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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.

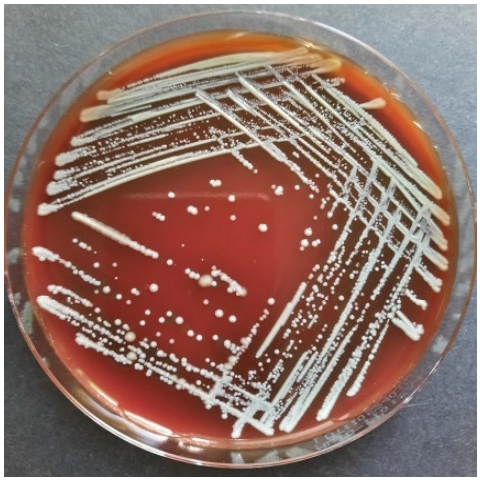

Figure 1. Columbia agar with 5% sheep blood at 24 hours after insemination

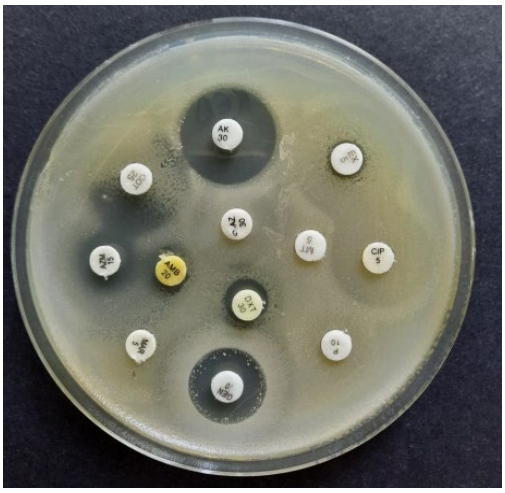

Figure 2. Antibiogram on Mueller-Hinton agar

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.

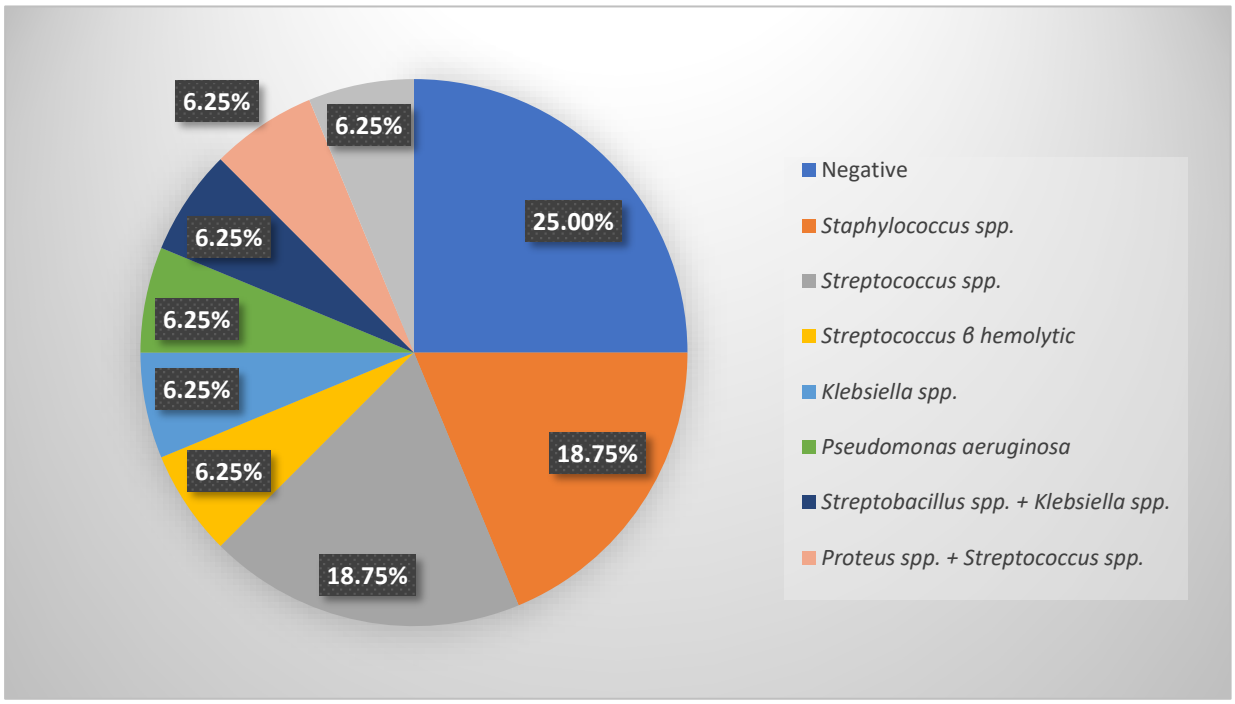

Figure 3. Results of the microbiological examination

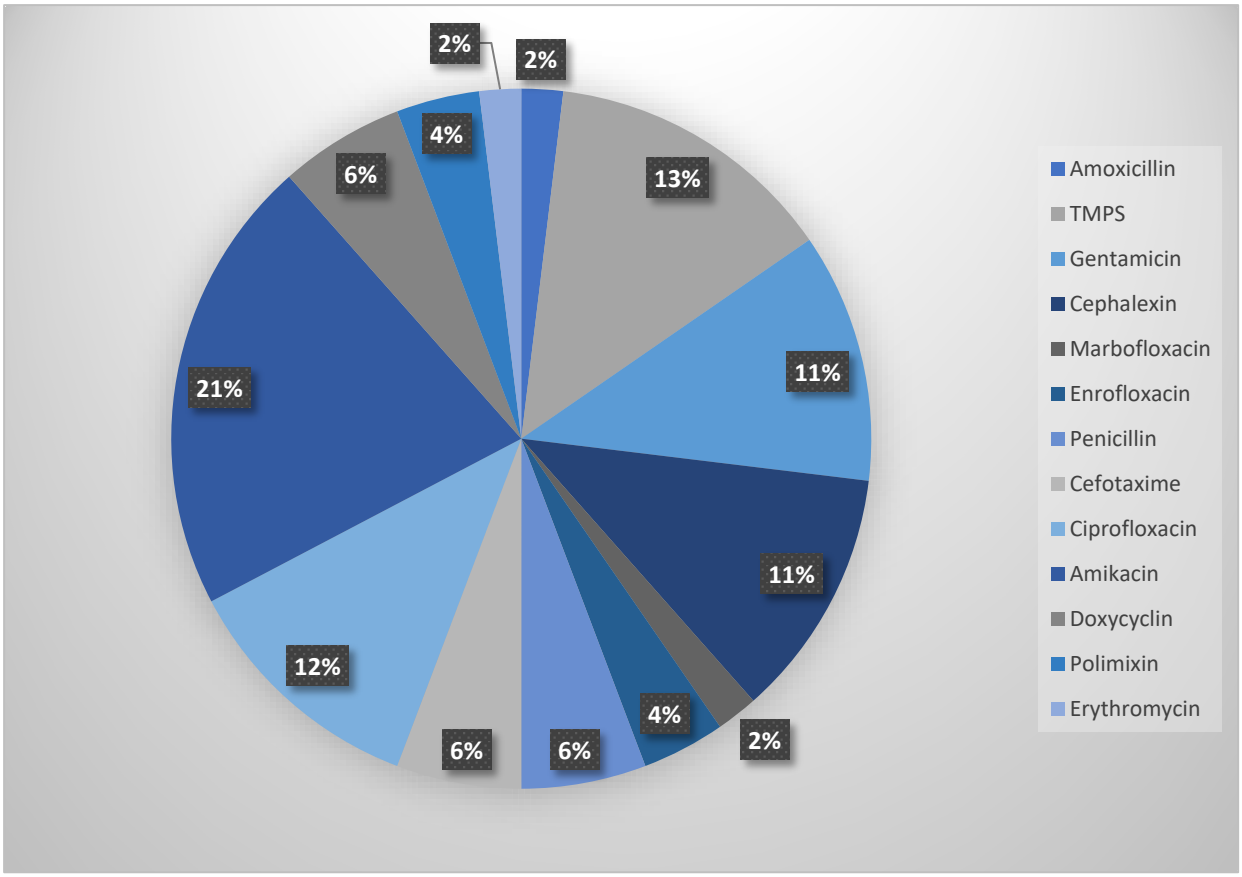

Figure 4. Results of the antibiotic sensitivity testing and drug efficiency

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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Article

The Impact of Parity on Dairy Cows Colostrum Quality

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Abstract: One of the main indicators of colostrum quality is represented by its immunoglobulin content, protein molecules responsible for the calf's passive immunity. The objective of this study was to investigate the quality of colostrum according to the concentration of total proteins and γ-globulins, as well as the influence that the number of parturitions has on its quality. Twenty colostrum samples, collected from primiparous (n =10) and multiparous cows (n = 10), from two different dairy cow breeds, were analyzed by the ultraviolet spectrophotometric method. The results of the study showed an increase in colostrum quality depending on the number of parturitions only in cows of the Romanian Black-Spotted breed, , γ-globulins concentration increasing from an average of 63,72 g/l in the case of primiparous cows to 116,32 g/l in multiparous cows. In the case of Holstein cows, colostrum quality was not influenced significant by parity. This study underlines the need to expand research on the influence of individual factors on colostrum quality.

Keywords: colostrum; immunoglobulins; parity.

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1. Introduction

The colostrum is the secretion of the cow mammary gland during the first days following calving. [1]. It is composed of a range of compounds that are very important to the health and productivity life on calves, including nutritional elements, antimicrobial and growth factors, cytokines, and immunoglobulins [2]. Colostrum composition and quality exhibit significant variability due to multiple factors, including parity, individuality, the duration of the dry period in cows, heat stress [3], gestational nutrition during pregnancy, and the subsequent metabolic and hormonal profiles [4-5], as well as the overall puerperium and its potential complications [6]. The quality of colostrum is characterized by adequate concentration of immunoglobulins, which is essential for the newborn and his immunity, as ruminants are agammaglobulinemic at birth due to the synepitheliochorial placenta [6]. The latter prevents the passage of immunoglobulins, making them vulnerable to infectious disease [7]. Muller and Ellinger [8] concluded that parity determined a significant difference in colostrum IgG content between heifers' offspring and cows'progenies in their third or later parities, emphasizing the importance

of parity in this regard. To estimate the quality of a colostrum, its IgG content is assessed, researchers suggest that it is qualitative when the content of these immunoglobulins is at least 50g/l [9]. Immunoglobulin values below 50g/l denote an inadequate colostrum in terms of quality and predispose the calf to failure of passive transfer (FPT) [10]. Acquiring passive immunity, the calf depends, in addition to ingesting colostrum in sufficient quantity and quality, on the absorption of immunoglobulins along the intestinal wall before stopping intestinal transport which can vary between 24 to 36 hours after birth [11]. FPT is defined by a serum IgG concentration of <10 mg/ml in neonatal calves aged 24 to 48 h [12-13]. However, thresholds, including the aforementioned value, are highly reliant on the selected technique, with several indirect or direct methods being available for FPT assessment [14-15]. Due to its association with significant economic losses [13], alternative treatments for FPT have been proposed, such as plasma transfusions [16]. However, this approach may present challenges, particularly in finding suitable donors, as intensive testing for highly prevalent pathogens like bovine herpesvirus becomes compulsory [17].

2. Materials and Methods

2.1. Experimental group

A total of 20 dairy cows were involved in this study, sourced from farms in Ilfov County, situated in the southeast region of Romania. To ensure the validity of the findings, the cows were divided into experimental groups. These groups consisted of 10 Holstein breed cows and 10 Romanian Black-Spotted cows, with an equal distribution of each breed, primiparous (n=10) and multiparous (n=10) cows. All cows were scheduled to calve in February 2023. The number of lactations in multiparous females varied between 2 and 4 lactations, and the average milk production for the last lactation was 9867 l for the Holstein cows from the farm 1 and 4337 l for the Romanian Black-Spotted breed, housed at the farm 2. A week before calving, the cows are moved to the maternity area where they are kept until the time of complete recovery. After calving, the calves are separated from the cows immediately after consuming the colostrum.

2.2. Clinical examination

The cows included in the study underwent a brief clinical assessment, during which vital signs were measured, the condition of the mucous membranes was evaluated, and the mammary gland was examined. Thermometric readings revealed body temperatures within physiological ranges, with slightly elevated heart and respiratory rates, which is typical for the final stages of gestation. The mucous membranes exhibited a species-specific color indicative of good health. Upon examination of the mammary gland, no pathological alterations were observed, and there was no tenderness upon palpation, nor were there any temperature irregularities.

