PhD THESIS

Diagnosis and management of dental disease in pet rabbits

(SUMMARY OF THE PhD THESIS)

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INTRODUCTION

The domestic rabbit (*Oryctolagus cuniculus*) is originally from the Iberian peninsula, but roughly 2000 years ago, the Romans spread them to other parts of the world, and in the 12th century, the Normans brought them to England and Ireland. The Romans raised the rabbits in „gardens with walls", which were called „leporaria". Between the 5th and 6th centuries AD, French monks began the process of domesticating rabbits, this process had been completed around the 17th century. Through domestication rabbits became a source of food, pets and even characters in Victorian-era books such as „Alice in Wonderland", „Peter rabbit" and „Brer rabbit" (BATCHELOR, 1999; SANDFORD, 1996).

During the Second World War, due to the acute shortage of food, the breeding of rabbits for meat consuption gained momentum (SANDFORD, 1996), an aspect that is maintained even today. Out of the more than 70 recognized rabbit breeds, most are kept as pets, but recently they are also used as animal models for research purposes.

Wild rabbits are very selective regarding the elected type of food, preferring the consumption of soft, energy-rich grasses in order to reduce the time allocated to feeding and to be as little exposed to predators as possible (BAKER et al., 2005). Pet rabbits are relatively easy to raise, if the microclimate conditions and optimal nutrients are ensured. They adapt well to captivity and are not inhibited by the presence of humans. Pet rabbits prefer to consume energy-rich food in the form of pellets, in favor of rough grasses that would ensure proper dental use (PREBBLE JENNIFER and MEREDITH ANNA, 2014).

As a consequence of this fact, pet rabbits are prone to develop systemic pathologies such as dental disease, gastrointestinal stasis or even obesity, that can subsequently cause pododermatitis and behavioral problems. If rabbits have the choice between pellets and extruded food, they often choose the extruded one as it takes longer to chew. This means that a proper diet must take into consideration both their caloric needs and the time they spend chewing (MARTIN LOUISE et al., 2021).

To ensure good health and well-being, rabbits should undergo at least an annual general physical examination completed by a thorough examination of the oral cavity and teeth (CROSSLEY and AIKEN, 2004; CAPELLO, 2006). Among the diseases of the pet rabbits, dental disease is one of the most common and requires constant veterinary care (HARCOURT-BROWN FRANCES, 2007). Oral examination of most pets, but also of new companion mammals is done with some difficulty, especially in rabbits and rodents, due to the particular morphology of their oral cavity and the way their teeth grow (CAPELLO, 2016). Establishing a positive diagnosis regarding the state of health or illness of an

animal or a group of animals requires a correct, complete and objective examination. In order to achieve this the clinician must know and respect the mandatory stages of the examination: the rational use of preparatory measures for carrying out the clinical examination in optimal conditions; establishing the work methodology which means creating a plan that is clear, precise and adapted to each species.

The aim of this research was to update the specialized literature regarding morphology, raising and accurate care of pet rabbits, but also to identify the most relevant methods for diagnosing dental disease in this species and to establish an interventional protocol with clear steps to follow and personalised for each patient. At the same time, we aimed to customize the methods of approach and restrain in relation to the size of the rabbits, to establish, based on the morphometry of the skull and the facial index, certain values to be considered as benchmarks to identify the predisposition of an individual to certain dental pathologies and to bring back into discussion the importance of the bacteriological examination and the antibiotic sensitivity testing in the therapy of dental disorders.

For this aim, the following general and specific objectives were established:

General objectives are the following:

1. establishing the inclusion and the exclusion criteria of the biological material that will be included in this study;
2. organization of the research design and formation of experimental groups;
3. adapting the stages of preventive care, diagnosis and treatment from small companion animals and transferring them to pet rabbits;
4. stablishing a well-defined and reliable work protocol regarding the examination of leporids in general and pet rabbits in particular;
5. performing imaging examinations in the diagnosis of dental disease and acknowledge their limits according to the encountered dental pathology;
6. evaluating the predisposition concerning he developement of certain dental pathologies in pet rabbits;
7. exploring and using new surgical methods in the treatment of dental disease in pet rabbits;
8. emphasizing the value and importance of microbiological examination and antibiotic sensitivty testing in periodontal infections in rabbits.

