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Article

Microbiology of Dental Disease in Pet Rabbits

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Abstract:. Maintaining the health and hygiene of the oral cavity is an essential condition to prevent dental diseases, both in humans and animals.This study aimed to present the importance of prevention techniques and treatment options for dental disease in pet rabbits. The research was focused on 16 rabbits that had been diagnosed with dental disease based on clinical and paraclinical examination, we obtained samples from the dental injury site using a sterile cotton swab and followed up with bacteriological examination and antibiotic sensitivity testing for identifying the bacteria and the resistances. Out of 16 samples sent to the laboratory for testing, 4 were negative (25%), showing no bacterial growth, from the rest of the samples the following bacterial strains were identified: 18,75% *Staphylococcus spp.*, 18,75% *Streptococcus spp.*, 6,25% *Streptococcus β hemolytic*, 6,25% *Pseudomonas aeruginosa*, 6,25% *Klebsiella spp*. 3 cases presented with multiple-strain infection as follows: 6,25% *Streptobacillus spp.* and *Klebsiella spp.*; 6,25% *Proteus spp.* and *Streptococcus spp.*; 6,25% *Pseudomonas spp.* and *Streptococcus spp*. After obtaining the antibiotic sensitivity test results, we found that the most efficient drug was amikacin, no bacteria presented resistance to this medicine, and it was followed by trimethoprim/sulfa (TMPS) and ciprofloxacin. All the identified bacterial strains presented resistance to amphotericin and clindamycin. Antimicrobial resistance and the limited availability of veterinary-use-approved drugs constitute strong arguments that sustain the importance of this study in the management of dental disease in pet rabbits.

Keywords: rabbits, dental disease, bacteriology, antimicrobial resistance, treatment

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1. Introduction

Periodontal and endodontic disease in pet rabbits manifests in the form of periapical infections which often can lead to osteomyelitis and the appearance of odontogenic abscesses. In case of malocclusion, the pressure exerted on the occlusal surface of the cheek teeth raises the susceptibility to the occurrence of acquired dental disease. Crown elongation creates more interdental space and weakens the alveolar ligaments which can be invaded by pathogenic microflora. Jaw abscesses present a major health issue, they are considered inflammatory reactions caused by pyogenic strains of bacteria that are immune to phagocytosis due to the polysaccharides residing in the capsule of the abscesses [1, 2]. Odontogenic abscesses are frequently localized in the submandibular or maxillofacial region if the oral mucosa presents lesions caused by dental spikes or the periapical infection cannot be contained by the self-defense mechanisms of the host [3]. Mandibular abscesses usually appear due to intra-alveolar infections originating at the site of incisors or cheek teeth. The exposed apex of the tooth is the most affected zone and they often are presented with retrograde displacement. These types of infections are rarely detected in time because the rabbits do not manifest clinical symptoms at this stage. The subtle intraosseous changes can be detected only by radiological examination or by the use of computed tomography. Progressive bone resorption caused by the pyogenic bacteria will lead to the formation of typical mandibular abscesses around the severely damaged and infected incisor or molar with noticeable growth issues. The whole mandible can be compromised and destroyed in extreme cases [4]. A frequently met complication in these cases is the hematogenous or

lymphatic transmission of the infection that can lead to the apparition of secondary abscesses in the thoracic or abdominal cavities, in extreme cases the infection can also reach the cranial cavity [2]. Rabbits affected by intrathoracic suppurative processes will manifest dyspnea, general weakness, and apathy. Superficial abscesses can also appear and they create large cavities under the skin after they reach maximal size the skin will tear and a fetid, purulent secretion will drain to the exterior of the body. In an advanced stage of malocclusion, the superior molars and the last superior premolar due to the continuous growth of the rabbit teeth will become longer and will start presenting curvature towards the cheeks causing the apex to also be displaced and reach the retro-orbital zone. The apexes that reach the immediate proximity of the eyeball can cause local irritation, pain, and general discomfort during mastication. If these irritating factors persist and are associated with an apical infection, they can lead to the formation of retrobulbar abscesses. The infection around the orbital region can extend from the apex of the teeth to the soft tissue surrounding the eye and also can include the lacrimal gland. Inflammation of the peri and retro-orbital space presses the eyeball out of the socket causing the third eyelid to prolapse. Incomplete closing of the eyelids due to the eye protrusion can further lead to keratitis, and after a few days if left untreated, depending on the grade of the protrusion, uveal tract infection can appear. All these factors tailored together can cause panophthalmia or ptosis of the eye [4]. Treating abscesses in rabbits can be a challenge for practitioner veterinarians due to the encapsulated nature and the poor level of penetration of antibiotic drugs into the abscess cavity. Management of these abscesses usually consists of surgical ablation followed by antibiotic therapy both locally and systemic [5]. Excessive use of antibiotics concerns both human doctors and veterinarians because of the rising levels of resistant bacteria which became a global problem for all the species [5].

