right and left pericardial sites (PCS) for pericardial and pleural fluid and estimation of volume status (Lisciandro G. R., 2011).

The Vet BLUE protocol aims to determine the condition's aetiology in animals with dyspnoeic syndrome. The technique involves the qualitative and quantitative assessment of the presence of A lines, B lines and lung signs (shred sign, nodule sign and tissue sign) in less than 90 seconds. This method has a high sensitivity for ruling out left-sided congestive heart failure in dogs (88%) and in cats (96%) (Lisciandro G. R., 2017).

The radiological examination of cats experiencing dyspnoea is controversial. It is well known that the patient needs to maintain a lateral, dorso-ventral or ventro-dorsal recumbence. Therefore, it is advisable to delay this procedure until the cat is hemodynamically stable or performed with the animal under general anesthesia,

[1] Clinic Departament, Faculty of Veterinary Medicine, University of Life Sciences "Ion Ionescu de la Brad", Iași, Romania

securing the airways. Regardless, the two imaging methods are complementary. The main advantage for radiologic examination is the possibility of having an overview of the thorax, including the ribcage and the profound structures.

MATERIAL AND METHOD

This retrospective observational study included cats presented for acute onset of respiratory distress at the Veterinary Teaching Hospital, between March 2021 and May 2022. Written consent for all procedures was obtained from the owners. Cats were included if the ultrasonographic evaluation of the thorax using either the T-FAST or Vet BLUE protocol was available. Patients were excluded from this study if the stored examination was incomplete.

All cats were subjected to a physical examination prior to LUS. The time of onset, breathing rate, breathing pattern and mucosal color have been assessed. If necessary, the cats received oxygen supplementation and/or opioid analgesic (Butorphanol) before and during the examination.

The ultrasound examination was carried out using two devices (General Electric LOGIQ V5 Expert ultrasound machine, equipped with a microconvex transducer 7.5-10 MHz, linear transducer 6-12 MHz and phased array transducer 2.4-8 MHz, and the SonoScape E1V ultrasound machine equipped with a microconvex transducer 4-13 MHz).

T-FAST ultrasound examination was performed without patient preparation, therefore the position of the cat remained unchanged, the fur was not trimmed and ultrasonographic gel was not applied, but CTS, PCS and hepato-diaphragmatic (HD) sites were degreased using medicinal alcohol. These steps were subsequently performed if, after the initial evaluation, thoracentesis was performed.

Vet BLUE ultrasound examination was carried out inconstantly after clipping the fur. Compared to the T-fast method, animals were examined in sternal recumbency or standing, to allow evaluation of both hemithoraxes.

Regardless of the protocol of choice, LUS was performed using a microconvex or phased array probe positioned perpendicular to the pleural space, using high frequencies in order to allow assessment of surface structures.

For each patient, the following findings in LUS had been tracked: presence of A and B lines, presence of pleural or pericardial effusion, the shred, nodule, organ and barcode sign. The A lines represent reverberation artifacts and appear horizontal, parallel, and equidistant to the pleura. They generally appear in healthy individuals, but could appear obliterated due to B lines or reinforced in pneumothorax. The B lines represent comet tail artefacts starting that appear perpendicular to the pleural line and move with the pleura during breathing. The presence of 1-2 B lines per intercostal space is normal in clinically healthy animals. Pathologically, they are associated with pulmonary edema and interstitial lung pathologies. These lines may converge, giving the appearance of a wet or white lung (Lichtenstein D. A., 2007). The shred sign is associated with consolidation and peripheric air bronchogram perceptible on radiography. The nodule sign is present when discrete nodules are consolidated or infiltrated. The organ sign indicates the presence of abdominal herniated organs in the thoracic cavity. The barcode sign is typically found in patients with pneumothorax.

Twenty-eight cats underwent radiological examination of the thorax, either before or after the ultrasonographic evaluation, in order to obtain an overview of the thoracic cavity. The X-ray examination has been included in the study if at least two orthogonal chest images had been obtained. Chest X-rays have been performed using two machines, DR MAXIVET 400HF and Intermedical Basic 4006 equipped with mobile arm using phosphor plates and the X-CR Smart Exam digital system.

RESULTS AND DISCUSSIONS

Thirty-nine cats met the inclusion criteria. The population comprised the following breeds: domestic shorthair (n=26), Persian (n=6), British Shorthair (n=3) and one of each Russian Blue, Birman, Siamese, Norwegian forest. Twenty-three cats were male and sixteen females, with unassessed reproductive status. The mean age and standard deviation of the population was 6.44±5.09 years, and the mean bodyweight and standard deviation of the population was 3.48±1.01 kg.

Based on the aetiology, the patients have been divided into six groups, as following: traumatic conditions (25.64%; n=10), primary cardiological disorder (28.2%; n=11), primary respiratory disease (12.82%; n=5), neoplastic disease (25.64%; n=10) and unknown aetiology (7.69%; n=3). The latter group consists of animals that had incomplete history, animals whose owners declined further investigations or animals that had succumbed prior to complete the required investigations. Nonetheless, these patients were not excluded from the study, but rather express a limitation regarding the accessibility for advanced diagnostic imaging, such as endoscopy or Computed Tomography (CT).

The correlation between the aetiology and dyspnoeic syndrome in cats is consistent with the literature. To the author's best knowledge, there is only one study assessing the aetiology of the dyspnoeic syndrome in cats presented in an emergency setting, which concludes that the most common associated diseases are cardiac diseases

(37.7%), primary respiratory diseases (32.2%), neoplastic (20%) and traumatic conditions (8.9%) (Swift S., 2009).