2.3. Colostrum sample collection

For the present descriptive study, colostrum samples of 20 cows from two different cattle breeds were collected in sterile vials by farmers in two farms in Ilfov, Romania. Approximately 50 ml of colostrum samples were collected from the first milking after parturition (3,9±2,7h after parturition; range from 0 to 10 h) and immediately frozen at -20°C until analysis.

2.4. Laboratory Determination of Colostrum Quality

The amount of total proteins, especially immunoglobulins in colostrum is relevant for its quality. Thus, their quantification offers us the possibility of qualitative evaluation of the milk secretion that plays a role in the transmission of passive immunity [18]. The colostrum quality measurement technique used in this study was ultraviolet spectroscopy, a rapid and accurate laboratory-based method [19].

The initial stage in processing the collected samples involved a gradual thawing process, allowing them to thaw slowly at room temperature to prevent protein denaturation. From each thawed sample, a quantity of 10 ml was transferred into Falcon tubes, after which the samples were centrifuged at 3500x g for 5 min to remove the fat layer and isolate whey from the casein pellet. After centrifugation, 0,5 ml of the whey portion was aliquoted from the lower third of the tube and placed in Eppendorf tubes. Subsequently, whey samples were diluted 1:10 with 0,9 % NaCl solution and incubated at 47°C for 10 minutes.

For quantitative determination of Total Protein (TP), 20 µl of each sample was mixed with a Cu 2+ ions-reagent in order to form a chelate with peptide bonds of protein. Subsequently, the sample was incubated for 20 min at 37°C. The quantitative assessment was acquired through analyzing color intensity, a process conducted using a spectrophotometer (Spectrophotometer UV/Vis DLAB SP-UV1000) at a wavelength of 540 nm. To measure the immunoglobulin content of colostrum, a quantity of 20 µl of the sample was mixed with 18,5% Na_2SO_4 solution reagent. The sample was incubated for 15 minutes at a temperature of 37°C. The intensity of the turbidity formed was measured at a wavelength of 450 nm.

3. Results and discussion

3.1. Quality of colostrum samples

The colostral concentration of immunoglobulin was adequate (≥50 g/l) in 90% of samples from primiparous cows. Only one cattle had colostrum of suboptimal quality (< 50 g/l), with a concentration of 48,90 g/l immunoglobulin. In the case of cows with multiple parities, the colostrum quality was of high quality, with γ-globulin concentration varying between 95,90-129,80 g/l, for 90% of calves. Both Holstein and Romanian Black-Spotted, had immunoglobulin concentration above 100g/l, indicating superior colostrum quality.

3.2. γ-globulin concentration variability in relation to parity

In this study, the Holstein breed did not show an increase in colostrum TP or immunoglobulins with the increase in the number of parturitions. Moreover, testing of colostrum samples from multiparous cows indicated lower immunoglobulin concentrations than in first-calving cows, but the number of total proteins was higher compared to primiparous cows (Table 1). Parity, however, seems to cause an increase in the amount of immunoglobulins within the Romanian Black-Spotted. When determining the concentration of colostrum γ-globulins and TP in these subjects, the values of samples from primiparous cows indicated a lower quality colostrum compared to that collected from multiparous cows. Both the number of immunoglobulins and that of total protein increased with the number of parturitions (Table 2).

Table 1. Values of total protein and γ-globulin (g/l) in colostrum samples collected from primiparous and multiparous cows of the Holstein breed.

	PRIMIPAROUS COWS		**MULTIPAROUS COWS**	
	Total protein	γ-globulin	Total protein	γ-globulin
1	602.6	89.10	420	95.9
2	659	97.10	848	100.2
3	502	101.4	747	104.1

4	1040	139.9	1576	104.6
5	937	152.5	1040	107.1
AVR	748.12	116	926.2	102.38
SEM	205.21	25.28	382	3.92

AVR=Average

SEM=Standard Error of the Mean

Table 2. Values on total protein and γ-globulin (g/l) in colostrum samples collected from primiparous and multiparous cows of the Romanian Black-Spotted breed.

	PRIMIPAROUS COWS		**MULTIPAROUS COWS**	
	Total protein	γ-globulin	Total protein	γ-globulin
1	310	48.9	1224	109.9
2	350	60.2	1080	108.9
3	366	67.9	1234	112.9
4	552	63.7	1370	120.1
5	724	77.9	1445	129.8
AVR	460.4	63.72	1270.6	116.32
SEM	155.94	9.49	126.59	7.97

The average concentration of immunoglobulins in colostrum from primiparous cows of the Holstein breed was 116±25,28 g/l, higher than in the case of multiparous cows of this breed, where the immunoglobulin average was 102,38±3,92 g/l (Figure 1). On the other hand, in cows of the Romanian Black-Spotted, the quality of colostrum increased as they had more calvings. The primiparous recorded an average of colostrum immunoglobulins of 62,32±9,28 g/l, an amount that was almost double in the milk samples from multiparous, the average concentration in these females being 116,32±7,97 g/l (Figure 2).

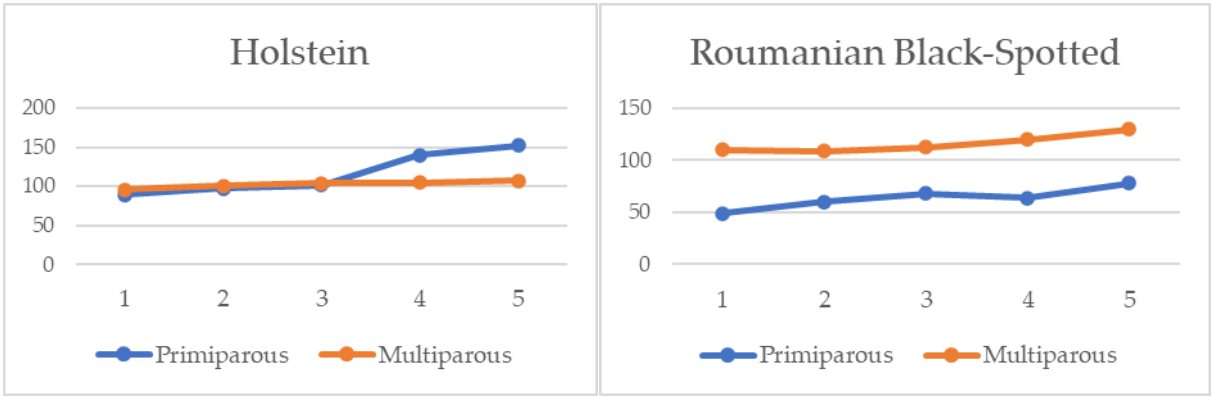

Figure 1 and 2. Graphical representation of the Holstein (1) and Romanian Black-Spotted breed (2) concentration of immunoglobulins (g/l).

Under the influences of breed, differences were observed in terms of colostrum quality. Primiparous Holstein cows exhibited an average concentration of γ-globulins in the investigated samples of 116±25.28 g/l, significantly higher than that of primiparous cows from the Romanian Black-Spotted who had an average IgG concentration of 62.32±9.28 g/l at first parturition.

In the case of cows from the Romanian Black-Spotted breed, the quality of colostrum from multiparous cows has improved, both in relation to primiparous cows from this breed, and compared to cows in their second or third parturition from the Holstein breed, recording average values of 116.32±7.97 g/l immunoglobulins (Figure 3).

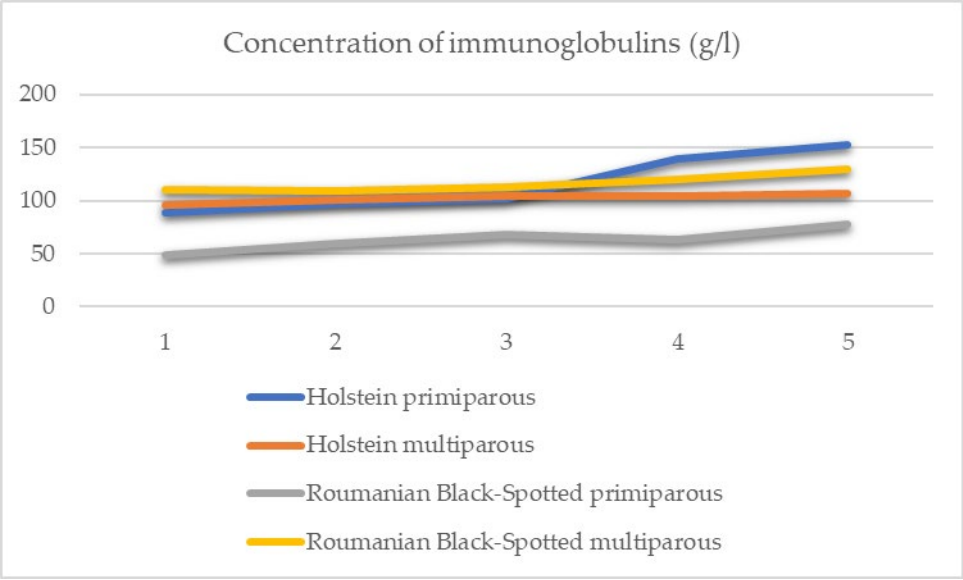

Figure 3. Graphical representation on immunoglobulins values depending on breed and parity

Most studies show that parity has a major impact on the percentage of immunoglobulins in colostrum, research by Muller and Ellinger supports the fact that multiparous cows produce colostrum with a higher number of immunoglobulins than first-calving cows [8] Gulliksen et. al. also confirm that there is a significant difference in colostrum quality between primiparous and multiparous cows [3]. In this study, parity had an influence on colostrum quality only in the farm with cows of the Romanian Black-Spotted breed, the value of γ-

globulins doubling in multiparous cows. Regarding the Holstein cows studied, the number of parturitions did not affect the improvement of colostrum, the average concentration of immunoglobulins being lower than in the case of primiparous cows of this breed.

Previous studies support the fact that with the increase in the number of parturitions, the quality of the colostrum is also improved. This aspect may suggest the possibility that this increase in the number of immunoglobulins in ruminant colostrum is due to the repeated exposure of these animals to various pathogens, ultimately leading to the formation of antibodies that are transmitted to the mammary gland and from there to the milk secretion [20]. The lower amount of γ-globulins in the colostrum of primiparous cows may be due to the insufficient development of the mammary gland. Older research concludes that the reduced development of the mammary gland hinders the ability of antibodies to pass through, so their milk secretion is poorer in immunoglobulins [21].

4. Conclusions

The main objective of this study was to investigate the influence of parity on colostrum quality. In order to achieve this objective, 20 colostrum samples were taken immediately after calving, from two breeds of dairy cows, both primiparous and multiparous. The quality of colostrum in relation to parity improved significantly in cows from the Romanian Black-Spotted breed, with the amount of γ-globulin quantified from samples from multiparous cows reaching double values compared to females at their first parturition. As for the Holstein breed, the increase in the number of parturitions had no effect on the number of γ-globulins. However, the colostrum quality was adequate, in a concentration above 50g/l in both breeds of dairy cows, regardless of the number of parturitions. Only one female showed concentration of immunoglobulins below the allowed limits.

In conclusion, this study demonstrates the role of parity in improving colostrum quality in cows from the Romanian Black-Spotted breed. Thus, our study underlines the need to expand research on the influence of individual factors on colostrum quality.

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CVJ CLUJ VETERINARY JOURNAL

Article

Histological evaluation of the common carotid arteries in the goat (*Capra Hircus*)

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Abstract: Fragments of the common carotid arteries were collected from 8 goats that had succumbed to accidents. These samples underwent histological processing, involving fixation and embedding in paraffin. Sections, 5 μ in thickness, were then stained using the Trichrome Goldner method for overall assessment and Verhoeff staining for elastic tissue analysis. Upon using Trichrome Goldner staining, it was observed that goat carotid arteries exhibited a typical appearance of muscular arteries, with the thickest layer being the media, primarily composed of muscular components. Verhoeff staining further revealed a well-represented elastic tissue presence within the adventitia, exhibiting a distinct arrangement. Both staining methods indicated that goat common carotid arteries possess tunics comparable in thickness to muscular arteries, with a predominantly muscular media. However, the adventitia was notably distinguished by a significantly higher concentration of elastic tissue compared to typical muscular arteries. Consequently, with these findings, we propose classifying goat common carotid arteries as musculoelastic transitional arteries.