All the aforementioned elements highlight the importance of the microbiological examination as a step that cannot be skipped in establishing an etiologic diagnosis and an efficient treatment plan. Even if these infections appear secondarily, they need to be treated accordingly since many bacterial strains identified in dental disease also carry zoonotic potential and that is notable in the global context of antibiotic resistance.

2. Materials and Methods

In this study, 16 dwarf breed pet rabbits were included , males and females with ages between 2 and 7 years with an average of 5 years, the age of the animals was determined by declaring it by the owners, which were diagnosed with acquired dental disease. The applied methods consisted of clinical and paraclinical examination. Initially, the rabbits underwent a general clinical examination followed by a rigorous examination of the oral cavity and the teeth. The samples were collected from all 16 pet rabbits presented with dental disease and then sent to a private laboratory for testing The paraclinical examination included the bacteriologic and bacterioscopic examination of the collected samples, small portion of the excised abscess capsule and the whole extracted tooth, using the following instruments: sterile cotton swab, heparinized vacutainer tubes, Columbia agar with 5% sheep blood, MacConkey agar medium, Mueller-Hinton agar dish, antibiotic disc reagent, insemination loop, Bunsen gas burner, incubator, dyes for Gram staining, microscope and slides. Columbia Agar with 5% Sheep Blood is a highly nutritious universal medium for the isolation and cultivation of fastidious and non-fastidious microorganisms from clinical samples. McConkey for the identification of the bacterial strain Escherichia Coli, knowing that the rabbit is a cecotroph. Dental infections in leporidae caused by anaerobic germs are limited, and most of the drugs used have digestive side effects and cause post-therapy dysbiosis.

The collection of the biological samples was done during surgery with the help of a sterile cotton swab from the abscess cavity and soft tissue, bone, tooth, and capsule fragments were also extracted. The samples were collected in the sterile swab container and heparinized vacutainers and kept refrigerated at 2-4°C for 24 hours. The microbiological examination took place in the laminar air flow chamber to provide aseptic conditions, the samples were inseminated on the blood and MacConkey agar mediums (Figure 1). The petri dishes were incubated at 37°C temperature for 24 hours. Slides were prepared from bacterial colonies grown in the culture medium, and Gram staining was performed to enable bacterioscopic examination than bacterial colonies were then isolated for antibiotic sensitivity testing using the Kirby-Bauer disc-diffusion technique. Each isolated strain was suspended in nutrient broth up to 0.5° optical density using the McFarland scale. The dishes containing Mueller-Hinton agar then were flooded with the prepared broth. The excess amount of nutrient broth was eliminated and the dish was left to dry. This was followed by placing the antibiotic disc

reagent on the agar. The antibiotic drugs used in the sensitivity testing are highly relevant in small mammals' clinical activity. The prepared Mueller-Hinton agar dishes were then kept again for 24 hours at 37°C temperature. The reading of the antibiogram (Figure 2) consists of measuring the diameter in millimeters of the total inhibition zone. The results classify in sensitive, resistant, partial inhibition, and partial inhibition with resistant colonies. Antibiotics used for testing (microtablets and antibiotic strength) was: amoxicillin 30 mcg (30 μg) AML, TMPS – 25 mcg (23.75 mcg/1.25 mcg) – Co-trimoxazole (Suslfa/trimethoprim) COT, gentamicin 10 mcg GEN, cephalexin 30 mcg CL, marbofloxacin 5 mcg MAR, enrofloxacin – 10 mcg ENR , penicillin G - 10 mcg P, cefotaxime - 30 mcg CTX, ciprofloxacin – 5 mcg CIP, amikacin – 30 mcg AK, clindamycin – 2 mcg CD, amphotericin B – 20 mcg AMB, doxycyclin – 30 mcg DXT, polymixin B – 50U PB, erythromycin – 10 mcg E.. The owners of the rabbits signed their agreement to this study.