The T-FAST protocol was used for hemodynamically unstable cats, sometimes simultaneously with the performance of other necessary medical procedures in order to stabilize the animal, such as pleurocentesis. If the initial examination detected the presence of B lines, the Vet BLUE protocol was implemented. For hemodynamically stable patients a complete LUS was performed. The T-FAST protocol were performed according to the initial recommendations (Boysen S. R., 2013). Changes introduced in the authors' last article (Lisciandro G. R., 2021) were evaluated when the Vet BLUE protocol was performed or during the cardiological examination. As the point-of-care protocols became more popular, the feasibility of the methods became uncontestable. However, the multitude of steps included in the T-FAST protocol distract the examiner from to the original purpose of the examination method, they complicate the working method and lengthen the examination time, which could be detrimental when dealing with dyspnoeic cats.

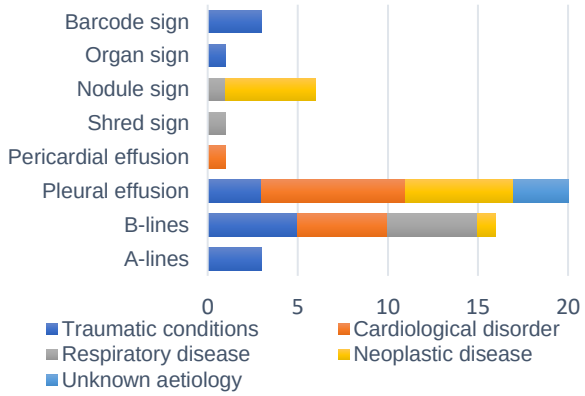


Figure 1 – Graphical representation of ultrasonographic findings in association with the aetiology of the disease

The correlation between LUS findings and aetiology for the studied population is depicted as stacked bar chart in *figure 1*.

The patients included in the trauma group revealed either normal findings during T-FAST (30%) or pathological findings, such as B-lines as a result of pulmonary contusion (50%), pleural effusion due to hemothorax (30%), barcode sign due to pneumothorax (30%) and organ sign due to transdiaphragmatic hernia (10%) (*figure 2*). The trauma resulted in unknown conditions (n=4) or due to free fall (n=2), dog bites (n=2) and hit by car (n=2).

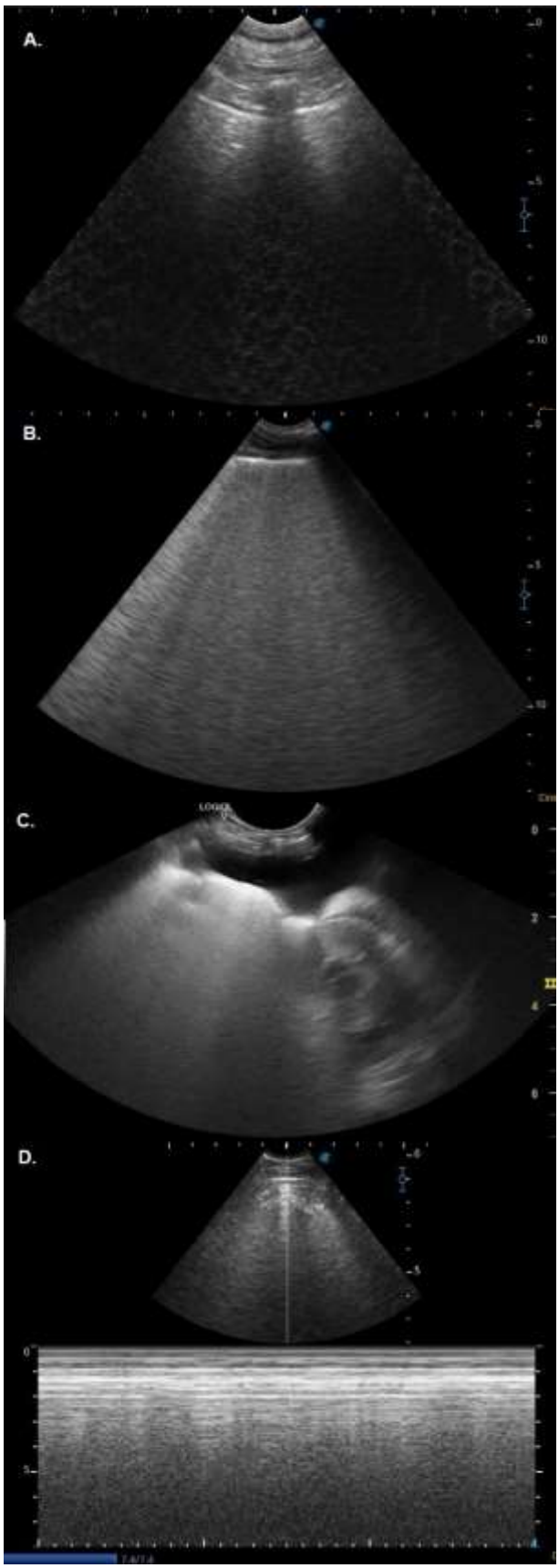


Figure 2 – LUS findings in trauma patients. A – A lines; B – B lines; C – pleural effusion; D – barcode sign;

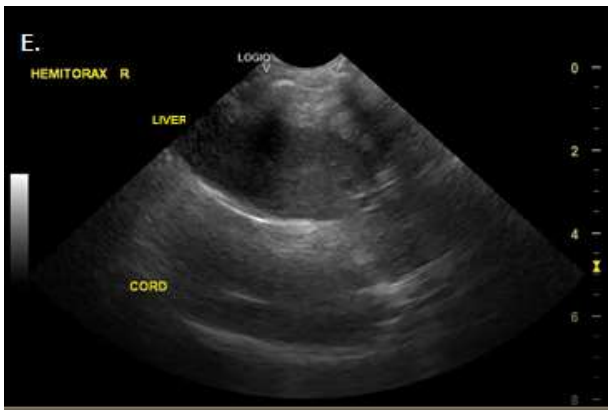


Figure 2 – LUS findings in trauma patients. E – organ sign.