Keywords: common carotid arteries, muscular component, elastic component

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1. Introduction

Arteries are broadly categorized into two types: elastic and muscular. Large-diameter arteries, such as the aorta and its primary branches, are classified as elastic arteries due to their predominant elastic media composition, despite also containing muscle and connective tissue [1]. In these arteries, the elastic component not only prevails but also exhibits a distinctive arrangement. Specifically, the elastic component of large arteries comprises multiple concentric elastic lamellae, interspersed with muscle and connective tissue. Examples of elastic arteries include the aorta, brachiocephalic trunk, common carotid artery, subclavian artery, and common iliac artery [2].

Arteries play a crucial role in supplying organs with blood and essential nutrients. The initial arteries emerging from the heart invariably experience high pressure. To withstand this stress, they are rich in elastic tissue and have a lower concentration of smooth muscle. Elastin, a key component of the arterial wall, provides elasticity, allowing arteries to expand and contract, thereby adjusting their diameter. The elasticity of the walls of large arteries enables them to maintain a relatively steady pressure gradient, even as the heart functions as a pump. Specifically, arterial wall elasticity facilitates the dampening of pulsations and the equalization of blood flow through passive expansion and elastic recoil. As arteries extend away from the heart, they progressively branch into smaller vessels characterized by a greater proportion of smooth muscle and reduced elastic tissue [3].

All vertebrates and invertebrates possess closed circulatory systems, though the properties of their components vary to accommodate species-specific physiological

pressure ranges. In each instance, the elasticity of vessels arises from the parallel arrangement of elastic and rigid connective tissue elements within their walls.

In ruminants, the common carotid arteries originate from the bicarotid trunk at an acute angle and ascend along the tracheal margins to the cranial end of the cervical region. There, they bifurcate into two branches: the external carotid artery, which supplies structures in the head, and the internal carotid artery, responsible for supplying the brain and its derivatives [4]. In contrast, other species such as equines and carnivores have common carotid arteries that terminate in three branches: the internal carotid, external carotid, and occipital artery [5]. These differences in arterial branching patterns seem to be influenced by various factors, including the animals' dietary habits and locomotion requirements. Animals adopt different postures for feeding based on their locomotion needs and specific feeding habits. Some animals feed with their heads raised to reach elevated branches, while others feed with their heads lowered to access food at ground level. Ruminants present a unique scenario, as they spend several hours daily in rumination, during which they typically hold their heads up [6]. To accommodate the hemodynamic needs associated with locomotion and feeding habits, animals have adapted through specific arrangements of vessels serving various anatomical regions. The goat, for instance, engages in extensive movement while foraging, gathering food with both upward and downward head movements, and dedicating significant time to rumination compared to monogastric animals.

Considering these factors, we deemed it appropriate to conduct morphological investigations on the common carotid arteries supplying the head and neck of goats. Our aim was to identify any adaptive structures that may have developed in response to the unique characteristics of this animal with distinctive feeding habits.

2. Materials and Methods

The biological material used consisted of 8 common breed goats, deceased from various causes. The study was conducted based on the approval of the Bioethics Committee of the USAMV Cluj-Napoca, under decision no. 403 dated 29.09.2023, adhering to the recommendations of the World Organization for Animal Health. An anatomical dissection was performed to gain access to the carotid arteries, after which arterial fragments were collected for histological investigations. The collected pieces were fixed in 10% formalin solution, dehydrated in increasing concentrations of ethyl alcohol (70%, 95%, absolute), clarified with 1-Butanol, and embedded in paraffin. Sections with a thickness of 5 μm were prepared, which were stained using the Trichrome Goldner method for general topographic aspects and Verhoeff staining to highlight elastic structures. The histological preparations were examined under an OlympusBX41 microscope, equipped with a digital camera.

3. Results

Structurally, the common carotid arteries exhibit a prominent media layer, constituting 65-70% of the total thickness of the arterial wall. The intima consists primarily of endothelium and a relatively thin endothelial layer. At the boundary between the intima and media, a thick and undulating internal elastic lamina is observed. The media is composed of smooth muscle cells predominantly arranged in a circular fashion (Figures 1 and 3), interspersed with discontinuous elastic lamellae that are relatively thin and spaced apart. Within the broad interstices between these lamellae lie delicate elastic fibers oriented obliquely and transversely, creating an impression of an elastic network anchoring the components together. The internal elastic lamina is thick and undulating, while the external lamina comprises 2-3 thin elastic lamellae. Overall, the structural characteristics of goat common carotid arteries closely resemble those of typical muscular arteries. However, a notable difference lies in the adventitia, which constitutes approximately 25% of the arterial wall thickness and contains numerous elastic lamellae. These lamellae exhibit a distinctive circular arrangement, reminiscent of the arrangement observed in the middle layers of elastic arteries (Figures 2 and 4).

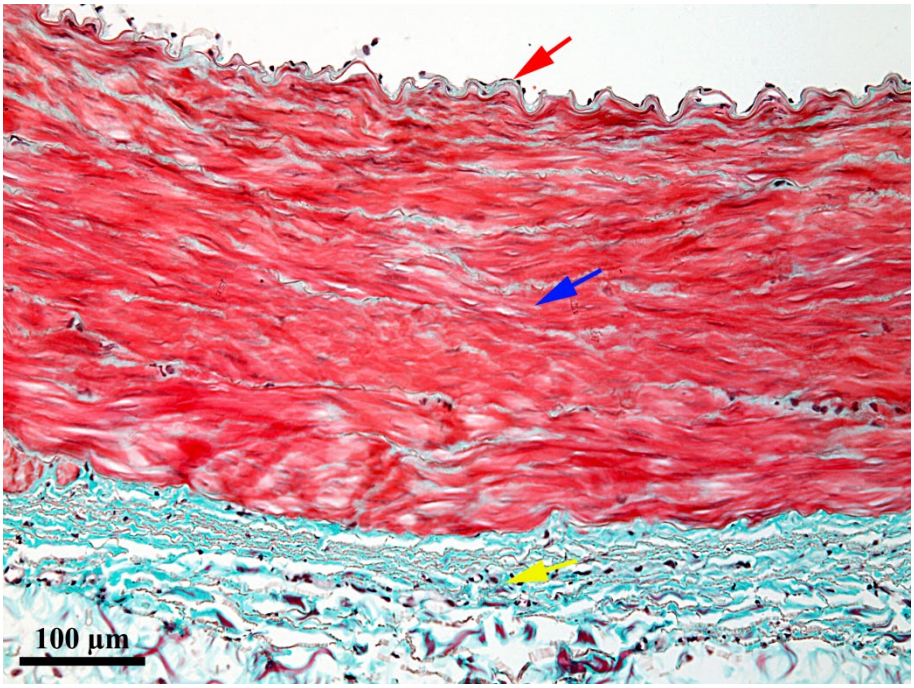

Figure 1. Right Carotid Artery (Goldner's Trichrome staining); red arrow - intima; blue arrow - media; yellow arrow – adventitia.

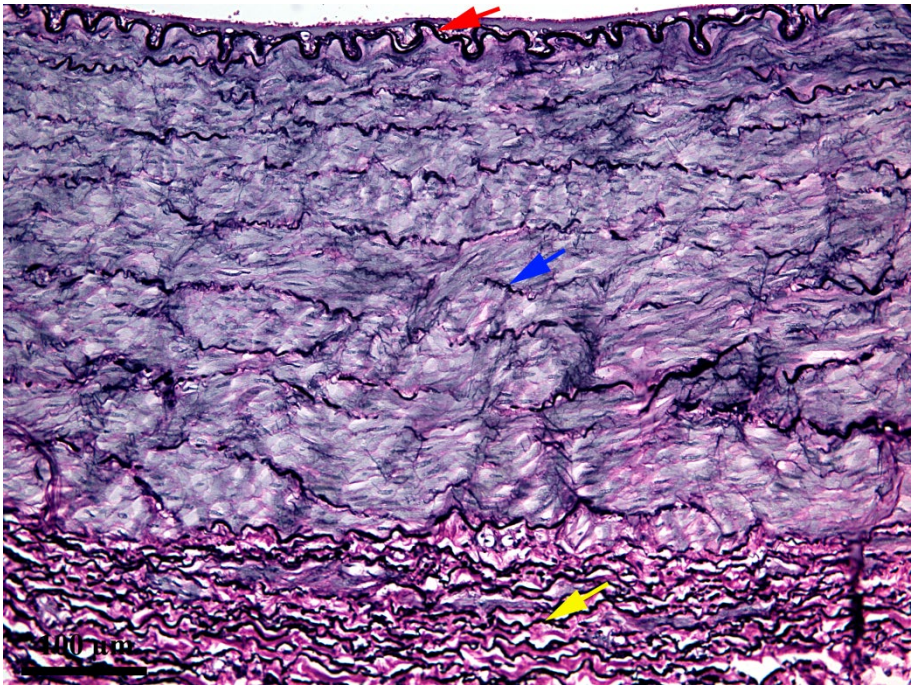

Figure 2. Right carotid (Verhoeff staining); red arrow – internal elastic limiter; blue arrow – elastic fibers on average; yellow arrow - elastic fibers in the adventitia.

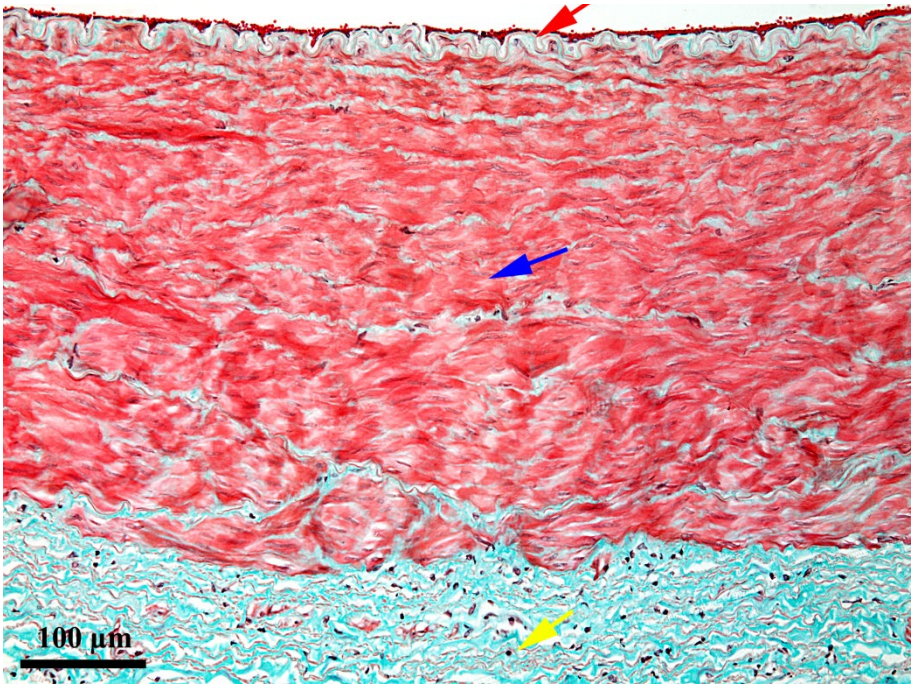

Figure 3. Left carotid (Goldner's Trichrome staining); red arrow – intima; blue arrow - media; yellow arrow – adventitia.

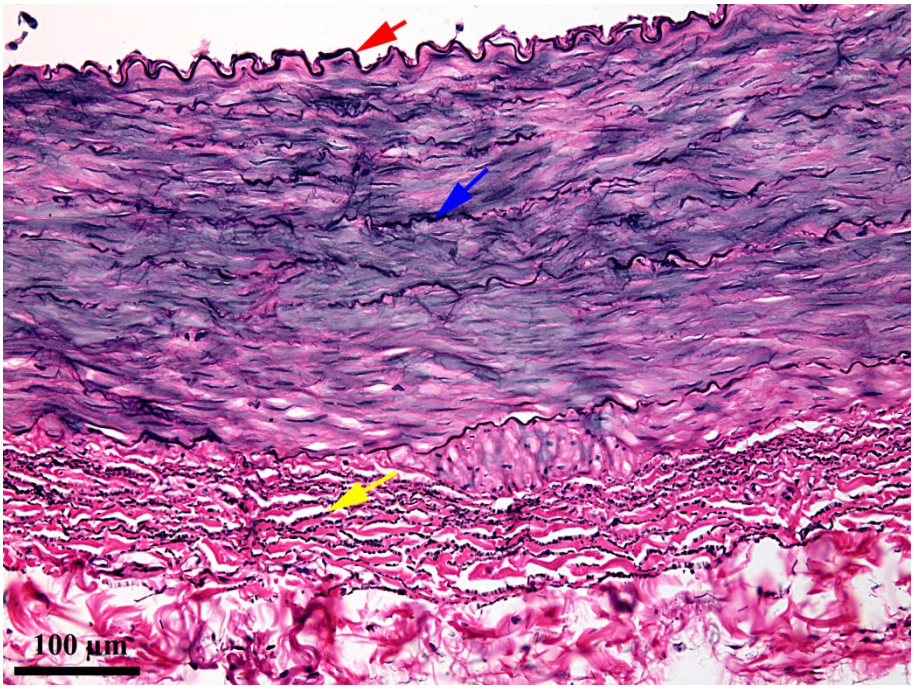

Figure 4. Left carotid (Verhoeff staining); red arrow – internal elastic limiter; blue arrow – elastic fibers on average; yellow arrow - elastic fibers in the adventitia.