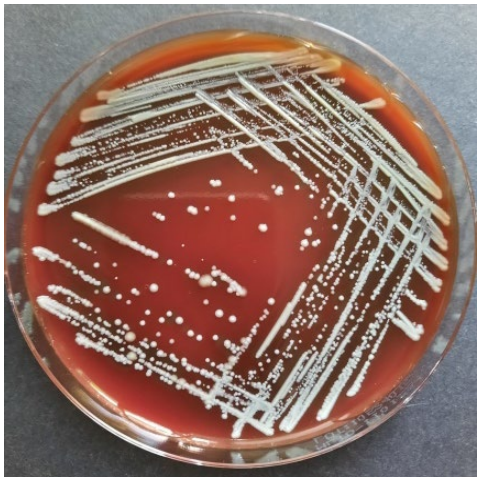

Figure 1. Columbia agar with 5% sheep blood at 24 hours after insemination

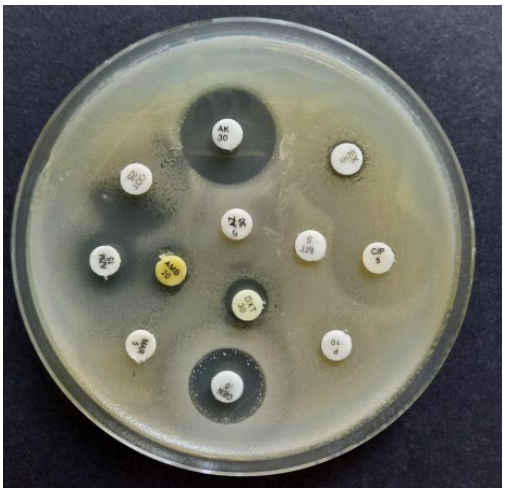

Figure 2. Antibiogram on Mueller-Hinton agar

3. Results

Out of 16 samples sent to the laboratory for testing, 4 were negative (25%), showing no bacterial growth, from the rest of the samples the following bacterial strains were identified: 18,75% *Staphylococcus spp*, 18,75% *Streptococcus spp*, 6,25% *Streptococcus β hemolytic*, 6,25% *Pseudomonas aeruginosa*, 6,25% *Klebsiella spp*. In the aforementioned 8 cases, only one bacterial strain was identified but 3 cases were presented with multiple-strain infection as follows: 6,25% *Streptobacillus spp.* and *Klebsiella spp.;* 6,25% *Proteus spp.* and *Streptococcus spp.*; 6,25% *Pseudomonas spp.* and *Streptococcus spp*. (Figure 3). To identify the bacterial strains *Klebsiella spp*. and *Proteus spp.*, the special medium TSI (Triple Sugar Iron) was used, for the species *Proteus spp., Pseudomonas spp., Staphylococcus spp.* the UTI medium (Chromogenic UTI medium) was used, and for bacterial strain *Streptococcus ssp.,* Columbia agar medium with the addition of 5% ram blood was used. Out of all the antibiotics used in the sensitivity testing, amikacina was the most efficient, no strain was resistant to this drug. The second most efficient drug was trimethoprim/sulfamethoxazole (TMPS) followed by ciprofloxacin. The identified bacterial strains presented no sensitivity at all to amphotericin and clyndamycin (Figure 4). The interpretation of the antibiogram was done according to EUCAST requirements. The results of the microbiological and sensitivity testing including the grade of the resistance can be found in Table 1.