The second group consists of cats with primary cardiac conditions. LUS findings revealed B-lines associated with cardiogenic pulmonary edema (45.45%), pleural effusion (72.72%) and pericardial effusion (9.09%) (*figure 3*). All cats underwent a complete cardiological examination including cardiac ultrasonography after the initial assessment. The definitive diagnostic was feline hypertrophic cardiomyopathy (n=8) and one of each nutritional dilated cardiomyopathy and tricuspid dysplasia.

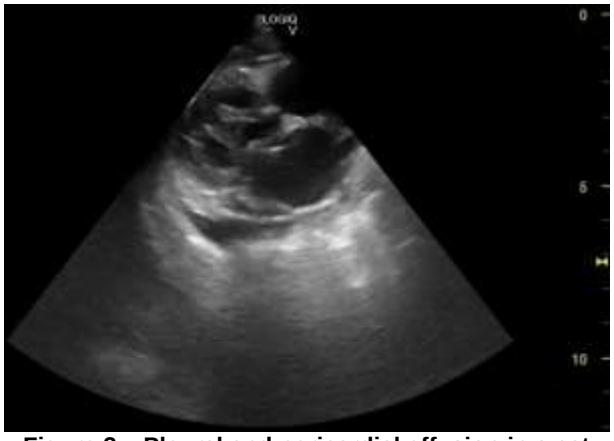


Figure 3 – Pleural and pericardial effusion in a cat diagnosed with unclassified hypertrophic cardiomyopathy.

The third group consists of cats with primary respiratory disease. The importance of point-of-care LUS for this group derives from the necessity to rapidly differentiate primary cardiogenic disorders from primary respiratory ones, as well as to identify patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS). ARDS is a life-threatening condition where the lungs cannot provide enough oxygen to the vital organs. ALI and ARDS are secondary disorders caused by a severe inflammatory reaction, the origin of which may be the lung or a site distant to the lung. A diagnosis is based on the presence of four main criteria: the acute onset (less than 72 hours), the presence of a primary underlying disease that causes enough inflammation to set up a reaction in the lungs, evidence of pulmonary capillary leak without evidence of increased hydrostatic pressures and evidence of inefficient gas exchange (Boiron L., 2016). The most important and practical diagnostic tests include a thorough history, thoracic radiography, echocardiography, and arterial blood gas analysis (Wilkins P. A., 2007). However, cats have the tendency to mask all clinical signs, therefore the owners will not be able to identify minor changes in the breathing rate or pattern. This results in belated presentation at the veterinarian, frequently in an emergency setting. By assessing the PCS, a differential diagnostic can be proposed prior to other more invasive diagnostic procedures that might increase the stress level of the cat and even worsen the dyspnoea, such as collecting blood samples. The LUS findings for this group revealed the presence of B-lines (100%) constantly associated with interstitial lung patterns on chest radiography, shred sign (20%) (*figure 4*) and nodule sign (20%). The definitive diagnostic was aspiration pneumonia (n=2), feline asthma (n=1), aelurostrongylosis (n=1) and one owner declined supplementary investigations. Based on the hypoxia level, one cat has been diagnosed with ALI, 3 with ARDS and one was unassessed.

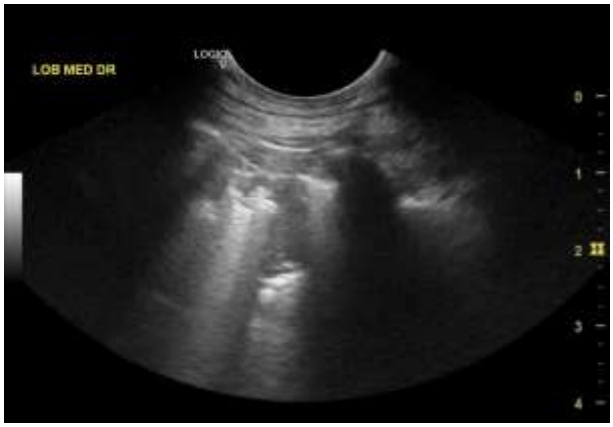


Figure 4 – Severe shred sign in a cat with aspiration pneumonia

The fourth group consists of cats with neoplastic disease. LUS findings revealed pleural effusion (60%), nodule sign (50%) (*figure 5*) and B-lines (10%). The definitive diagnostic consisted of mesothelioma (n=4), lymphoma (n=2), thymoma (n=1) and three owners declined fine needle aspiration and cytological investigations.

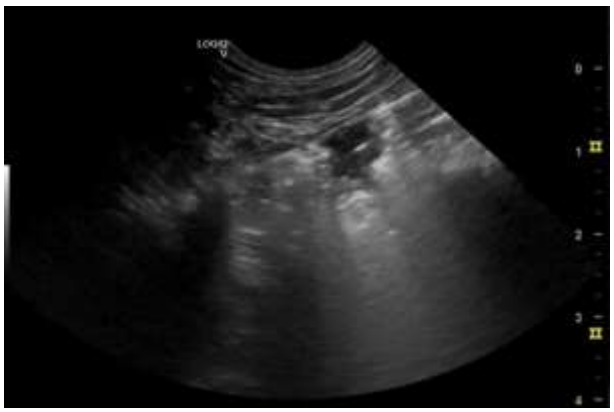


Figure 5 – Nodule sign in a cat with mesothelioma

Three cats have been presented with pleural effusion of unknown aethiology. No other abnormal findings have been detected with LUS of chest radiography. Pleurocentesis was performed, revealing transudate in two cats and chylothorax in one. The owners declined further investigations.