Specific objectives:

1. establishment of clinical and radiological diagnosis in dental pathology in pet rabbits;

2. carrying out craniometric measurements in the Flemish giant breed and in dwarf pet rabbits regarding the predisposition of a patient to develop certain dental pathologies;
3. performing a microbiological study on dental pathology in pet rabbits
4. description of the stages in the surgical interventions in rabbits diagnosed with primary or secondary dental disease
5. establishing work methods in the management of dental disease in pet rabbits, as well as prevention and therapy with medication of dental diseases.

STRUCTURE OF THE DOCTORAL THESIS

The thesis entitled „Diagnosis and management of dental disease in pet rabbits" consist in 131 pages written according to the current editing norms at academic and national level established by the doctoral school of veterinary medicine. The doctoral thesis is structured in two parts consisting of 10 chapters and it contains 62 figures, 7 tables and 114 bibliographic references.

The first part of the doctoral thesis entitled "Current state of knowledge" is structured in 3 chapters and comprises 21 pages. This part synthesizes information regarding the taxonomy of leporids, anatomical and physiological aspects of the oral cavity, as well as information on the structure of the dentition in rabbits and the peculiarities of elodont, hypsodont, lophodont and aradicular teeth **(Chapter 1)**. It also includes the presentation of the most frequent dental pathologies encountered in leporids, represented by conditions of the incisors, premolars, molars and odontogenic abscesss formation. Among the diseases associated with the oral cavity the most common were congenital, viral, bacterial diseases, wet dermatitis, electirc burns, diseases of the tongue, of the gums, of the head and salivary glands and neoplastic disease **(Chapter 2)**. The last chapter of this part **(Chapter 3)** is devoted to the description of the surgical and drug treatmeant applied in the dental pathology of rabbits, with strict references to general and loco-regional anesthesia, to infraorbital and mental nerve block, to crown resection and tooth extraction.

The second part of the doctoral thesis is allocated to original research. This is structured in 7 chapters and comprises 87 pages. Each chapter is dedicated to a research activity, established before the actual start of the experiments. The studies are preceded by the working hypothesis, the purpose and objectives of the research. Before starting the research a bioethical file was prepared regarding compliance with the rules for the protection and welfare of animals used for scientific purposes, according to the European Directive of 22.09.2010/63/EU and the national law 43/2014. The approval

of the bioethics committee was obtained by decision no. 204 of 12.03.2020. The non-biological materials used were classified into 4 categories: instruments for clinical examination, for surgical interventions, for imaging examination and for microbiological examination. The methods applied were the following: clinical examination of the patient, examination of the oral cavity and dentition, imaging represented by radiological examination, computed tomography, stomatoscopy, crown resections, dental extractions, marsupialization and microbiological examination completed by carrying out the antibiogram.

RESULTS OF THE ORIGINAL RESEARCH

Chapter 4 of the thesis is assigned to **Study I**, entitled: *"Clinical and radiological diagnosis in dental disease in pet rabbits"* and aimed to identify and apply the most adequate methods of approach, restraint, handle and the most relevant diagnostic methods in dental disease in pet rabbits. A total of 29 male and female rabbits of different breeds and ranging in age from 1 year to 10 years, with and average of 7 years old, were included in this study. The rabbits were examined in the NAC clinic within FMV-USAMV Cluj-Napoca. In 19 cases, after the general clinical examination, serial dental radiographs were performed for a detailed view of the dention and in 10 cases head CT was performed for a more thurough examination of the skull. From the total of 29 rabbits included in the study, 1 rabbit (3.44%) was diagnosed clinically healthy, and 28 rabbits (96.56%) were diagnosed with various dental conditions. 7 rabbits were diagnosed with diseases of the incisors (24.13%), 17 with disorders of the premolars (58.62%) and 4 with problems at the level of the molars (13.79%). The highest percentage of the diagnosed dental conditions were represented by diseases of the premolars at 58.62%. The classification of cases had as its main criterion the tooth from which the process of dental disease was initiated. Each case presented a diverse dental pathology, affecting one or more teeth that showed both primary and secondary conditions. In addition to the presented dental conditions, a number of 15 rabbits were also diagnosed with abscesses located in the mandible and maxilla (51.72%). Most of these abscesses developed secondary to periodontal infections, and one patient was also diagnosed with a retrobulbar abscess of odontogenic origin.