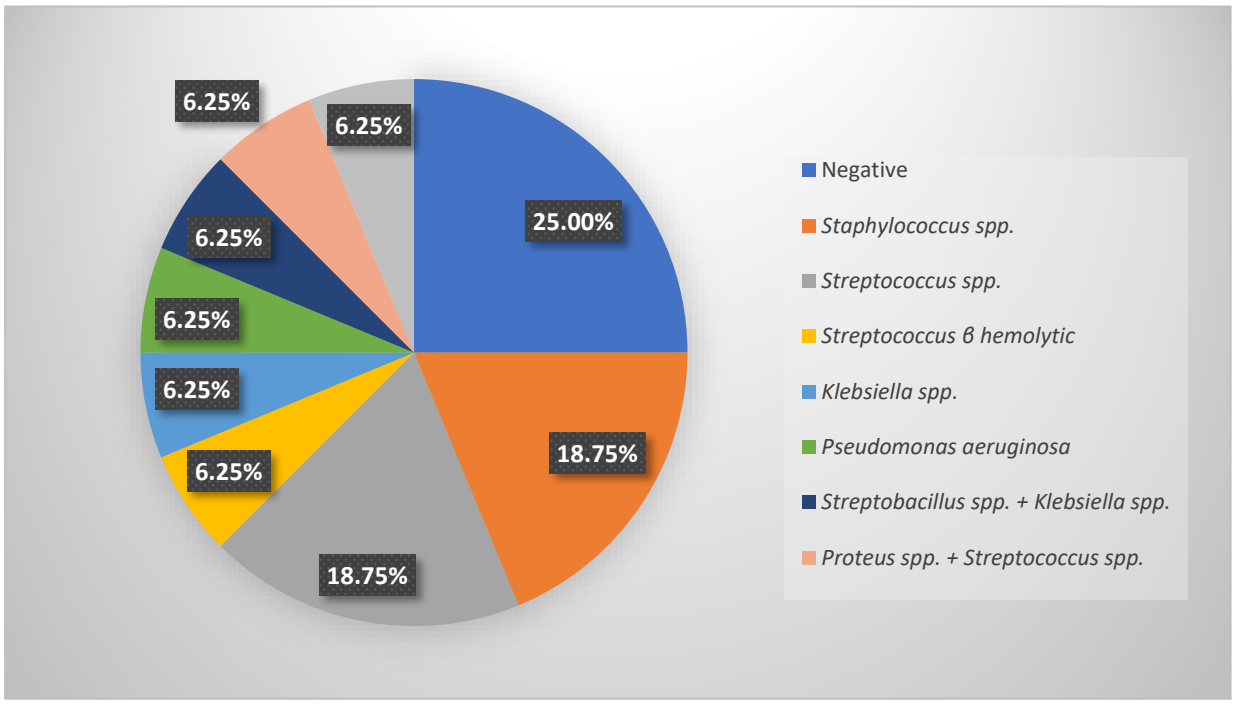

Figure 3. Results of the microbiological examination

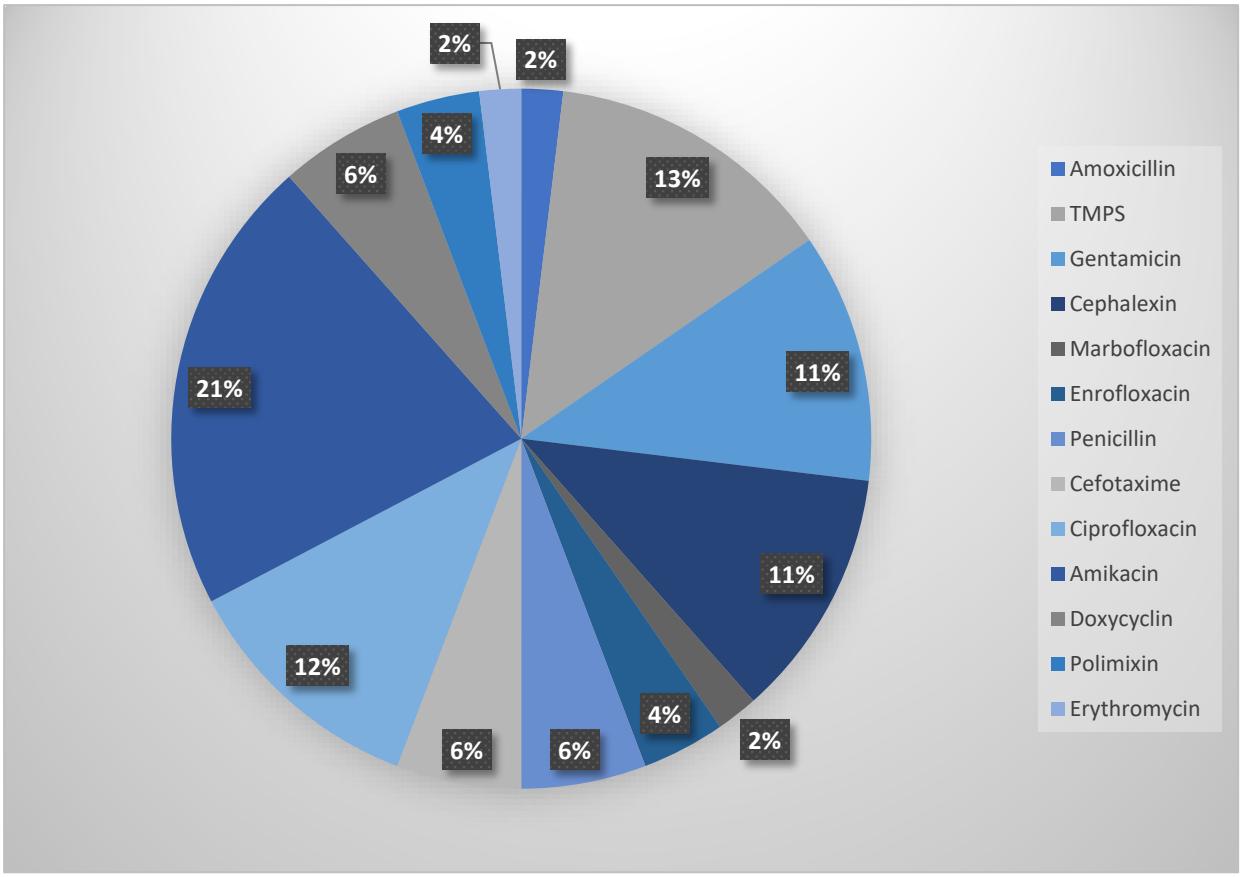

Figure 4. Results of the antibiotic sensitivity testing and drug efficiency

Table 1. Antibiotic sensitivity test results

Nr	Antibiotic / Bacterial strain	Amoxicillin	TMPS	Gentamicin	Cephalexin	Marbofloxacin	Enrofloxacin	Penicillin	Cefotaxime	Ciprofloxacin	Amikacin	Clindamycin	Amphotericin	Doxycyclin	Polymixin	Erythromycin
1	*Staphylococcus spp*	34	22	21	40	21	23	38	24	-	-	-	-	-	-	-
2	*Staphylococcus spp*	-	-	27	21	-	20	R	21	15	26	-	-	-	-	-
3	*Streptococcus spp*	-	18	22	-	-	-	-	-	R	20	R	R	17	-	-
4	*Staphylococcus spp*	-	31	26	-	-	-	-	R	21	27	-	-	22	-	-
5	*Pseudomonas aeruginosa*	-	11	17	R	-	-	-	R	R	21	-	R	-	R	-
6	*Streptococcus spp*	-	16	-	R	-	-	R	-	12	21	-	R	-	18	-
7	*NEGATIVE*															
8	*NEGATIVE*															
9	*NEGATIVE*															
10	*Pseudomonas aeruginosa, Streptococcus spp*	R	15	-	14	-	-	R	R	R	20	R	-	R	-	25
11	*Streptobacillus Klebsiella spp*	-	20	-	21	-	-	21	27	29	21	R	R	21	-	-
12	*Klebsiella spp*	R	R	-	R	R	R	-	R	27	19	-	-	R	-	-
13	*Proteus spp Streptococcus spp*	R	R	R	R	R	R	R	R	20	10	R	R	R	R	R
14	*Streptococcus β hemolytic*	R	-	-	21	-	-	R	-	18	19	-	R	-	17	-
15	*Streptococcus spp*	R	R	27	35	-	-	26	R	-	22	-	-	-	-	-
16	*NEGATIVE*															

4. Discussion