Chest radiographies have been obtained for twenty-eight cats using standard protocols. The localizations of abnormal findings are shown in *figure 6.*

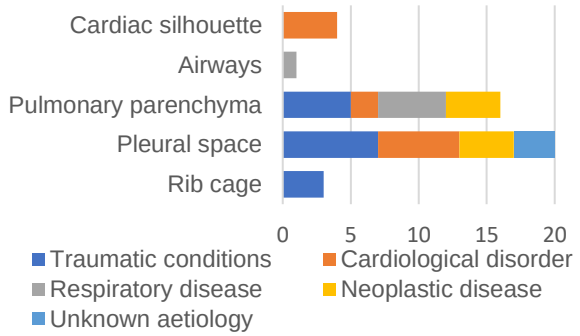


Figure 6 – Graphical representation of affected anatomical segments diagnosed using X-ray in association with the aetiology of the disease

The fact that most of the abnormalities have been associated with the pulmonary parenchyma and pleural space in dyspnoeic cats is thus not surprising. The ultrasonographic examination has increased sensitivity when assessing these segments (Richards J. R., 2017), therefore their description was not considered an objective of this paper. However, the importance of obtaining an overview of the thorax is underlined by pathologies associated with the rib cage, airways or cardiological diseases.

Three cats with thoracic trauma had multiple rib fractures. For these patients, pain management has been implemented in order to increase the patient comfort and decrease the respiratory stress. One cat with primary respiratory disease revealed severe bronchial pattern on X-ray. Due to the fact that ultrasounds can only assess the superficial structures of the thorax, this method is limited in diagnosing airway disease. Thoracic radiography is insensitive for identification of mild or moderate cardiac changes associated with feline cardiomyopathy (Virginia Luis Fuentes, 2020). However, severe cardiomegaly was observed in four cats.

CONCLUSIONS

Lung ultrasonography is a non-invasive, stress-free and fast examination method with high sensibility and specificity for diagnosing cats with difficulty breathing. Based on the findings, a suitable management protocol can be implemented and a differential diagnostic can be obtained.

ACKNOWLEGMENTS

This research received no external funding.
The authors report there are no competing interests to declare.

REFERENCES

Boiron L., H. K. (2016). *Risk factors, characteristics, and outcomes of acute respiratory distress syndrome in dogs and cats: 54 cases.* Veterinary Emergency and Critical Care, 1-7. doi:10.1111/vec.12819

Boysen S. R., L. G. (2013). *The use of ultrasound for dogs and cats in the emergency room: AFAST and TFAST.* Veterinary Clinics of North America: Small Animal Practice, 773-797.

Lichtenstein D. A. (2007). *Ultrasound in the management of thoracic disease.* Journal of Critical Care Medicine, S250-S261.

Lisciandro, G. R. (2011). *Abdominal and thoracic focused assessment with sonography for trauma, triage, and monitoring in small animals.* Journal of Veterinary Emergency and Critical Care, 104-122.

Lisciandro, G. R. (2017). *Vet BLUE® Lung Ultrasound in Small Animals - The New Way to Do Respiratory Distress.* Southwest Veterinary Symposium. Spicewood, TX, USA.

Lisciandro, G. R. (2021). *Abdominal and thoracic focused assessment with sonography for trauma, triage, and monitoring in small animals.* Journal of Veterinary Emergency and Critical Care, 104-122.

Richards J. R., M. J. (2017). *Focused Assessment with Sonography in Trauma (FAST) in 2017: What Radiologists Can Learn.* Radiology, 30-48.

Swift S., D.-M. J. (2009). *Aetiology and outcome in 90 cats presenting with dyspnoea in a referral population.* Journal of Small Animal Practice, 446-473.

Tseng L. W., W. L. (2000). *Approach to the patient in respiratory distress.* Clinical Techniques in Small Animal Practice, 53-62.

Virginia Luis Fuentes, J. A. (2020). *ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats.* Journal of Veterinary Internal Medicine, 1062–1077.

Wilkins P. A., O. C. (2007). *Acute lung injury and acute respiratory distress syndromes in veterinary medicine: consensus definitions: The Dorothy Russell Havemeyer Working Group on ALI and ARDS in Veterinary Medicine.* Journal of Veterinary Emergency and Critical Care, 333-339.

LONG TERM MONITORING OF A CAT WITH IDIOPATHIC MEDIASTINAL CYST

Andreea Cătălina TURCU[1], Radu Andrei BAISAN[1], Eusebiu Ionuț CONDURACHI[1], Vasile VULPE[1]

Corresponding author: Andreea Cătălina Turcu catalina.andreea.turcu@gmail.com

Abstract

Mediastinal cysts are previously described as incidental findings in cats. A nine years old, 2.8 kg, Persian cat was presented to out Veterinary Teaching Hospital (VTH) for mild dyspnea. Chest radiographies, revealed an incapsulated soft tissue opacity in the cranial mediastinum. Thoracic lung ultrasonography (LUS), evinced the presence of a cyst-like structure. Furthermore, the cat was subjected to complete cardiological examination and complete blood analysis. Echo-guided drainage followed by cytological examination was carried out in order to reach a definitive diagnostic and to improve the general status of the patient. No other medical treatment has been pursued. Monthly evaluations have been performed during a period of five months, and the drainage was repeated one more time until the complete resolution of the pathology. No signs of relapse have been noticed over a period of two years. Only four cats diagnosed with idiopathic mediastinal cyst have been mentioned in the literature. To the author's best knowledge, this is the first case report which describes the dynamic evolution of an idiopathic mediastinum cyst in Persian cat presented for mild dyspnoea.