4. Discussion

The common carotid arteries, crucial for supplying blood to the head and neck regions, are large elastic arteries [7, 8, 9]. They bifurcate at the carotid sinus into the internal carotid artery, serving the brain, and the external carotid artery, supplying the neck and face [10, 11].

The anatomical positioning of the goat's common carotid arteries is notably unique, situated within a highly mobile region and in close proximity to the external environment. This anatomical context demands continuous blood flow despite external stresses, particularly the significant movements of the head and neck during food collection, whether upward or downward from branches. Adaptations in the structural

composition of goat common carotid arteries have occurred to meet these demands for both quantitative and qualitative blood supply.

To optimize blood flow, the average goat common carotid artery possesses a relatively thick media primarily comprised of smooth muscle cells arranged predominantly in a circular pattern. Unlike typical elastic arteries characterized by a predominantly elastic media, goat common carotid arteries exhibit a media where smooth muscle cells are the predominant feature. While elastic lamellae are present, they are fewer in number, discontinuous, and spaced apart compared to elastic arteries. The media is demarcated from the intima by a thick, undulating elastic lamina, akin to typical muscular arteries, alongside a thin outer elastic lamina.

Overall, the structural characteristics of goat common carotid arteries resemble those of muscular arteries, despite a slightly greater representation of elastic lamellae. However, they differ significantly from elastic arteries in terms of the number and arrangement of elastic lamellae. Based solely on media structure, these arteries could be classified as muscular arteries**.**

A noteworthy observation pertains to the adventitia, which shares a comparable thickness with muscular arteries but differs slightly in structure. The distinct appearance of the adventitia in goat common carotid arteries stems from a substantial presence of elastic tissue, manifested by elastic lamellae arranged approximately concentrically. These lamellae exhibit relative polymorphism in thickness, and their arrangement partially mirrors that of elastic arteries, albeit with greater and uneven spacing between them. This conjunctive-elastic composition of the adventitia in goat common carotid arteries stands apart from the thin, predominantly collagenous structure of elastic arteries and the thicker but structurally distinct adventitia of muscular arteries. The notable disparity lies in the significantly greater abundance of elastic tissue.

In our assessment, this unique adventitial structure in goat common carotid arteries does not seem to arise solely from adaptation to internal stresses imposed by the blood column traversing their lumen. Instead, adaptations primarily occur at the structural level within the intima and media to address internal demands. We believe that the development of a well-defined adventitia with abundant elastic tissue arranged in a specific pattern could stem from adaptation to external stresses, likely due to the anatomical positioning within a highly mobile region.

In response to external stresses, the adventitia of goat common carotid arteries has adapted by significantly increasing the elastic component, thereby primarily providing elasticity to these arteries. Consequently, the adventitia acts as an elastic buffer, ensuring the integrity of the arterial wall despite exposure to external stresses.

Considering both the structure of the media and the adventitia, goat common carotid arteries cannot be classified as typical muscular arteries or typical elastic arteries. Instead, they exhibit characteristics of transition arteries, combining features of both muscle and elastic arteries.

Similar findings, albeit not identical, have been reported in related species such as sheep. Martonos et al. [12] observed that in the average carotid artery of Țurcană breed sheep, the internal elastic lamina is wavy and easily distinguishable compared to the deeper elastic lamellae. Additionally, there is a slightly increased number of elastic lamellae, likely influenced by the gradual change in vessel caliber and anatomical-topographical distance from the heart [13]. Interlamellar bridges, consisting of elastic fibers crossing the interlamellar space and anchoring the elastic lamellae together, have also been identified [12]. These bridges are hypothesized to play a significant role in maintaining interlamellar spaces against the pressure of blood flow in the vascular bed [12], a phenomenon previously observed in human aortic segments [14]. Martonos et al. [12] classify the common carotid artery of sheep as elastic arteries, albeit with certain distinct aspects. However, this classification does not align with our recommendation for goat common carotid arteries, despite the high similarities observed between the two species. Differences in interpretation may account for these discrepancies.

Differences in arterial elasticity are also evident in animals living in unique environmental and feeding conditions. Large whales provide a striking example, with their aortic arch exhibiting exceptional elasticity while the descending aorta displays minimal elasticity [2]. The aortic arch in these animals possesses an extraordinary capacity for expansion, effectively ensuring elastic compliance throughout the arterial tree, whereas the descending aortic segments have thinner walls and are approximately 30 times stiffer than the aortic arch [15]. The remarkable expansion capability of the aortic arch in large whales enhances the aorta's volume capacity and likely aids in maintaining blood flow during diving bradycardia [16, 17].

To estimate elastic, viscous, and inertial moduli, Bia et al. [18] developed various methodologies, through which they discovered that carotid arteries exhibit higher relative levels of collagen and smooth muscle compared to the ascending and descending aorta. Moreover, over time, the relative value of elastin was found to be significantly lower ($P < 0.05$).

5. Conclusions

Considering the thickness of their tunics comparable to muscular arteries, a predominantly muscular media, and a distinctive presence of elastic tissue within the adventitia, we classify goat common carotid arteries as transitional, musculoelastic arteries. We believe that this unique structure reflects adaptation to the hemodynamic demands specific to goats, influenced by their anatomical disposition and the functions they serve.

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Article

Clinical and radiologic diagnosis in dental disease in pet rabbits

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Abstract: Rabbits became more and more popular pets these last few years. For assuring their good health and wellbeing both the owners and the veterinarians are equally responsible. Sudden change in diet or inadequate nutrients, poor husbandry or stressing factors can all cause complex systemic pathologies to appear that often start at the oral cavity and the teeth. The aim of this study was to identify and apply the most accurate methods of approach, restrain, examination and diagnosis in dental disease in pet rabbits. This study included 19 rabbits, one of them clinically healthy and 18 were identified with dental disease. The patients had multiple affections but we categorized them according to the primary disease. 6 of them presented with pathologies of the incisors (31.57%), 10 had issues starting with the premolars (52.63%) and 2 with the molars (10.52%). The highest rate was 52.63% represented by the ones that presented dental disease at the premolars. The most met pathology in this study was odontogenic abscess formation in 11 out of 19 cases (57.89%), in most cases the abscesses appeared secondary due to periodontal infections. Knowing the specific features of the oral cavity, of the approach and the restraining methods as well as the clinical and imagistic diagnosing can assure proper management of dental disease in pet rabbits.

Keywords: rabbits, dental disease, restraint, radiologic examination, management

1. Introduction

These days the domesticated rabbit (*Oryctolagus cuniculus*) is considered an excelent animal companion but at the same time they are also used as laboratory animals and also some specific breeds are raised in farms settings for meat production. Research in the last few years shows that in order to ensure the pet rabbits wellbeing and health they need to be committed for annual health checkups, to examine the whole body completed with a full clinical examination of the head and the oral cavity and the teeth [4, 5]. Knowing the specific morphology of the head, the mouth and the dentition of the rabbits is essential in the management of dental disease. Rabbits teeth are elodont (they grow throughout their entire life) and hypsodont (high-crowned teeth that exceeds the gum line) so they need to chew constantly for proper dental wear. The teeth, the periodontal ligaments and the bone structures are active tissues and they all change in relation to dietal changes, behavioral modification or environmental changes. Development of periodontal disease is plurifactorial and includes: genetic disorders, breed predisposition, traumatic events, defective treatment and neoplasia. Other morphological aspects that predispoze rabbits to development of dental disease are a narrow and elongated oral cavity and a massive tongue with evident

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lingual protuberance. The upper lip of the lagomorphs is split, the diastema is long and they have heterodont dentition with hypselodont and lophodont cheek teeth. The enamel of the incisor teeth is white due to the lack of pigments. The occlusion of the cheek teeth is the anisognathus type what means that the lower jaw is narrower than the upper one. As a consequence of this type of jaw the occlusion changes during chewing and the grinding of the food only happens at one side at a time. During the mastication process the occlusion changes 120 times in a minute [15]. The rabbit's teeth are continuously growing throughout their entire life, the incisors grow approximately 2-2.5 mm/week and the cheek teeth 2.5-3 mm/month. Important factors that contribute to the normal ware of the teeth are time spent consuming food, type of the nutrients and the abrasiveness of the diet [12, 13]. Elodont teeth, as met in rabbits, have different anatomy and they are singular, homogenous elongated structures inside and outside the alveolus. The apex of the teeth remain largely open and they never form a "root" so they are considered aradicular. The germinative tissue represented by the pulp and the dental sac is positioned at the level of the apex and they continue to produce dental tissue from ameloblasts, odontoblasts and cementoblasts. There is a noticeable difference between the clinical and the reserve crown. The clinical crown is smaller and visible outside the alveolus while the reserve crown is the main structure of the teeth and is situated under the gum, intraosseous. Both of the crowns together form the long anatomical crown (*Corona anatomica*) or the body of the tooth (*Corpus dentis*) [1]. The dental formula is as follows: I (2/1), C (0/0), PM (3/2), M (3/3) = 28. The superior incisors have a specific placement in 2 rows: 2 well developed primary incisors followed by 2 secondary smaller ones. They have no canine teeth so the diastema is long. The premolars and the molars are placed in close proximity of the cheeks, the clinical crown is visible on the exterior of the gingiva and the reserve crown is situated under the gum line [9]. The mastication process begins with a vertical action of the incisor teeth, the edge of the lower incisors slide along the occlusal surface of the superior primary incisors. Once the food reaches the cheek teeth, the mandible proceeds with a lateral action of chewing. For this specific movement the mandible is first retracted from the initial position separating the incisors and aligning the molars. The temporomandibular joint acts as a pivot on the side that is currently active in chewing with the masseter and the pterygoid muscles activated only on one side at a time [17].

Dental disease in rabbits is divided in two big categories: hereditary and acquired. The most frequent hereditary dental diseases are prognatism and brevignatism especially in the dwarf breed pet rabbits due to genetic modification to obtain a more desired look which led to the shortening of the skull much like in brachycephalic dog breeds. A shorter nasal bone, abnormal curvature or shorter mandible can lead to elongated incisors and secondary excessive growth of the cheek teeth. Acquired dental disease is more frequent and there are also 2 big groups: traumatic and non-traumatic lesions. Traumatic acquired dental disease is caused usually by accidents and is represented by fractures of the teeth, the skull or contusion of the facial bones and structures that compromise the dental structures. Non-traumatic injuries often are caused by inadequate food, insufficient dental wear or periodontal infections. They can also be seen after thermal burns caused by faulty crown resection, calcium of vitamin D deficiency or neoplasia. Specific literature shows that the most common dental pathology in pet rabbits is odontogenic abscess formation [8, 10, 11].

2. Materials and Methods

In this study the biologic materials were represented by 19 pet rabbits, male and female, aged between 1 and 10 years of age that were submitted to the NAC (New Animal Companion) clinic of the Faculty of Veterinary Medicine, Cluj Napoca.

It is highly recommended that before the clinical examination, the patients need to be placed on the table in the transportation box and to be left there for a few minutes to get used to the smell, the lights, the voice of the veterinarian. The examination table has to be covered with a non-slippery material that is easily cleaned and disinfected, and if possible on top of that to place an absorbent mat, a towel or a blanket.

The non-biologic materials used in the study were represented by the TEMCO GRX radiography machine, classic stomatology instruments and specific ones used for rodents.

The applied work methods were represented by: approach of the patient and restraint followed by clinical examination which entailed: inspection, palpation, percussion, auscultation and thermometry, respecting all the semiological requirements [16]. The approach and the restraint of the pet rabbit has some specific features that differ from the ones used in farm animals. A very important part of the restraint is constant support of the spine and the lumbar area by fixing the hind limbs. The muscles of the posterior train are well developed and the brutal extension of the legs, the propulsion force or the twisting of the spine can cause luxation or even fracture of the vertebrae, lumbo-sacral fracture or fracture of the spine [6, 7]. If possible, it is recommended to avoid lifting the rabbits off the ground, but if it is necessary the best way is to support the body and fix the hind limbs with one hand while holding the sternum and supporting the chest with the other hand. For examining the head and the oral cavity, a towel can be used to wrap the patient in it. This towel or a blanket can be used also if the rabbit is very aggressive to lift it out of the transport container and then other different restraining methods can be applied (Fig. 1).