Data from the literature regarding periodontal infections in rabbits show that the very first etiologic agent involved was the strain *Actinomycetes* from the order *Antinomycetales*. This is a Gram-negative prokaryotic organism identified by Frostowicz and Frelik in the Chadronian (Eocene) lagomorph, *Megalus* from Pipestone Springs, Montana, United States [6].

In our study, we did not identify any of these microorganisms. Tyrell and colleagues identified[7] in his study on 12 rabbits a wide range of bacterial strains involved in dental pathology causing mandibular and maxillofacial abscesses in rabbits. These were the following: *Fusobacterium nucleatum, Prevotella heparinolytica,*

Prevotella spp., Peptostreptococcus micros, Actinomyces israelii and *Arcanobacterium haemolyticum* [7]. Another study conducted by Gardhouse and team [8] on a significant number of 48 rabbits shows identified aerobic bacteria: *Pseudomonas aeruginosa, Streptococcus spp., Staphylococcus spp.,* as well as anaerobic bacteria like *Fusobacterium spp., Peptostreptococcus spp., Bacteroides spp.,* involved in odontogenic abscess formation. Mixed infections containing anaerobic and aerobic bacteria in 73% percent of the cases also contained 3 or more bacterial strains [8]. The results obtained by us in this study did not confirm the data from the specialized literature regarding the pathogenic bacterial species that caused the dental infections described in the two studies, with the exception of Streptoccus spp. and Pseudomonas spp. strains.