Key words: idiopathic mediastinal cyst; cranial mediastinum; dyspnoea; thoracic radiography; lung ultrasonography

INTRODUCTION

A nine years old, 2.8 kg, spayed female Persian cat has been presented to our VTH for asynchronous breathing pattern and tachypnea associated to stressful situations. The clinical signs have been observed by the owner a week prior to presentation. The cat had no history of associated pathologies. At time of presentation, the animal was alert and responsive. Physical examination revealed normal body condition score, normal-colored mucosal membranes and capillary refill time (CRT) less than two seconds, normal findings during palpation of the lymph nodes. The animal presented a respiratory rate (RR) of 42 rpm, heart rate of 220 bpm, oxigen saturation (SpO_2) of 85% and arterial pressure of 181/89 mmHg (SAP/DAP). Pulmonary auscultation revealed absent breath sounds on the cranial half of the thorax and decreased cardiac sounds. Based on the findings, a pleural space occupying lesion has been suspected.

CASE PRESENTATION

Written consent for all the following medical procedures has been obtained from the owners. The first intention procedure was oxygen supplementation administered via nasal cannula. Lateral (LL) and dorso-ventral thoracic (DV) radiographies have been obtained using the Intermedical Basic 4006 machine equipped with mobile arm using phosphor plates and the X-CR Smart Exam digital system. On the lateral view, an ovoid shape with soft tissue opacity occupied the cranial half of the thoracic cavity. A mass effect affected the tracheal silhouette, which narrowed the angle between the tracheal bifurcation and the thoracic spine. The opacity overlapped the cranial contour of the heart. The area of the tissue opacity assessed on the right LL view was 33.8 cm^2. The lung parenchyma of the diaphragmatic lobes had a normal radiological aspect (*figure 1*).

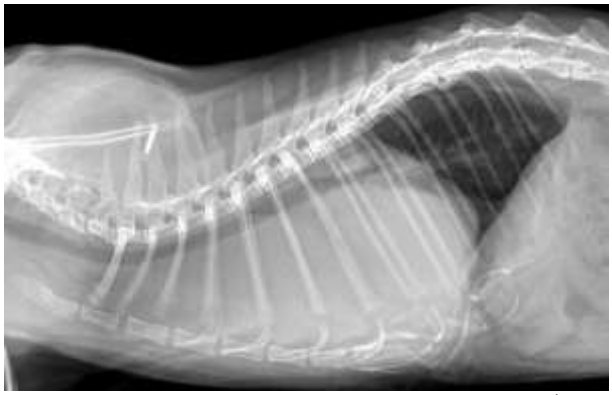


Figure 1 – Right LL thoracic radiography, 1st evaluation. Soft tissue opacity creating a mass effect on the trachea and heart

[1] Clinic Departament, Faculty of Veterinary Medicine, University of Life Sciences "Ion Ionescu de la Brad", Iași, Romania

On the DV view, the right hemithorax appeared occupied entirely by soft tissue opacity, with the heart silhouette displaced towards the left hemithorax (*figure 2*). The maximal measured dimensions of the mediastinal soft tissue opacity mass were 105/53/46 mm.

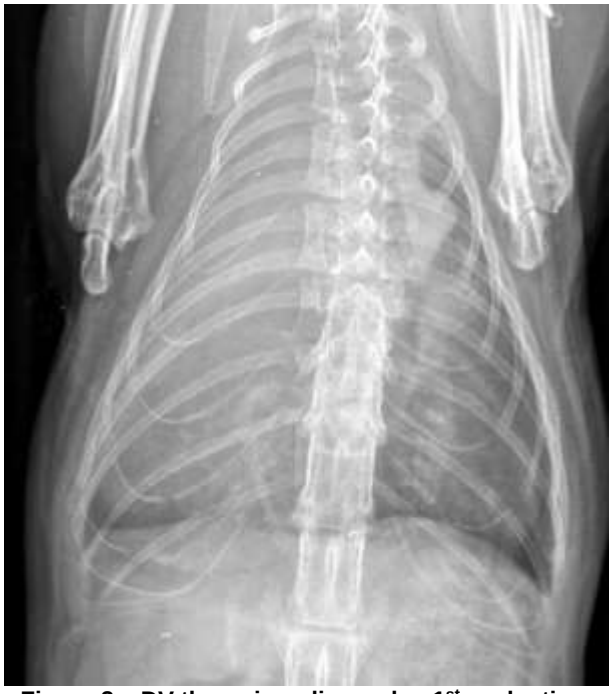


Figure 2 – DV thoracic radiography, 1st evaluation. Soft tissue opacity occupying the right hemithorax

Subsequently, LUS was performed using the General Electric LOGIQ V5 Expert ultrasound machine (equipped with a microconvex transducer 7.5-10 MHz, linear transducer 6-12 MHz and phased array transducer 4-8 MHz). An ovoid, thin walled, single lumen, anechoic-filled structure localized in the cranial mediastinum has been identified when assessing the right hemithorax, with a maximal diameter measured cranial to the heart base of 5 cm (*figure 3*). No pleural effusion was detected. Computed tomography (CT) was suggested, but owners declined. We consider this a limitation of the study, due to the fact that no CT results correlated to idiopathic mediastinal cyst have been reported.

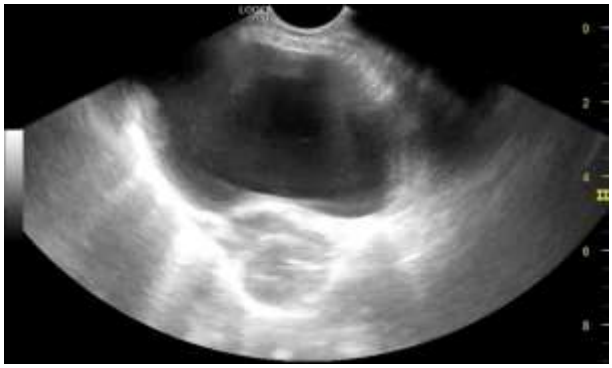


Figure 3 – Initial LUS findings. Anechoic-filled ovoid structure in the cranial mediastinum

Ultrasound-guided drainage was performed using a 5 cc syringe and a 21 G 3/4 inch needle. At the end of the procedure, a residual volume was not removed, observed as an anechoic triangular shape cranial to the base of the heart, with a maximal depth of 2.6 cm. The aspirate obtained sixty milliliters of clear fluid with a specific gravity <1.010, refractive index <1.3360 and no protein contain (figure 4). Cytological examination was negative for the presence of cellular contents.