Fig. 1. Restraining methods for manipulation, lifting and transportation

When the burrito restraining method is used (Fig. 2), the rabbit is placed on the towel or blanket in sternal recumbency followed by covering the hind limbs and the back of the animal. The towel then is folded and fixed around the neck gently. This method enables the veterinarian to examine the head, the ears and the mouth of the rabbit. A big advantage is that oral medication can be also given during this restrain and the patient cannot move abruptly.

Fig. 2. Burrito restraining technique

Proper examination of the head is a very important step in the general clinical examination. When done correctly, it can provide essential information to establish a proper diagnosis.

Inspection from a distance is the first step of the examination of the head. We evaluate the symmetry of the face, abscesses appear usually only on one side of the mandible or the maxilla. Asymmetry of the face can also be caused by neurological diseases like facial nerve paralysis with ptosis of the eyelids or the lips. In most cases epiphora can be caused by elongated upper incisors or premolars and even wet dermatitis of the skin around the eye can be seen due to the constant irritation [9]. Fetid smell can guide the diagnostician to consider the existence of abscesses or other suppurative processes with bacterial involvement. In certain situations with dental disease or persistent pain, most of the rabbits have bruxism, presented by a grinding sound of the teeth that the practitioner can easily identify. Dental disease can induce lethargy, apathy, behavioral changes, lack of self-cleaning or changes of the appetite.

Palpation of the rabbit face needs to be done very cautiously due to the discomfort it causes. Often when evaluating the bone structures and palpating any present modifications the rabbits may react and retract but that is not always caused by the presence of pain. Uneven surface of the head, or any kind of asymmetry of the skull has to be properly investigated. The methodology of palpating the head (Fig. 3) is as follows: Initially we palpate the ventral and lateral surface of the mandible to check for any kind of formation of soft or hard tissue like abscesses, ossification processes, bone remodeling due to excessive growth of the lower cheek teeth; then we follow with palpation of the nasal bone and the maxilla to identify any dental disease associated with elongation of the upper teeth or odontogenic abscess formation. Palpation of the cheek area is not recommended before an inspection of the oral cavity due to the possible formation of dental spikes that cause discomfort and pain that interferes with the evaluation and the interpretation of the symptoms. Then we proceed with the palpation of the zone around the eyes, we evaluate symmetry and protrusion of the eye globes and the temporomandibular joint. In lop eared rabbits the external auditory canal has to be palpated in a descending direction after lifting of the earlobe.

Fig. 3. Palpation of the rabbit head

Examination of the oral cavity can be done using the classical stomatology tools, or the specialized ones developed for rabbits and small rodents (Fig. 4). The rabbit is often awake for this part of the exam but in cases where it is needed the patient can be under general anesthesia too. Because of the length and narrowness of the mouth, two types of speculums are needed when examining the patient under anesthesia: one for the cheeks and one for the mouth to keep it open. The instruments needed for the inspection of the oral cavity are: mouth gag, cheek dilator, cotton swabs, cheek spatula, otoscope, periodontal probe, dental mirror, hemostat, light source, pediatric laryngoscope and containers for biological samples.

Fig. 4. Instruments for examining the oral cavity of the rabbit

When not in use, the inferior incisors should be positioned between the first and the second row of the superior incisors (Fig. 5). The normal physiological shape of the incisors should be a chisel-shape. The labial surface of the incisors should be white and smooth to the touch, any modification of color, shape, roughness, or presence of horizontal ridges may indicate pathological processes.

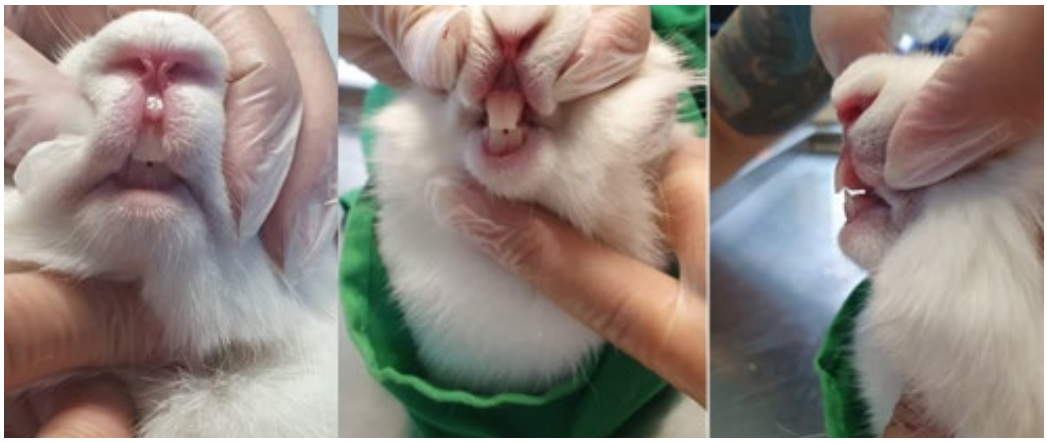

Fig. 5. Examination of the nose and occlusion of the incisors

For examining the inside of the mouth, the veterinarian with one hand fixes the head of the rabbit and lifts the upper lips while gently sliding the otoscope or the laryngoscope inside the mouth with the other hand (Fig. 6).

Fig. 6. Examining the oral cavity and the teeth with the otoscope or laryngoscope

Depending on the size of the rabbit, the lower cheek teeth have a short clinical crown while the superior premolars and molars are barely visible at the level of the gum line. The occlusal surface of the premolars and molars is at a 10° angle [19]. Any kind of modification needs to be identified like: changes in the angle of the occlusal surface, uneven surface, changes in the length of the clinical crown, dental spike formation, lesions of the surrounding soft tissue, ulcerations, excessive amount of saliva in the mouth, halitosis, absence of teeth, high dental mobility, presence of purulent material or hemorrhage. Often is needed to clean the mouth using a cotton swab before trying to examine the oral cavity because food and other kind of substances can remain stuck between the teeth and the mucosa or tongue [14].

Performing radiological imaging is recommended when during the clinical examination certain modifications are found like: deformity of the head, asymmetry of the skull, abscesses, malocclusion or metabolic diseases are suspected: osteodystrophy or secondary hyperparathyroidism. A high quality radiologic image can help the clinician to establish therapeutic protocol more efficiently and to elaborate a more precise prognosis. The most important aspect of obtaining a high quality image is based on the positioning of the patient on the radiology table. In rabbits when they are well restrained and docile an overall image can be made but usually it is recommended that the rabbits undergo general anesthesia or sedation at least. When the patient is weakened

anesthesia can carry unnecessary risks and it is not indicated until the patient is stabilized and well hydrated. A radiological examination usually consists of two exposures: one latero-lateral and one dorso-ventral but in most cases these are not satisfactory due to the superimposition of multiple tissue layers. Research shows that a minimum of 4 different exposures are needed to fully asses dental disease in rabbits, as shown in the 19th case included in this study on a clinically healthy rabbit (Fig. 7). The aforementioned two exposures need to be completed with an oblique exposure at 40° angle on each side to visualize the mandibles and the apex of the lower cheek teeth.

Fig. 7. Mandatory exposure of the head, case 19:
A-latero-lateral; B-dorso-ventral; C-oblique-right ; D-oblique-left

Out of these 4 exposures, the most relevant information can be obtained when the patient is positioned correctly on the latero-lateral one, to apply the reference line system developed by Bohmer and Crossley [3]. On the latero-lateral view (Fig. 8 A) in rabbits' normal occlusion, there should be no dental structures outside the line that connects the proximal extremity of the nasal bone to the occipital protuberance. Another reference line parallel with the dorsal line connects the rostral extremity of the hard palate to the tympanic bulla at 1/3 of its height. This second line marks the occlusal surface of the cheek teeth. Although there are 6 upper cheek teeth and only 5 lower ones, the length of the occlusal surfaces is roughly the same. In healthy rabbits, the apexes of the lower cheek teeth should not penetrate the osseous ventral lamina of the mandible. The jaw should have a constant width and homogenous structure at the level of the lower premolars. In the dorso-ventral exposure (Fig. 8 B) a series of reference lines can be traced starting with 2 lines that connect the lateral limit of the superior incisors down to the median angle of the mandible on the same side. Another set of symmetrical lines connect the lateral limit of the tympanic bulla to the lateral extremity of the incisor on the opposing side. No other dental structures should be visible in the areas traced by these set of lines except for the apexes of the superior premolars that normally have a curved shape [2].

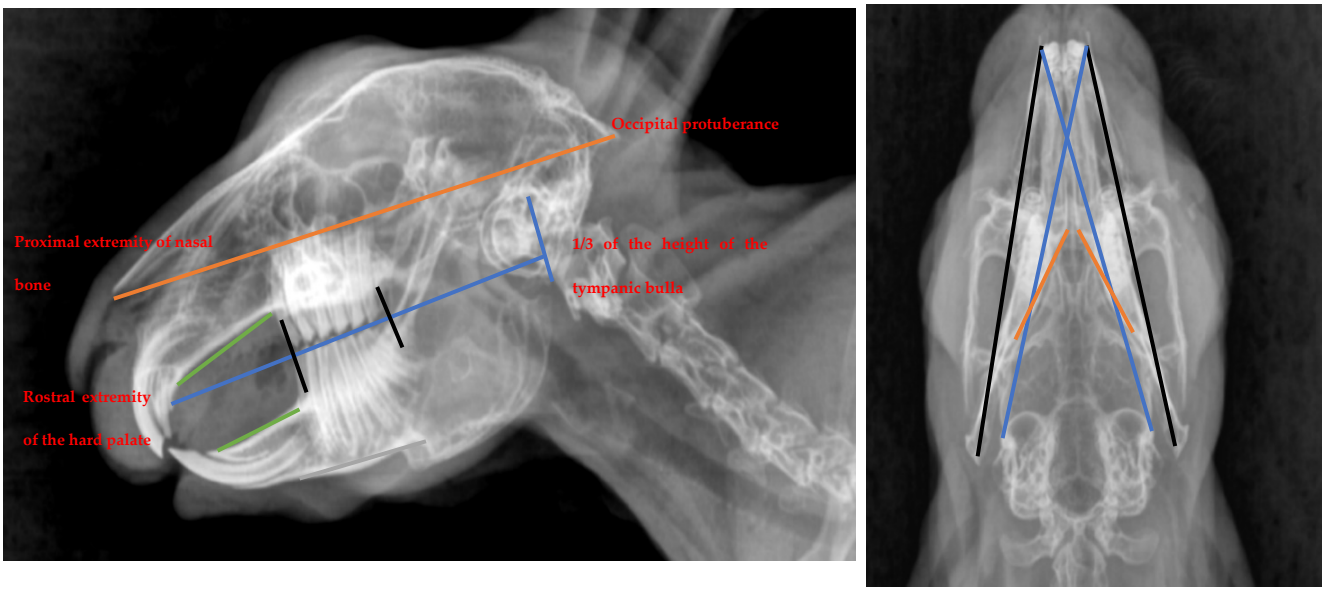

Fig. 8. Reference lines on the radiologic image: A- latero-lateral exposure; B- dorso-ventral exposure

3. Results

In this study were included 19 rabbits, 1 of them (5.26%) was clinically healthy, and 18 were diagnosed with dental disease. 6 of the rabbits had primary dental disease at the level of the incisors (31.57%), 10 of them had problems with the premolars (52.63%) and 2 presented pathology in the molars (10.52%). The highest percentage was represented by 52.63% with diseases of the premolar cheek teeth (Fig. 9). The classification criteria was based on the type of teeth that initiated the development of the dental disease. Each of the cases except for the healthy one, presented multiple disorders, primary and secondary modifications as well. The most frequently identified pathology in this study was odontogenic abscess formation in 11 of the 19 cases (57.89%). Most of these abscesses developed secondarily to periodontal infections.

