Biological response to endodontic treatment in one versus two-visit: a systematic review and meta- analysis of animal studies

Raimundo Sales de Oliveira Neto
cdraimundoneto@hotmail.com
University of São Paulo – USP
Thais de Moraes Souza
University of São Paulo – USP
Stefani Jovedi Rosa
University of São Paulo – USP
Rodrigo Ricci Vivan
University of São Paulo – USP
Murilo Priori Alcalde
University of São Paulo – USP
Heitor Marques Honório
University of São Paulo – USP
Marco Antonio Hungaro Duarte
University of São Paulo – USP

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Abstract

OBJECTIVES

To perform a systematic review of animal studies that compared the histopathological characteristics between teeth with apical periodontitis after endodontic treatment in one or two visits.

MATERIAL AND METHODS

This systematic review was registered on the International Prospective Register of Systematic Reviews (PROSPERO) – CRD42022340849. Studies were collected from PubMed/MEDLINE, LILACS, EMBASE, Livivo, SciELO, Web of Science, Scopus, and Cochrane Library and manual and gray literature searches. Animal studies that evaluated histological characteristics after endodontic treatment of teeth with apical periodontitis in one or two visits were included. Risk of bias analysis of the included studies was performed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool. Data synthesis of the included studies with quantitative data was performed, and meta-analysis was conducted with the Comprehensive Meta-Analysis software, using the random effects model and odds ratio (OR).

RESULTS

Eighteen studies met the inclusion criteria (Kappa = 0.891). Meta-analyses indicated values in inflammatory infiltrate intensity with effect size of 5.5% (95% CI: 0.020–0.148; p < 0.001), periodontal ligament thickness: 25.6% (95% CI: 0.134–0.487; p < 0.001), dentin resorption: 13% (95% CI: 0.015–1.141; p = 0.066), cementum resorption: 7.1% (95% CI: 0.015–0.325; p = 0.001), bone resorption: 1.4% (95% CI: 0.002–0.130; p < 0.001), mineralized tissue resorption: 42.8% (95% CI: 0.110–1.671; p = 0.222), biological apical sealing: 13.1% (95% CI: 0.055–0.314; p < 0.001), and presence of microorganisms: 10.3% (95% CI: 0.014–0.747; p = 0.025).

CONCLUSIONS

The two-visit endodontic treatment, using calcium hydroxide-based intracanal medication, resulted in better biological repair characteristics.

CLINICAL RELEVANCE:

A two-visit endodontic treatment with calcium hydroxide-based intracanal medication yields superior histopathological outcomes.

INTRODUCTION

Apical periodontitis is characterized by inflammation and destruction of the periapical tissues associated with infection of the root canal system [1, 2]. This inflammatory reaction stimulates the host immune response, degrading the extracellular matrix and mineralized tissues [3]. Endodontic treatment is performed to reduce the number of microorganisms below a certain threshold and prevent the invasion of new microorganisms to allow the repair of periapical tissues [4, 5]. In this sense, biomechanical preparation of the root canal, with instrumentation and irrigation protocols, plays a fundamental role [6, 7]. However, the literature has no consensus on whether intracanal medication between sessions is required.

Calcium hydroxide is the most common intracanal medication used between sessions of endodontic treatment because it stimulates mineralization and has antimicrobial effects [8, 9]. Intracanal medication has been recommended in the endodontic treatment of teeth with apical periodontitis because microorganisms persist in the root canal system after biomechanical preparation [10, 11]. Some clinicians recommend treatment in a single visit for time and cost reasons and to avoid contamination between appointments [12].

Systematic reviews evaluating the effectiveness of endodontic treatment in one or more visits show no differences in success between protocols. The studies assess outcomes based on clinical and radiographic signs [13–15]. However, in teeth with apical periodontitis, microscopic and radiographic findings may diverge [16], and the long-term persistence of apical periodontitis is associated with dysregulation of the immune system and alterations in inflammatory factors in the circulation [17]. Thus, this study aimed to systematically review animal studies that compared, at a histological level, the biological repair characteristics of teeth with apical periodontitis that received endodontic treatment in one or two visits.

MATERIAL AND METHODS

Protocol and registration

This systematic review followed the Preferred Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines [18]. It was registered in the International Prospective Register of Systematic Reviews (PROSPERO) - CRD42022340849 (SUPPLEMENTARY FILE 1).

Review question and eligibility criteria

The PICOS strategy was defined as follows: “P” teeth with apical periodontitis treated endodontically, “I” two visits, “C” one visit, “O” histological response (intensity of inflammatory infiltrate, thickness of periodontal ligament, incidence of biological apical sealing, dentin, bone and/or cementum resorption, and presence of microorganisms), (S) animal studies. Thus, the key question was: "Does the histological response differ between animal teeth with apical periodontitis treated endodontically in one or two visits?".

Regarding the intervention, only studies that used calcium hydroxide as intracanal medication were considered, regardless of vehicle and brand, time between sessions, instrumentation technique, irrigating solution used in biomechanical preparation, and obturation material and technique. Studies that reported single-visit treatment were considered for the comparator, regardless of the irrigating solution, instrumentation protocol, obturation technique, and material used for root canal filling. Regarding treatment outcome, studies that provided quantitative and/or qualitative data on the intensity of inflammatory infiltrate, periodontal ligament thickness, incidence of dentin, bone, or cement resorption, and presence of microorganisms after endodontic treatment by histological evaluation were included.