Treatment protocols are limited in dental disease in rabbits complicated by anaerobic bacteria due to the restrained compatibility of drugs available to rabbits and they can cause secondary digestive side effects and dysbiosis following treatment so the patients need to be monitored carefully. Studies confirm that aminopenicillins, clindamycin, and erythromycin administered orally can cause severe enterotoxemia and dismicrobism [8].

Metronidazole, chloramphenicol, and penicillin G can be used in parenteral administration as a systemic treatment for anaerobic bacterial infections. Azithromycin also proved efficient in these types of infections according to literature [15]. Metronidazole is a great option for treating dental infections in rabbits due to its potential to easily penetrate tissues, including bone tissue, and has a satisfactory absorption rate when administered orally. The recommended dosage is 20 mg/kg per dose once a day at least for 6 weeks after orofacial surgery, based on the antibiotic sensitivity test results [9].

Ward's study[10] describes using polymer gel based on doxycycline (Doxirobe™) as being an ideal alternative to fill the cavities or fistulas created by abscesses. The physical properties of the gel allow us to apply the gel in liquid form and then while it solidifies it cuts off the communication between the organism and the exterior environment while distributing the antibiotic drug in the affected zone. It is easily removed during the control of the patient or replaced if needed [10].

Craniometric measurements can also play an important role in identifying rabbits predisposed to develop dental disease either acquired or hereditary and also can be a factor in selecting individuals for breeding dwarf pet rabbits [11].

In topical treatment of extensive bone defects in patients presenting dental disease who underwent multiple teeth extractions, measuring the skull alongside radiography and computed tomography examinations can help put in place a proper treatment protocol [11]. In these cases, there are multiple available solutions like calcium hydroxide or antibiotic-impregnated polymethylmethacrylate (AIPMMA). The antibiotic-impregnated beads in many cases succeed in stabilizing the bone structure and controlling the infection, but in extensive bone defects, with larger cavities, these beads have a lower efficiency rate [10].

AIPMMA beads are a recommended therapy for creating antibiosis in extensive bone damage due to multiple teeth extraction and abscess extirpation. The polymethylmethacrylate will be covered in connective tissue in a short amount of time and the antibiotic will only penetrate a 3 mm distance from the bead, so placing this inside the capsule of an abscess is not efficient [12]. The impregnated antibiotic is chosen based on the antibiotic sensitivity test results.

Based on our results from this study, all our patients presenting with dental disease received medication. The antibiotic administration protocol was based on the study by Fisher and Jenifer Graham, (2018) and the study by Taylor et al., (2010) applied to rabbits with dental abscesses [13, 14]. The therapy protocol was dependent on the grade of the sensitivity shown in the bacterial strains with the selected antibiotic drug in the following dosage: amikacin 10 mg/kg IV or SC administration once a day for a minimum of 10 days,

trimethoprim/sulfamethoxazole 30 mg/kg PO twice a day for 10 to 14 days and ciprofloxacin 15 mg/kg twice a day for 10-14 days. Patients that presented different sensitivity test results were treated with doxycycline 4mg/kg PO twice a day for two weeks, penicillin G at 40000 U/kg IM once a day for 10 days, enrofloxacin 5 mg/kg PO or SC twice a day for 10 days and marbofloxacin at 2 mg/kg SC once a day for 7-10 days. We did not use amphotericin nor clindamycin at all in our study due to bacterial resistance.

5. Conclusions

Our study reconfirms the major importance of bacteriological examination and antibiotic sensitivity testing in identifying the etiological agents and efficiently treating dental disease in pet rabbits.

Our results shine a light on the significance of the sensitivity of bacterial strains that cause apical infections in pet rabbits to correctly manage the treatment containing antibiotics, an aspect that today is not routinely performed in all veterinary medical offices. In the oral cavity of rabbits, a wide range of bacteria already exists and because they may carry zoonotic potential, they present a high risk for the owners as well. Species from the genus *Streptococcus spp.* and *Staphylococcus spp*., are commensal and opportunistic species that can cause respiratory tract infections in people with a weakened immune system.

Considering the growing number of pet rabbits and their predisposition to dental disease and odontogenic abscess formation, an accurate treatment plan based on medical evidence is the most important in ensuring good health and wellbeing in the rabbit patient.

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to preexisting conditions in the dogs, which included recommended euthanasia based on previously obtained consent from the owners.

Data Availability Statement: For further information, please contact the corresponding author via email.

Conflicts of Interest: The authors declare no conflict of interest.

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