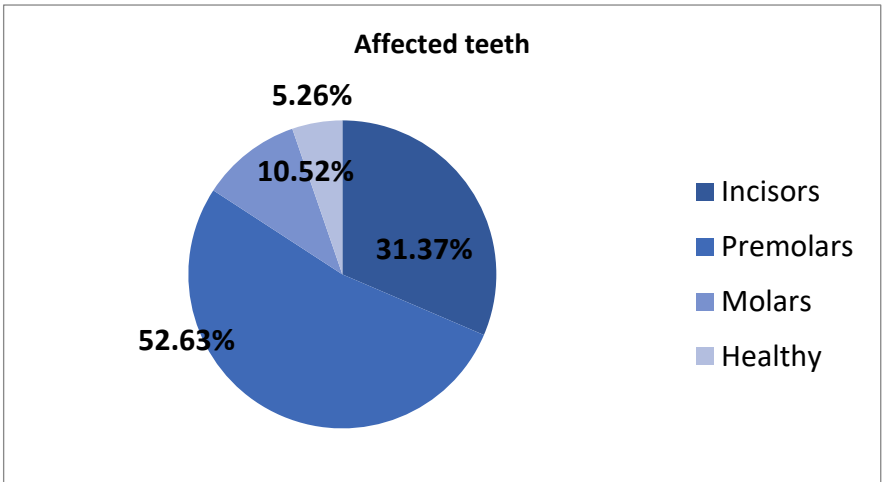

Fig. 9. Classification of dental pathology (%) based on the type of the teeth primarily affected

4. Discussion

The cases included in this study were classified in two big categories: hereditary dental disease, like prognathism, and acquired dental disease like traumatic lesions, abscess formation, malocclusion, or metabolic bone disease. We had 2 cases identified with congenital dental disease: cases 1 and 2. Case 1, female, 1 year old, diagnosed with prognathism with extreme curvature of the superior incisors. In this case resection of the clinical crown of all the incisors was used to try to correct the malocclusion (Fig. 10 A). The second case was represented by a male rabbit, 6 months old, also with prognathism but also a secondary elongation of the premolars both superior and inferior and osteodystrophy of the upper primary incisors (Fig. 10 B). The rabbit underwent surgery to resect the crowns of the incisors close to the gum, to shorten the clinical crown of the cheek teeth to correct occlusion. After 6 months, he still presented malocclusion of the incisors, but the occlusal surface of the cheek teeth was improved (Fig. 10 C).

Fig. 10. A- Maxillary prognathism, case 1; B- Maxillary prognathism with osteodystrophy of the upper incisors, case 2; C-. 6 months after crown height reduction, case 2

We diagnosed 16 cases of acquired dental disease out of the 19 cases. They will be presented here based on the type of the teeth that started the pathological process. Cases 3, 4, 5 and 13 presented with primary acquired dental disease of the incisors. Case 3, male, 4 years old, suffered from fractured superior incisor due to some level of metabolic bone disease expressed by osteolysis and odontogenic abscess formation at the first lower premolar (Fig. 11 A). Surgical treatment concluded extraction of the upper incisors and the first two lower premolars on the left side, then followed by marsupialization of the abscess. Both cases 4 and 5 were identified with odontogenic abscesses at the inferior incisors. Case 4, female, 1 year old, presenting jaw abscess with the starting point at the right inferior incisor (Fig. 11 B). The abscess was marsupialized and the affected incisor was extracted. Case 5, male, 8 years old, presented with abscess at the mandible due to periodontal infection of the lower incisors (Fig. 11 C). In this case both of the inferior incisors were extracted and then proceeded to the marsupialization of the abscess. Case 13, male, 2 years old, diagnosed with apical elongation of the inferior incisor that pushed the first lower premolar and dislocated it and then led to abscess formation because the osseous lamina of the mandible was injured (Fig. 11 D).

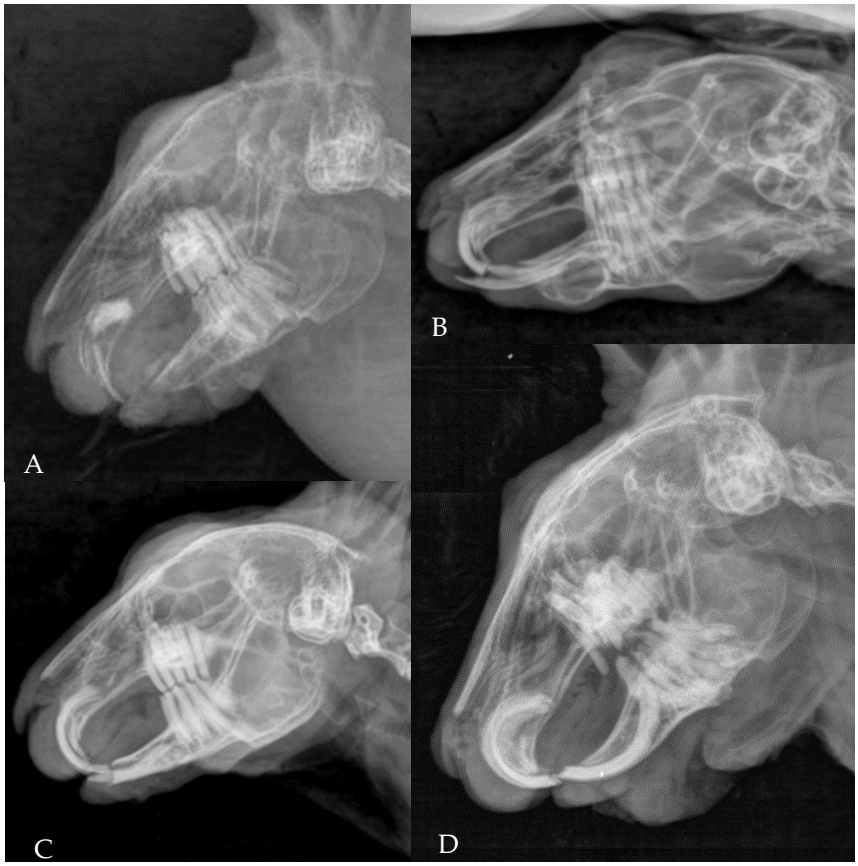

Fig. 11. A- Fractured superior incisor, case 3; B- Mandibular abscess at the right lower incisor, case 4; C- Periodontal infection of the lower incisors with abscess formation, case 5; D- severe malocclusion and mandibular abscess with elongation of the lower incisors, case 13

The premolars were the most frequently affected teeth in this study and their pathology included: crown elongation, odontogenic abscess formation, in some cases also secondary malocclusion of the incisors. Dental disease of the premolars usually appear due to insufficient wear most likely caused by inappropriate nutrition. Cases 6, 7, 9 and 10 were all identified with excessive growth of the premolars which later led to complications. Case 6, male, 3 years old, had elongated premolars with bone remodeling of the mandible which then caused secondary malocclusion of the incisors with exaggerated curvature and overgrowth (Fig. 12 A). We resorted to crown reduction of the incisors to the gum line, and also reduced the crown of the upper cheek teeth. The inferior premolars were extracted. Case 7, male, 2 years old, presented with slight malocclusion of the incisors due to overgrown cheek teeth (Fig. 12 B). Under general anesthesia the clinical crown of the incisors and the cheek teeth were shortened to give back a more natural occlusion. Crown elongation of the superior and the inferior molars in Case 9, male, 3 years old, condition a non-physiological positioning of the inferior molars and remodeling of the mandible (Fig. 12 C). The overgrown premolars were extracted. Case 10, female, 3 years old, elongated premolars led to penetration of the osseous lamina of the mandible and osteolysis. In this case too, the premolars were surgically extracted.

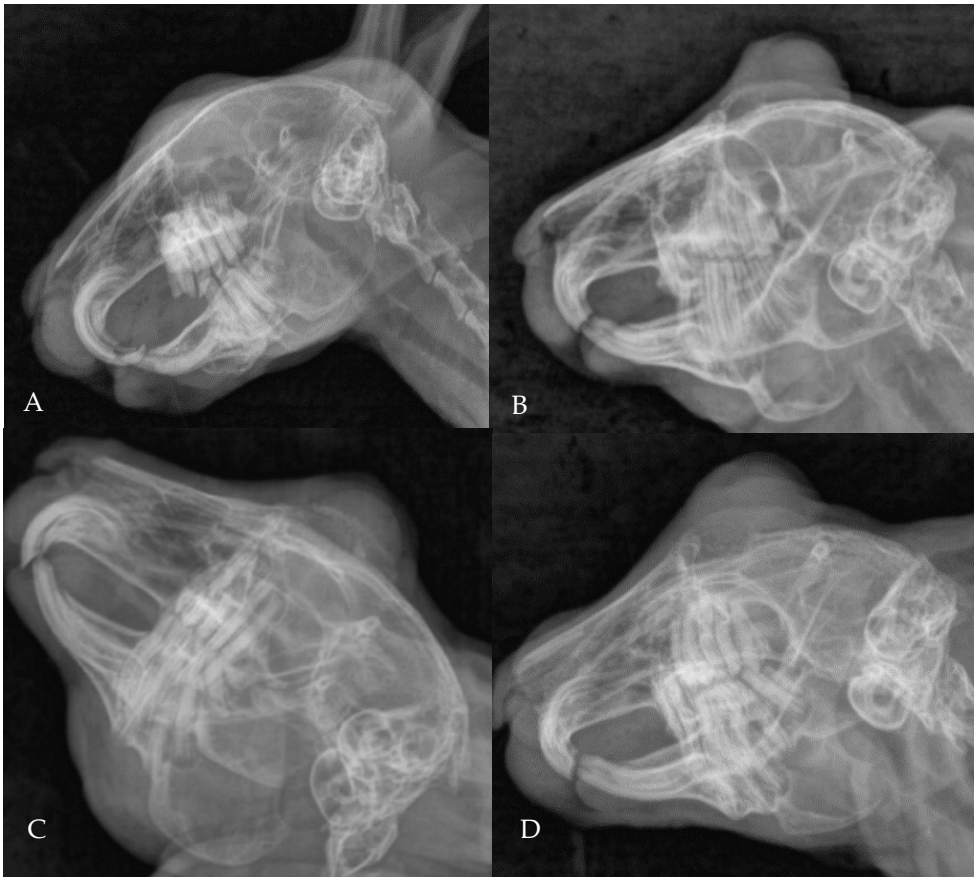

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Fig. 12. A- severe malocclusion, case 6; B- Slight malocclusion of the incisors due to overgrown premolars, case 7; C- Crown elongation of the premolars and bone remodeling, case 9; D- Overgrown inferior premolars causing remodeling and osteolysis, case 10

Out of all the diagnosed non-traumatic acquired dental disease most of the cases were represented by odontogenic abscess formation based on gum inflammation and periodontal infections. Cases 8, 11, 12, 14, 15 and 16 all were identified with abscesses originated at the level of the premolars and later presented complications like tooth fracture, osteolytic leasions and severe bone remodeling. Case 8, male, 5 years old, presented with fracture of the first inferior premolar, secondary malocclusion of the incisors, remodeling of the mandible and apical abscess that involved also the lower incisors. The maxillary incisors were reduced, and both lower incisors were extracted along with the fractured premolar (Fig. 13 A). Case 11, 3 years old male, with severe acquired dental disease. Presented with excessive growth of the mandibular cheek teeth, especially the premolars, with heavy mandibular bone remodeling that lead to abscess formation and secondary malocclusion of all the incisors. The incisors were resected with a dental burr using a diamond disc, the mandibular incisors on the left side were all extracted and the abscess was marsupialized (Fig. 13 B). Case 12, male, 1 year old, presented with a mandibular abscess that started at the apex of the first lower left premolar. The capsule of the abscess presented severe ossification, the superior premolars also were elongated and the lower molars presented pathological curvature of the reserve crown. Surgical treatment involved marsupialization of the abscess together with resection of the capsule, extraction of the affected premolar and leveling the occlusal surface of the cheek teeth with the help of the dental burr (Fig. 13 C).