Chapter 5 includes **Study II** of the thesis: *"Craniometric measurements of the Flemish giant rabbit and the dwarf pet rabbit skull (Oryctolagus cuniculus domesticus)".* Motivated by the increase in the number of pet rabbits and the wide variety of dental disorders, the aim of this study was to identify, based on the morphometry of the skull, using the 3D reconstruction of the CT images, certain morphometric indices of whose

values are to be considered benchmarks. Then, these standard values should be compared with the values of cranial indices from both clinically healthy rabbits and from those diagnosed with dental disease in order to be able to predict their predisposition to develop certain dental disorders and malocclusion. The biological material used in this research was represented by 4 Flemish giant pet rabbits, clinically healthy, aged between 1-1.5 years, with a body weight of 3.8-4 kg and 4 dwarf pet rabbits (Oryctolagus cuniculus domesticus) diagnosed with severe dental disease, aged 5-13 years and weighing 1.5-2 kg. The study was performed *in vivo*, and no specimens were sacrificed after the experiment was completed. Each rabbit included in the study underwent a general clinical examination followed by a thorough examination of the oral cavity and a head CT. After the CT examination, the obtained images were evaluated using digital image analysis software. The analysis and interpretation of the images demonstrated that there is no statistically relevant difference in the indexes measured at the level of the head between females and males, nor between clinically healthy giant German rabbits and dwarf rabbits diagnosed with dental disease. In contrast, the female rabbits from the group diagnosed with dental disease a significant difference was observed compared to the male rabbits from the same group regarding the average of the 19 distances measured at the level of the skull in the coronal, sagittal and axial planes and in the axial plane at the level of the mandible, females having higher values than males (p<0.05). The mean values of the measurements made in both the healthy rabbits and in the rabbits diagnosed with dental pathology, show clear differences in all the measured lines, except for the values measured at the level of the upper and lower occlusal surfaces. The calculated indexes showed no differences between the two categories of rabbits, except for the facial index (p<0.05). The facial index is the main criterion in differentiating the rabbit breeds, most of the dwarf pet rabbits belonging to the brachycephalic breeds. The mean values of the indices calculated in the healthy rabbits of the study revealed clear differences compared to the rabbits with dental pathology.

Chapter 6 contains **Study III:** "*Microbiology of dental disease in pet rabbits".* In this study the bacterial flora involved in periodontal infections in pet rabbits was evaluated and the importance of applying preventive methods in dental pathology was highlighted in order to bring back into discussion the value of the antibiotic sensitivity testing in the treatment of dental infections, considering the low tolerance to certain antibiotic therapy of the rabbits. The study included a total of 16 microbiological samples collected from 16 pet dwarf rabbits diagnosed with periodontal infections and odontogenic abscesses. The biological samples were collected intraoperatively, after which they were sent to the laboratory for bacteriological, bacterioscopic examination and antibiotic sensitivity testing. The bacteriological examination was performed from

the capsule of the dental abscess, respectively from the affected tooth, and the antibiogram was performed according to the classic requirements for identifying bacterial strains. Out of the 16 samples sent to the laboratory, 4 samples were negative and the following bacterial strains were identified from the positive samples: 18.75% *Staphylococcus spp.*, 18.75% *Streptoccus spp.*, 12.5% *Pseudomonas aeruginosa*, 12, 5% *Klebsiella spp.*, and 6.25% *Proteus spp,*. To identify *Klebsiella spp.* and *Proteus spp.*, the special medium TSI (Triple Sugar Iron) was used, to identify the strains of *Proteus spp.*, *Pseudomonas spp.*, *Staphylococcus spp.*, the UTI medium (Chromogenic UTI) was used, and for the bacterial strain *Streptococcus ssp.*, Columbia agar medium with the addition of 5% sheep blood was used. In most of the cases in this study, the dental infections were determined by a single bacterial strain, except for 3 cases in which multiple bacterial strains were the etiological agents. Among the antibiotics used in the antibiogram, the most effective antibiotic was Amikacin, which did not show any resistant colonies, followed by Trimethoprim/sulfamethoxazole (TMPS) and Ciprofloxacin. Amphotericin B and Clindamycin were the least effective, as no germs were sensitive to their action. The interpretation of the antibiogram was done according to EUCAST requirements. Based on the results obtained in this study, after carrying out the antibiotic sensitivity testing, antibiotic therapy was initiated in all patients with dental disease. Antibiotic treatment was applied according to the degree of sensitivity of the bacterial strains, so the treatment of choice was Amikacin at a dose of 10 mg/kg administered IV and SC every 24 hours, for a minimum of 10 days, followed by Trimethoprim/sulfamethazole at a dose of 30 mg/kg PO every 12 hours for 10-14 days and Ciprofloxacin at a dose of 15 mg/kg PO every 12 hours for 10-14 days. Amphotericin B and Clindamycin were not used in therapy because no bacterial strain was sensitive to their action. In addition to these antibiotics, Doxycycline was also used in a dose of 4 mg/kg PO and SC every 24 hours for 14 days; Penicillin G in a dose of 40,000 U/kg IM every 24 hours for 7-10 days; Cefalexin at a dose of 15 mg/kg SC every 12 hours for 7-10 days; Enrofloxacin at a dose of 5 mg/kg PO and SC every 12 hours for 10 days and Marbofloxacin at a dose of 2 mg/kg PO, SC every 24 hours for 7-10 days. These antibiotics were administered only to patients in whom bacterial strains sensitive to theim were identified.