Figure 4 – Macroscopic aspect of the fluid during drainage

After the procedure, the breathing pattern normalized, with a RR of 22 rpm and 98% SpO_2. The oxygen supplementation was stopped, and chest radiographies were repeated. On LL view, a triangular shaped soft tissue opacity was overlapped with the cranial aspect of the heart silhouette, with an area of 7.13 cm^2. The area of the structure decreased by 78.9% (*figure 5*). The maximal dimensions of the opacity decreased to 37/31/22 mm. No mass effect modified the topography of the trachea and the heart. No signs of reactive thoracic lymph nodes were observed.

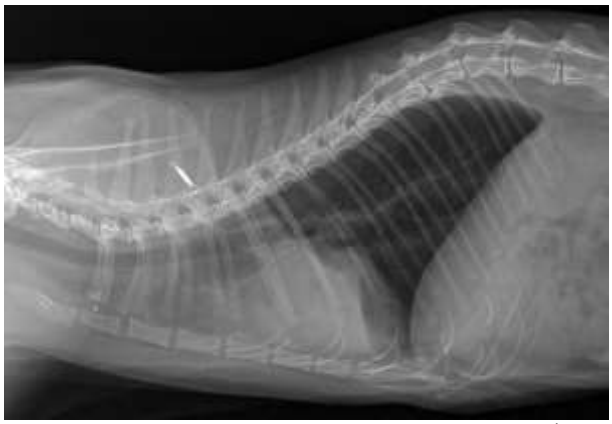


Figure 5 – Right LL thoracic radiography, 1st evaluation after aspiration. Decreased area of the soft tissue opacity

A complete cardiological examination revealed normal ECG and normal morphology of the heart. Blood analysis consisting of complete blood count and biochemical blood test were unremarkable. Based on the findings, the definitive diagnosis of idiopathic mediastinal cyst with a good prognosis has been established. No medical treatment was prescribed for the patient, and a reevaluation has been scheduled in 30 days. The owners have been informed about the possibility of

relapse, and were instructed to measure the RR of the cat three times a week, during sleep (Ohad D., 2013).

One month later, the owners described no signs of respiratory effort, absence of exercise intolerance and normal behavior. The physical examination revealed normal colored mucosal membranes and normal CRT, with a RR of 24 rpm and 98% SpO_2. Chest X-ray and LUS were repeated. On the LL view, a triangular shape with soft tissue opacity and an area of 8.77 cm^2 was overlapped to the cardiac silhouette. On ventro-dorsal view (VD), the mass overlapped the right cranial lung lobe and the right atrium. No mass effect was observed. The maximal dimensions of the cyst were 65/33/18 mm. Therefore, the comparison of the areas assessed on LL view show an increase of 23% (*figure 6*). LUS revealed a minor accumulation of fluid within the cyst, with a maximal depth of 2.85 cm measured cranial to the heart and 0.7 cm between the right atrium and thoracic wall. No other medical procedures have been carried out. A third monthly evaluation has been proposed.

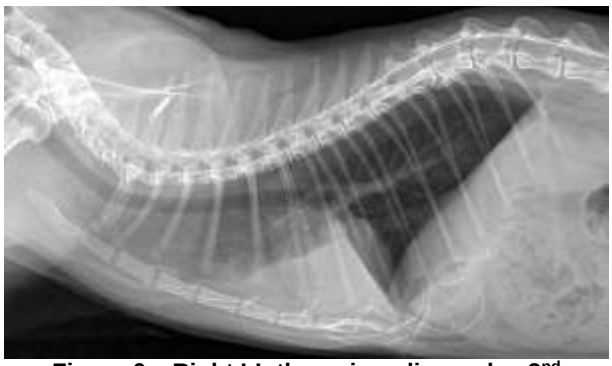


Figure 6 – Right LL thoracic radiography, 2nd evaluation

Thirty days later, the owners reported no changes in behaviour or respiratory pattern. The RR was 28 rpm, with constant 98% SpO_2. Compared to the previous examination, the LL chest X-ray presented a cranial rectangular accumulation of fluid additional to the triangular shape noticed before. No mass effect was detected. The maximal dimensions of the cyst were 78/32/22 mm, with an area of 13.01 cm^2 (*figure 7*). Therefore, the cyst increased by 82% in comparison to the area assessed after aspiration. LUS findings revealed an increase of maximal depth cranial to the heart to 4.2 cm and between the right atrium and thoracic wall to 1.7 cm. A second ultrasound-guided fine needle aspiration was proposed but the owners declined the intervention.

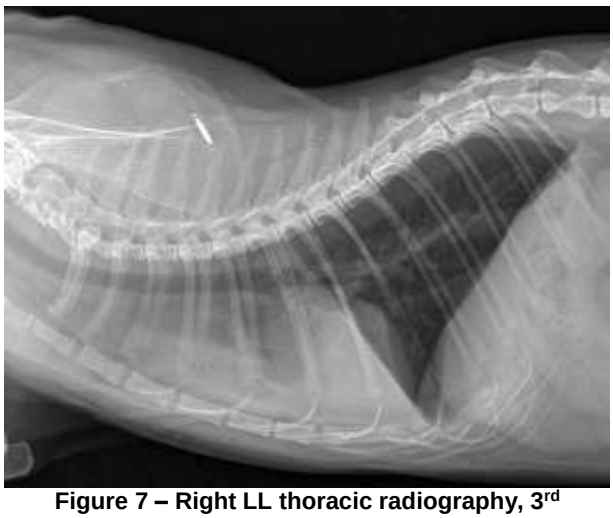


Figure 7 – Right LL thoracic radiography, 3rd evaluation

The fourth evaluation was carried out next month. The owners reported no signs of dyspnoea, however they subjectively assessed the cat as being less active. The clinical examination was normal, the areal comparison of the cyst dimension (17.32 cm^2) revealed a constant progress, with an increase of 142.98% compared to the area after aspiration (*figure 8*). The maximal dimensions of the cyst were 77/41/19 mm, creating a mass effect on the intrathoracic trachea and the heart. Ultrasound-guided fine needle aspiration was performed using the same protocol. A volume of 31 ml have been withdrawn with the same characteristics. Chest X-rays have not been obtained after the centesis.