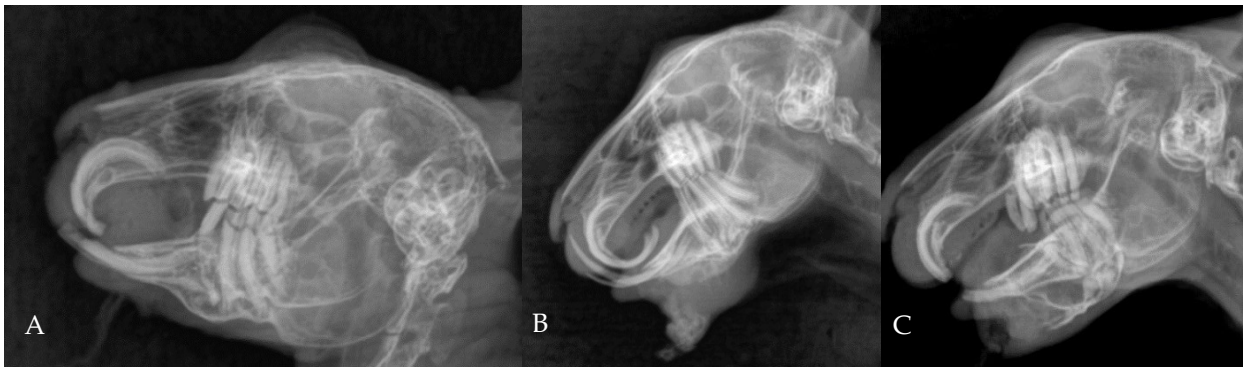

Fig. 13. A- Fracture of the first mandibular premolar and secondary malocclusion of the incisors, case 8; B- Mandibular abscess and secondary malocclusion of the incisors, case 11; C- Mandibular abscess of the mandible with ossified capsule and severe malocclusion, case 12

Cases 14, 15 and 16 also presented with mandibular abscesses with various etiology. Case 14, 1 year old male, diagnosed with abscess formation on the mandible due to periapical infection of the lower premolars on the left side with severe ossification of the abscess capsule and bone remodeling (Fig. 14 A). The affected premolars were extracted and the abscess was marsupialized after removing as much as possible of the capsule tissue. Case 15, male, 1 year old, diagnosed with dystrophy of the first lower premolar of the left side that caused lateral mandibular abscess formation due to the periapical infection. The abscess was drained and the affected premolar was extracted (Fig. 14 B). Case 16, male, 7 years old, presented with abscesses both on the mandible and the maxilla, due to chronic periapical infections of the premolars. The abscess capsule was completely ossified on the mandible (Fig. 14 C). The patient was euthanized as requested by the owner.

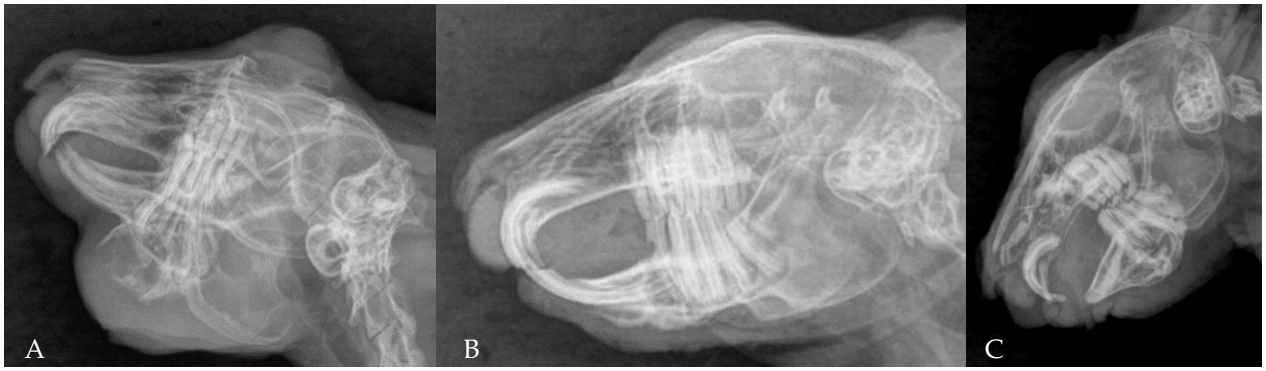

Fig. 14. Odontogenic abscess formation: A- Premolars left side of the mandible, case 14; B- First left lower premolar, case 15; C- Severe acquired dental disease with ossified abscess capsule, case 16

Primary pathology of the molars is quite rare, but in this study were included 2 of them presenting crown elongation and abscess formation: cases 17 and 18. Case 17, male, 2 years old, diagnosed with odontogenic mandibular abscess and elongation of the cheek teeth both superior and inferior (Fig. 15 A). Treatment involved surgical marsupialization of the abscess and crown reduction of the molars. Case 18, male, 3 years old, presented with caudal dislocation of the last lower molar and dystrophy of the tooth structure. In this case surgery was not

recommended, the owner was advised to improve the diet and add rough nutrients that help with dental wear (Fig. 15 B).

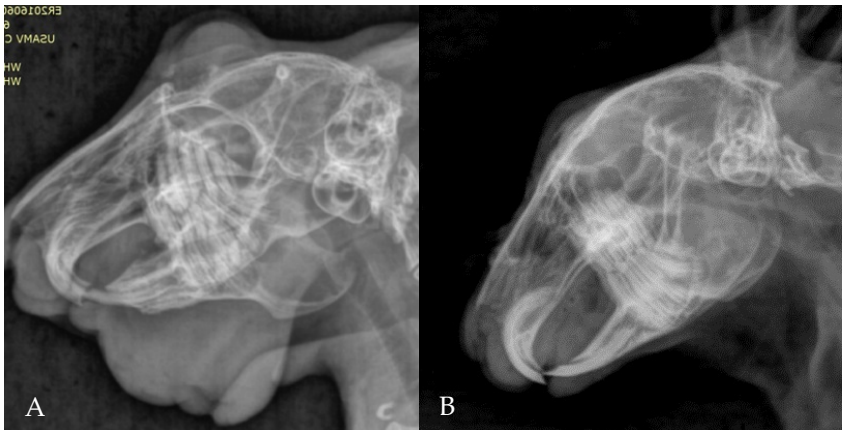

Fig. 15. A- severe elongation of the mandibular molars and abscess formation, case 17; B- Dystrophy of the dental structure of the last inferior molar, case 18

5. Conclusions

In our study after quantitative evaluation of the described dental pathology of rabbits, the results are similar to results obtained in the specific literature, which shows that the most affected teeth are the inferior premolars (43.33%). Using the reference lines as a method of interpretation the radiological images offers a better understanding and a higher chance of proper diagnosing of dental pathology in rabbits. Radiological examination in suspected dental disease is a gold standard method in identifying the primary pathology in stomatological disorders and it also aids in following up on the healing process as needed.

Author Contributions: Conceptualization, K-P.T.T. and I.P.; methodology, investigation, L.R., P.R.C. and M.S.M.; writing—original draft preparation, K-P.T.T.; writing—review and editing, M.T., L.B. and C.D.; supervision, I.P.; All authors have read and agreed to the published version of the manuscript.

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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Article

Prevalence of retained fetal membranes in a dairy cattle farm located in Mureș county, Romania

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Abstract: A clinical study was conducted in a cattle farm in Sângeorgiu de Mureş, Mureș County, Romania (46°34′35″N - 24°36′15″E, altitude 320 m) with a continental-moderate climate, average annual temperature of 8-9°C, and humidity of 86%. The study spanned January to December 2021, involving 240 cattle, including 105 adult cows. The population comprised three breeds: Baltata Romaneasca (n=89), Pinzgau (n=12), and Red Holstein (n=4), with age groups 2-4 years (n=57), 5-7 years (n=41), and >7 years (n=7). The study analyzed the prevalence of retained fetal membranes, dystocia, and endometritis by breed, age, calf sex, and season. Results showed a 13% prevalence of retained fetal membranes (14/105), 2% dystocia (2/105), and 4% endometritis (4/105). Among Baltata Romaneasca, retained fetal membranes was 15.73% (14/89), and Pinzgau had 1 case (8.33%). Dystocia was observed at 2.25% (2/89) in Baltata Romaneasca, and endometritis at 33.33% (4/12) in Pinzgau. Age influenced disease prevalence, with cows over 7 years showing higher rates: retained fetal membranes (28.57%) compared to younger groups (≤12.28%). Endometritis and dystocia followed similar age-related trends. Seasonally, retained fetal membranes cases were highest in spring (16.67%) and winter (14.29%), and lower in summer (9.25%). Endometritis was seen only in summer (12.5%), and dystocia in autumn. Retained fetal membranes was more prevalent in cows with male calves (18.18%) versus female calves (8%). Differences in endometritis and dystocia by calf sex were not significant.

Keywords: prevalence, retained fetal membranes, risk factors, Bălţată Românească, Romania

1. Introduction

In the most recent USDA National Animal Health Monitoring System survey, producers reported that 4.5% of dairy cows experienced retained fetal membranes [1]. The etiology of retained fetal membranes is influenced by factors such as hygiene, management practices [2], cow age and parity, nutrition, and calving conditions (stillbirth, single, or twin calving) [1].

Several risk factors contribute to this condition, including abortion (especially due to brucellosis or mycotic infection), dystocia, twin births, stillbirths, hypocalcemia, high environmental temperatures, advancing cow age, premature birth or induced parturition, placentitis, and nutritional imbalances, such as elevated prepartum serum nonesterified fatty acids. Cows with retained fetal membranes face a higher likelihood of developing metritis, displaced abomasum, mastitis, ketosis, and early-lactation culling. Additionally, their fertility in the subsequent lactation can be adversely affected. The economic impact of metritis is significant, with an estimated cost of $386 per case due to decreased milk production, longer intervals to the next pregnancy, increased risk of related periparturient diseases, and higher culling rates [3].

Risk factors for retained fetal membranes vary among different regions or countries because of differences in general management, environment, and herd health control conditions [4, 5]. In addition, effects of retained fetal membranes on

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reproductive performance have varied [6]. Our first objective was to determine the risk factors for retained fetal membranes by evaluating several factors: the breed and age of the cows, season, sex of the calves in Bălţată Românească cattle farm located in Mureș County, Romania.

2. Materials and Methods

The clinical study was conducted in a cattle farm located on the territory of Sângeorgiu de Mureş in Mureș County, Romania. Geographically, this area is located at 46°34'35"N - 24°36'15"E, an altitude of 320 m, on the left bank of the Mureș river. The climate is continental - moderate with an average annual temperature of 8-9°C and humidity 86%.

The study was conducted between January 2021 and December 2021, in this period of time the farm having a total of 240 cattle, including both adults and young stock.

Concerning the feeding regimen, at the cattle farm, animal nutrition is primarily sourced from existing stock resources year-round, maintaining the unaltered daily ration composition. Notably, green forage is abstained from in the diets of lactating cows.

At the farm, during the winter months, Romanian Spotted Cows are fed with hay that has an appropriate botanical composition to ensure superior nutritional value. During the summer, their diet is occasionally supplemented with fresh forage from the pasture. Additionally, the hay provided was periodically weighed to estimate the average consumption per fed cow. It was challenging to precisely determine the exact quantity of feed ingested by each individual bovine due to the collective maintenance system with unrestricted access to feed. For better handling, the hay was provided in the form of cylindrical bales with a diameter of 1.20 meters. It was made available to the cattle by placing it in a specialized feeding trough with 12 feeding stations located within the paddock where the taurine cattle are housed.

Experimental Animals

Research manuscripts reporting large datasets that are deposited in a publicly available database should specify where the data have been deposited and provide the relevant accession numbers. If the accession numbers have not yet been obtained at the time of submission, please state that they will be provided during review. They must be provided prior to publication.

105 adult cows were included in the study and their calving were followed for one year. First of all, the structure of the population was analyzed according to race and age. The cows in the analyzed population belonged to three breeds: Baltata Romaneasca (no. = 89), Pinzgau (no.= 12) and Red Holstein (no. = 4) (Figure 1).

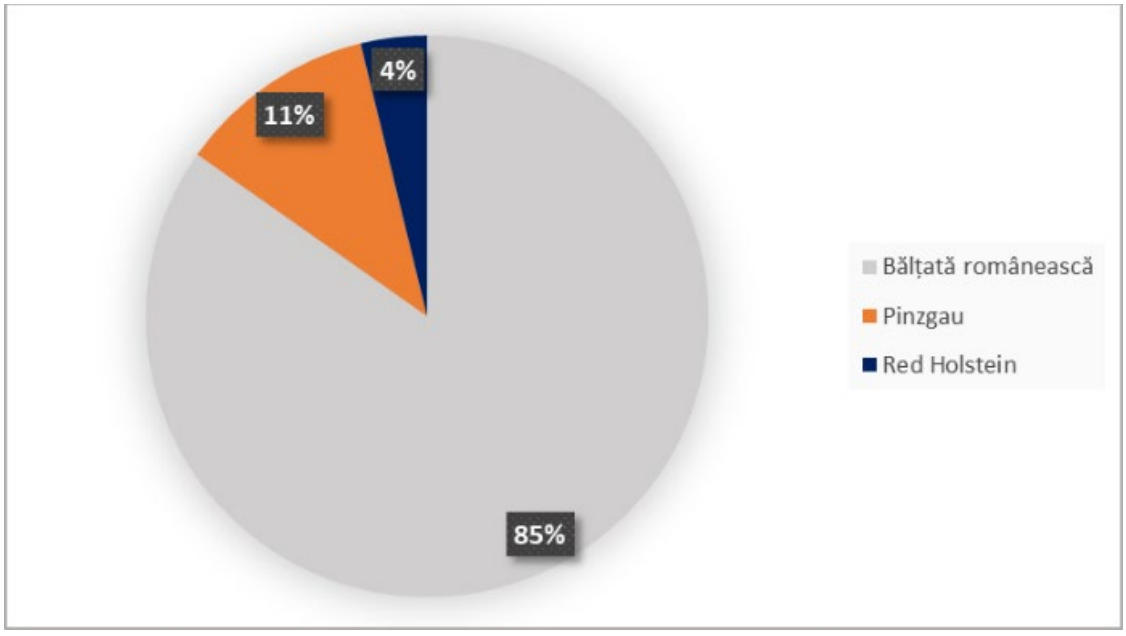

Figure 1. The structure of the studied population: the percentage distribution of the breeds.