Chapter 7 is devoted to **Study IV: "***Surgical treatment of dental abscesses and certain secondary diseases associated with dental disease in pet rabbits***".** A generous part has been dedicated to the description of surgical interventions and the process of marsupialisation in case of odontogenic abscesses. In the management of retrobulbar abscesses, the enucleation procedures of the eyeball were described, and in patients with serious dental conditions, the techniques of rhinotomy or even rhinostomy were presented. Patients diagnosed with dacryocystitis associated with dental disease were treated with a nasolacrimal duct catheterization procedure.

Chapter 8 includes Study V: "*Management, hygiene and diet plan and drug therapy in dental diseases in pet rabbits*". The management of dental disease in leporids requires an extremely rigorous organization of activities that includes medical planning, raising and basic caretaking, because the development of periodontal and dental pathology is multifactorial and includes genetic elements, breed predispositions, trauma, faulty treatments, fractures in the head or tumor formations of the head. Diet plays an extremely important role in ensuring the health of pet rabbits, but especially in ensuring the health of the oral cavity, teeth and gastrointestinal tract, due to the fact that they need a permanent source of plant fibres. In situations where rabbits have been diagnosed with anorexia, assisted feeding is of real use to supplement the nutrients required for a balanced ration. Patients with periodontal infections must also benefit from antibiotic treatment, but this will only be done after a microbiological examination and after antibiotic sensitivity testing. In the case of dental abscesses, the antibiotic therapy will be supplemented with a local therapy for a better management of healing in this type of pathology. Last but not least, we recall the importance of pain management and the application of systemic therapies in the event of secondary problems arising caused by periodontal disease.

In **Chapter 9** the conclusions and recommendations driven from the results of the present research are presented and **Chapter 10** is dedicated to the originality and innovative contributions of the thesis.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

In **Chapter 9** of the thesis, the general conclusions and recommendations are presented.

After the quantitative evaluation and statistical interpretation of the dental pathology in pet rabbits, the results obtained by us confirm the data from the specialized literature according to which the most affected teeth in this species are the premolars.

The radiological examination can be considered a reference examination in identifying the primary etiology of dental conditions in pet rabbits and in monitoring the healing process.

The interpretation of radiographic images, based on craniometric measurements, provides an increased degree of certainty in establishing the diagnosis of dental disease in pet rabbits.

The use of CT in the examination of the skull and teeth in pet rabbits has two great advantages: first, it provides the possibility of 3D reconstruction of images on the basis of which cranial indices can be measured to establish the degree of malocclusion and

predisposition to certain dental disease, and secondly, it provides conclusive data on tooth structure, adjacent bony structures and soft tissue.

In this research, it was demonstrated that there are no statistically significant differences in the values of the cranial indexes calculated in females compared to those calculated in males, except for the facial index.

The research carried out reconfirms the fact that, for the identification of etiological germs and in the antibiotic-based treatment of periodontal and endodontic dental diseases, hereditary or acquired in pet rabbits, the bacteriological examination and antibiogram should be part of the obligatory interventions.

In the oral cavity of pet rabbits there is a rich bacterial flora, and some of these bacteria have zoonotic potential which, under certain conditions, can cause infections with a high risk for the owner.

Considering the growing number of pet rabbits and their predisposition to certain dental pathology and the formation of odontogenic abscesses, the development of a precise therapeutic plan based on medical evidence, is the only way to ensure their health and well-being.

The vast majority of pet rabbit owners have little knowledge about their care, breeding and pathology, therefore it is recommended that practicing veterinarians, in addition to specialist interventions, also provide scientific information about the particularities of the species.

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