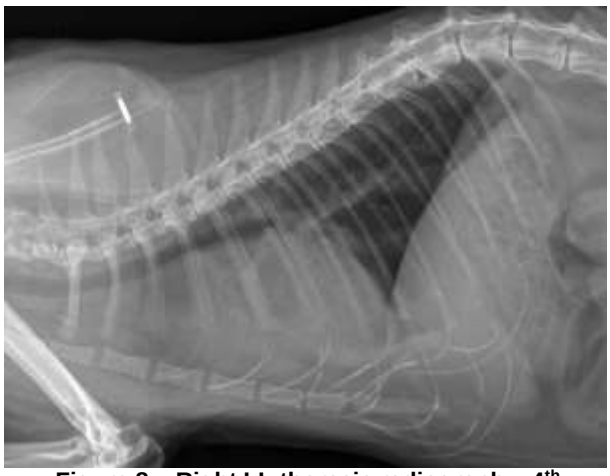


Figure 8 – Right LL thoracic radiography, 4th evaluation

One month later, during the fifth evaluation no abnormalities have been reported by the owners or noticed during physical examination. Chest X-rays have been performed using DR MAXIVET 400HF machine and the measurements have been obtained using the VXvue System. The maximal dimensions of the cyst were 41/10.2/18.2 mm, with an area of 2.09 cm^2 on LL view. The triangular soft tissue opacity overlapped the apex of the heart, with no abnormalities detected in the cranial half of the thoracic cavity (*figure 9*). LUS

detected no fluid interposed between the heart and thoracic wall.

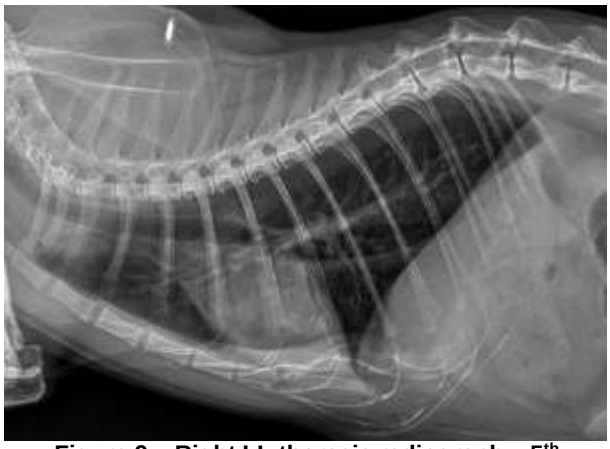


Figure 9 – Right LL thoracic radiography, 5th evaluation

The last recheck has been carried out two years after the initial diagnosis. The owners reported a less active lifestyle of the animal, without any discomfort observed. The respiratory pattern was normal, with a FR of 30 rpm and an SpO_2 of 99%. On the lateral chest X-ray no sign of enlargement of the cyst was observed. The maximal dimensions of the cyst were 21/9.5/13.7 mm, with an area of 1.29 cm^2 (*figure 10*). Age-related changes were observed such as increased sternal contact of the heart silhouette and ventral displacement of the tracheal bifurcation. On LUS no pathological changes were remarked in the cranial mediastinum. It is important to mention that the body weight remained constant throughout the five months, no lymph node enlargement has been detected during physical examination of imaging procedures and no pleural free fluid has been detected during LUS.