In terms of age, the following intervals were established: 2-4 years (no. = 57), 5-7 years (no. = 41) and >7 years (no. = 7) (Figure 2).

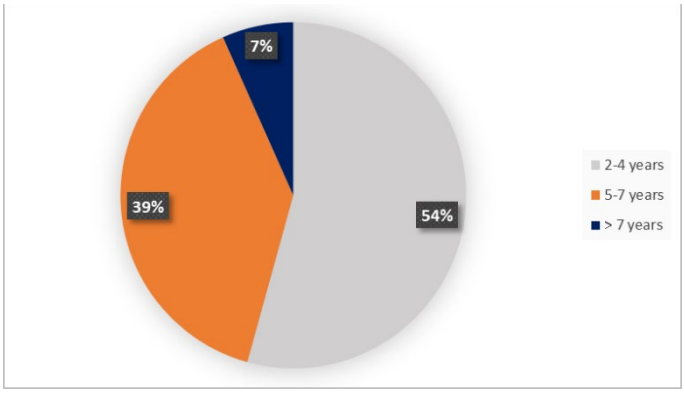

Figure 2. The structure of the studied population: the percentage distribution of age.

Data analysis

The prevalence of retained fetal membranes and other diseases was analyzed descriptively, depending on the breed and age of the cows, season, sex of the calves. For each analyzed variable, data reporting was done for the total number of calving within each category. The results were expressed in absolute and percentage terms (Table 1).

Table 1. Distribution of calvings according to breed and age of cows, season, sex of calves.

	TOTAL CALVING (no. = 105)	
BREED	No.	%
Balaţată Românească	89	84.76
Pinzgau	12	11.43
Red Holstein	4	3.81
AGE		
2 – 4 years	57	54.29
5 -7 years	41	39.05
> 7 years	7	6.67
SEASON		
spring	24	22.86
summer	32	30.48
autumn	21	20.00
winter	28	26.67
CALF SEX		
M	55	52.38
F	50	47.62

3. Results and Discussion

This study showed that the prevalence of retained fetal membranes accounted for 13 % of the population (14 out of 105), during the analyzed period, 2 dystocia (2%) and 4 cases of endometriosis (4%) were also diagnosed

(Figure 3). The current study found an overall prevalence of retained fetal membranes at 13%, which is lower than the 17.8% and 18.3% reported by Markusfeld, 1987 [7], Gaafar et al. 2010 [8], and Rahawy, 2021 [9], but higher than the incidences of 6.6%, 7.8%, and 10% reported by Bruun et al. 2002 [10] and Goff, 2006 [11]. The variation in retained fetal membranes incidences reported by different authors may be due to factors such as environment, breed, age, heredity, nutrition, immunity, and hormonal status.

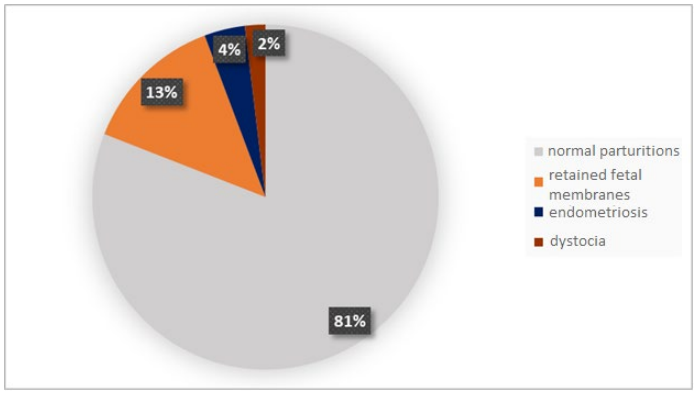

Figure 3. The prevalence of retained fetal membranes, endometriosis and dystocia.

The frequency distribution of retained fetal membranes was presented based on the characteristics of the cows (Figure 4; 5), season (Figure 6), sex of the calves (Figure 7).

From the point of view of the prevalence of retained fetal membranes, depending on the breed, in the case of Balţata Romanească (no. = 89) 13 cases (15.73%) were recorded, and for the Pinzgau breed (no. = 12) 1 single case. The other diseases were represented by dystocia with a level of 2.25% (no. = 2) in Baltata Romaneasca and 4 cases of endometriosis (33.33%) in the Pinzgau breed (Figure 4).

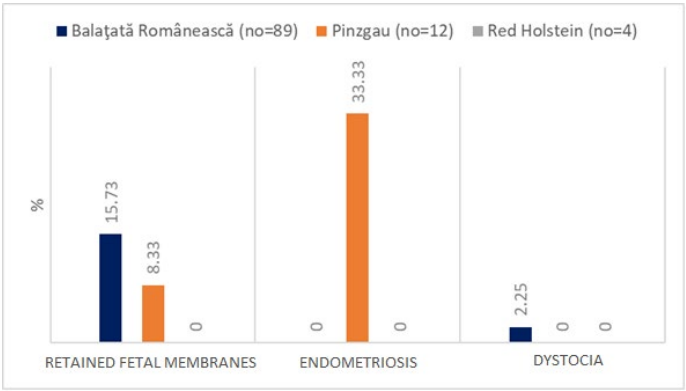

Figure 4. The prevalence of retained fetal membranes, endometriosis and dystocia based on breed of the cows.

The age of the cows was an important factor for all the diseases investigated, the higher percentages being observed in cows over 7 years old. In this category, the level of retained fetal membranes was 28.57%, while for the other categories it did not exceed 12.28%. The same dynamics were observed in the case of endometriosis and dystocia (Figure 5).

Older cows are more frequently associated with retained fetal membranes, older cows tend to have lower uterine muscle tone, contributing to dystocia [12], a known factor in retained fetal membranes [13]. Dairy cows older than five years a decline in their reproductive endocrine system [14], making them more susceptible to retained fetal membranes [15].

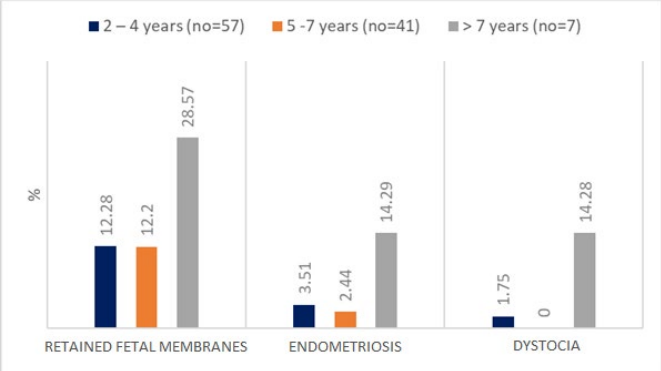

Figure 5. The prevalence of retained fetal membranes, endometriosis and dystocia based on age of the cows.

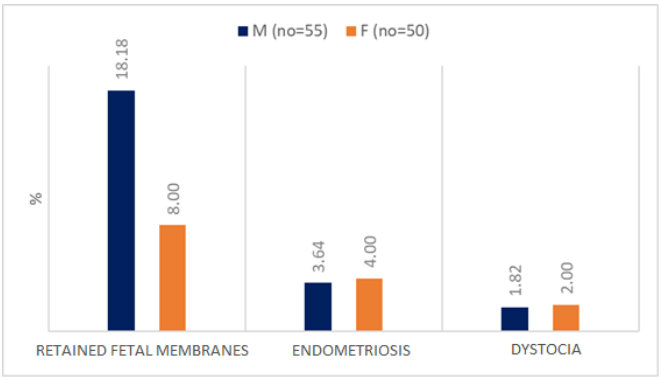

Figure 6. The prevalence of retained fetal membranes, endometriosis and dystocia based on sex of the calves.

The analysis of the influence of the sex of the calves on the prevalence of retained fetal membranes revealed the evolution of 10 cases (18.18%) in cows that had male calves and 4 cases (8%) in those with female calves. We consider that the differences recorded from the point of view of the evolution of endometriosis and dystocia are not significant (Figure 7).

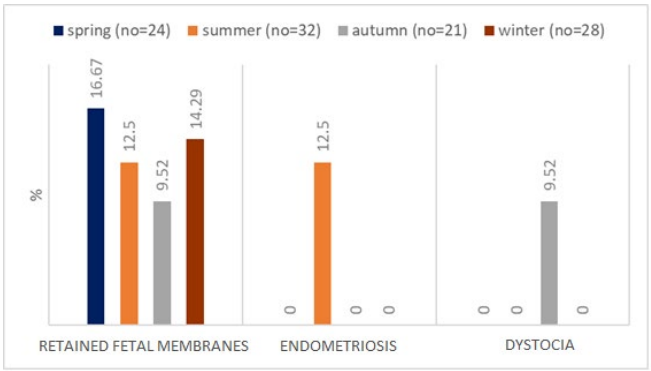

Figure 7. The prevalence of retained fetal membranes, endometriosis and dystocia based on season.

This study demonstrated that male calves are more likely to cause retained fetal membranes compared to female calves. Specifically, the incidence of retained fetal membranes was higher in cows with male calves (23.12%) than those with female calves (17.85%) [16]. However, the role of calf sex in retained fetal membranes incidence is debated [17], possibly due to different maternal factors associated with male and female calves, which remain unclear. Gestation length may be a more significant factor in retention of placental mebranes, as shorter gestation periods often lead to lighter offspring and a higher likelihood of retained fetal membranes [18]. Female calves typically weigh less (27.57 ± 0.54 kg) than male calves (30.71 ± 0.19 kg) [19], possibly due to fetal androgenic hormones from male fetuses influencing retained fetal membranes incidence [18]. These hormones can negatively

impact the hypothalamic-pituitary axis, reducing Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) production [20], which in turn lowers estrogen levels and increases postpartum progesterone, leading to retained fetal membranes [15].

Regarding the season, we state that most cases of retained fetal membranes were diagnosed in spring (16.67%), followed by the winter season (14.29%), while in spring and summer the percentage was 12.5 and respective 9.25. The evolution of endometriosis was observed only in summer (12.5%), and dystocia in autumn (Figure 6).

Different studies reported that the season of calving significantly influences the incidence of retained fetal membranes [21 - 23]. The summer calving season, in particular, is a major risk factor, with cows calving in summer being 2.84 times more likely to experience retained fetal membranes compared to those calving in spring. The high rate of retained fetal membranes in summer is likely due to heat stress, which hinders placenta expulsion [24]. This finding aligns with Fernandes et al. 2012 [25] but contrasts with Berglund et al. 2003 [26], who observed a higher retained fetal membranes rate in winter, attributing it to stillbirths, dystocia, and twinning. Additionally, Nobre et al. 2012 [22] noted that the rainy season increases environmental challenges for animals, making them more prone to retained fetal membranes.

However, calving's that occur in the summer [27] or during periods of heat stress [1] are linked to a higher incidence of retained fetal membranes. Conversely, Chassagne et al. 1996 [28] found a lower incidence of retained fetal membranes in the autumn. These differing results may be attributed to the varying temperature ranges or management environments across different countries or regions.

5. Conclusions

The study concluded that the prevalence of retained fetal membranes in the cattle population was significantly influenced by breed, age, season, and the sex of the calves. Retained fetal membranes was more common in the Balţata Românească breed (15.73%) compared to the Pinzgau breed (8.33%). Older cows (over 7 years) exhibited a higher incidence of all investigated diseases, with retained fetal membranes reaching 28.57% in this age group. Seasonal variation showed the highest occurrence of retained fetal membranes in spring (16.67%) and winter (14.29%). Additionally, cows that gave birth to male calves had a higher prevalence of retained fetal membranes (18.18%) compared to those with female calves (8%). These findings highlight the importance of considering multiple factors in managing and preventing reproductive health issues in cattle.

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