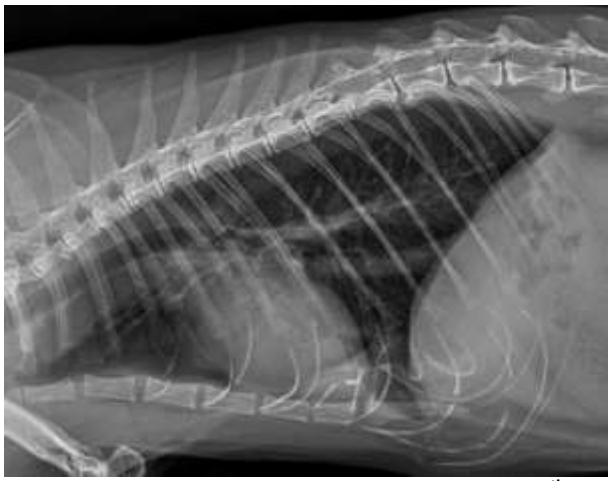


Figure 10 – Right LL thoracic radiography, 6th evaluation

DISCUSSIONS

The physical examination of cats can be challenging due to the fact that on one hand they have the tendency of hiding signs of acute and chronic pain and on the other hand the physiological signs of acute stress cand result in abnormal examination findings in otherwise healthy cats (Quimby J. M., 2011). Such signs include tachycardia, tachypnea, hypertermia and pupillary dilation. Cats may hyperventilate, breathing both rapidly and deeply, or pant from either stress or disease, creating difficulty differentiating cardiac or respiratory compromise from stress (Horwitz D. F., 2018). This cat expressed tachypnea and asynchronous breathing pattern, the latter being a symptom that is significantly associated with pleural space disease as single localization in both dogs and cats. In cats, this breathing pattern has also been associated with chest wall localization. This breathing pattern consists of inward movement of the abdominal wall during inspiration. Moreover, the association with decreased lung auscultation sounds is highly sensitive (99%) but not very specific (45%) for this localization (Sigrist N. E., 2011). A study focusing on nine cats diagnosed with cranial mediastinal cysts reports that eight of them had no signs of respiratory distress, whereas one expressed respiratory distress possibly correlated to associated pathologies (Zekas L. J., 2002). Another study focused on describing six cats with mediastinal cysts states that a cat with an associate diagnosis of feline infectious peritonitis experienced dyspnoea (Camero C. M., 2019). However, the RR and breathing pattern have not been mentioned for any of the patients. Two studies describe four cats with idiopathic mediastinal cyst, none of whom presented dyspnoea (Zekas L. J., 2002; Reichle J. K., 2000). Therefore, we conclude that due to the fact that respiratory signs are mild, they are easily misinterpreted as stress and might be ignored by owners or unexperienced staff. In our case, the low SpO_2 prior to aspiration correlated to the respiratory pattern were the best indicators of dyspnoea.

The age of the cats diagnosed with mediastinal cyst ranged between 9-17 years. For three of the cats diagnosed with idiopathic mediastinal cyst the signalments were not given (Zekas L. J., 2002), still one case report describes this pathology in a thirteen years old Domestic Shorthair cat (Ellison G. W., 1994).

The radiological examination of the thorax is insufficient in carrying a definitive diagnostic. Tissue and liquid opacities have a similar appearance, therefore the differential diagnostic for such findings localized within the cranial mediastinum should include neoplastic disease, such as lymphoma, thymoma, ectopic thyroid

tissue, heart-based tumors or metastatic neoplasia, or non-neoplastic disease, like abcess, granuloma, benign thymic hyperplasia, haemorrhage or cyst (Camero C. M., 2019).

Thoracic ultrasonography is performed in order to differentiate the abovementioned pathologies. It is a fast and stress-free diagnostic procedure that has been thorough described during the last years (Lin C. H., 2020). Uniformly hypoechoic masses have been associated with lymphosarcoma, neuroendocrine tumors, and pulmonary lymphomatoid granulomatosis, in contrast to complexe or heterogenous masses that are associated to mast cell tumor, lymphosarcoma, thymoma, thyroid carcinoma, melanoma and hemorrhage (Reichle J. K., 2000).

Depending on the general status of the animal, fine needle aspiration followed by cytological analysis is the standard procedure that needs to be performed in order to obtain a definitive diagnosis of idiopathic mediastinal cyst and to stabilize the patient. If the cyst has a tendency of recurrence, surgical excision must be considered followed by histological analysis (Rogers K., 1997). However, none of the cases reported in the literature underwent surgical excision. Necropsy findings have been reported in one study, where histological analysis noted that the cyst lining was non-ciliated and resembled mesothelium (Ellison G. W., 1994).

The prognosis for idiopathic mediastinal cyst is excellent (Camero C. M., 2019). However, we recommend serial evaluations, due to the fact that discrete clinical signs might mask the recurrence of the disease. In our case, significant changes were observed four moths after the initial assessment, with a peak value for the area of the cyst measured 150 days after the first drainage that required a second centesis. Over the next nineteen months the imagistic evaluation of the cyst marked no changes.

CONCLUSIONS

It is important to consider idiopathic mediastinum cyst in the differential diagnosis for cranial mediastinum pathologies. This pathology is underdiagnosed in veterinary medicine. Considering the fact that in all the reported cases, including this paper, conservatory procedures were sufficient for long-term management of the patients, the authors suggest thorough investigations when assessing mediastinal changes.

ACKNOWLEGMENTS

This research received no external funding.
The authors report there are no competing interests to declare.

REFERENCES

Camero C. M., Neumann Z. L., Garret L. D. (2019). *Long-term survival in six cats with mediastinal cysts.* Journal of Feline Medicine and Surgery, 998-1002.

Ellison G. W., Garner M. M., Ackerman N. (1994). *Idiopathic mediastinal cyst in a cat.* Journal of Veterinary Radiology & Ultrasound, 347-349.

Horwitz D. F., Rodan R. I. (2018). *Behavioral awareness in the feline consultation: Understanding physical and emotional health.* Journal of Feline Medicine and Surgery, 423-436.

Reichle J. K., Wisne E. R. (2000). *Non-cardiac thoracic ultrasound in 75 feline and canine patients.* Journal of Veterinary Radiology & Ultrasound, 154-162.

Zekas L. J., Aadam W. M. (2002). *Cranial mediastinal cysts in nine cats.* Journal of Veterinary Radiology & Ultrasound, 413-418.

Lin C. H., Lo P. Y., Lam M. C., Wu H. D. (2020). *Usefulness of Chest Ultrasonography in Predicting Diagnosis in Non-emergency Small Animal Patients With Lung Parenchymal and Pleural Disease.* Frontiers in Veterinary Science.

Sigrist N. E., Adamik K. N., Doherr M. G., Spreng D. E. (2011). *Evaluation of respiratory parameters at presentation as clinical indicators of the respiratory localization in dogs and cats with respiratory distress.* Journal of Veterinary Emergency and Critical Care, 13-23.

Ohad D., Rishniw M., Ljungvall I., Porciello F., Haggstrom J. (2013). *Sleeping and resting respiratory rates in dogs with subclinical heart disease.* Journal of American Veterinary Medical Association, 839-843.

**Quimby J. M., Smith M., Lunn K. F. (2011*). Evaluation of the effects of hospital visit stress on physiologic parameters in the cat.* Journal of Feline Medicine and Surgery, 733-737.

Rogers K., Walker M. (1997). *Disorders of the mediastinum.* Compendium on Continuing Education for the Practising Veterinarian, 69-83.