Databases and search strategy

Between June and August 2022, searches were carried out in PubMed/MEDLINE, Biblioteca Virtual em Saúde (BVS), EMBASE, Livivo, Web of Science, Scopus, and Cochrane Library databases. The gray literature was also searched by manually examining the reference lists of relevant studies and consulting the Brazilian Library of Dissertations and Theses.

The search strategy included terms related to population (teeth with induced apical periodontitis treated endodontically), intervention and comparator (one- or two-visit endodontic treatment), type of study (animal studies), and outcome (intensity of inflammatory infiltrate, thickness of periodontal ligament, incidence of biological apical sealing, dentin, bone or cement resorption, presence of microorganisms after endodontic treatment, histologically evaluated), which were combined with Boolean operators. The search strategy was adapted to the needs of each database. There were no restrictions on the publication year or language. (SUPPLEMENTARY FILE 2).

Study selection

The records retrieved from the databases were exported to the reference manager EndNote Web® (Clarivate, London, UK) to identify duplicates. In phase 1 of the review, two independent reviewers read the titles and abstracts of each article and excluded studies that did not meet the eligibility criteria. The full text of the studies pre-selected in Phase 1 was read to identify those that met the inclusion criteria (phase 2). Any discrepancies that arose were resolved by discussion between the reviewers and, if necessary, by discussion with an experienced third reviewer.

Data collection and extraction

One reviewer conducted the data extraction. Only data provided in the articles were considered. Extracted information included: first author, publication year, country, sample size (number of root canals), surgical diameter after biomechanical preparation, irrigating solution, characteristics of intracanal medication, time between sessions, method of intracanal medication insertion, obturation technique and sealer used, follow-up time, histological findings (quantitative and/or qualitative data on characteristics of inflammatory infiltrate, periodontal ligament thickness, incidence of biological apical seal, dentin, cementum or bone resorption, and presence of microorganisms after endodontic treatment).

Risk of bias assessment

The risk of bias in the studies was evaluated using the SYstematic Review Centre for Laboratory animal Experimentation (SYRCLE) tool [19]. Biases related to selection (sequence generation, baseline characteristics of animals, and allocation concealment), performance (random housing and blinding), detection, attrition, and reporting (random outcome assessment, blinding, incomplete outcome data, and selective reporting), and other sources of bias (contamination, new animals added to groups) were evaluated.

Evidence and statistical analysis

Information from eligible articles was compiled in a database and organized into a Microsoft Office Excel 2016 spreadsheet (Microsoft Corporation, Redmond, WA, USA) for tabulating statistical data. The findings were summarized in a narrative synthesis structured by outcomes of interest. Additionally, the quantitative data from the included studies were summarized, and a meta-analysis was carried out using the Comprehensive Meta-Analysis software (CMA, Biostat, Englewood, NJ, USA) using the random effects model and odds ratio (OR).

RESULTS

Search and study selection.

The searches were conducted in seven databases and the gray literature, resulting in an initial yield of 950 records, of which 466 were excluded as duplicates, 1 was not retrieved, and 483 were screened for titles and abstracts, leaving 24 studies eligible for full-text analysis (Kappa = 0.914). Of these, 6 were excluded after applying the inclusion criteria (Kappa = 0.891) and 18 studies [9, 20–36] were included in this systematic review (Fig. 1).

The reasons for the exclusion of articles in the second phase were: no calcium hydroxide-based intracanal medication was used [37], there was no one-visit treatment group to compare with two-visit treatment [38], the article was published by the same research group with the same population as another study [39], and the aim was regenerative treatment instead of conventional endodontic treatment [40].

Characteristics of the studies

The studies were published between 1995 and 2018, with the majority conducted in Brazil [9, 20–27, 29–36] and one in the United States of America [28]. All studies were performed in dogs, totaling 1365 root canals in the overall sample. Tables 1 and 2 show the extracted data from the included studies.

Table 1
Data extracted from included studies.

Author	Sample (root canals)	Final diameter	Solution	Time between visits	MIC	Insertion of MIC	Endodontic sealer	Obturation technique	Follow-up
Holland et al. [9] 2003 (Brazil)	60	#40	NaOCl 2.5%	07 days (Group II) e 14 days (Group III)	Calcium Hydroxide + Propylene Glycol	Lentulo spiral drill	Sealapex	Lateral condensation technique	180 days
Machado et al. [20] 2009 (Brazil)	36	#60	NaOCl 5.25%	15 days	Calcium Hydroxide + Sterile Distilled Water	Lentulo spiral drill	Endofill	Lateral condensation technique	30 and 90 days
Silveira et al. [21] 2007 (Brazil)	67	#40	NaOCl 2.5%	07 days	Calcium Hydroxide + PMCC + Glycerin	Lentulo spiral drill	Sealer 26	Lateral condensation technique	180 days
Leonardo et al. [22] 2006 (Brazil)	66	#70	NaOCl 5.25%	15 days (Group 2), 30 days (Group 3) and 180 days (Group 4)	Calen PMCC	27 G long needle attached to ML syringe	AH Plus	Lateral condensation technique	180 days
Paula-Silva et al. [23] 2009 (Brazil)	120	#60	NaOCl 2.5%	15 days	Calen	-	AH Plus Jet Mix	Lateral condensation technique	180 days
De Rossi et al. [24] 2005 (Brazil)	82	#70	NaOCl 2.5%	15 days	Calen + chlorhexidine 1%	27 G long needle attached to ML syringe	AH Plus	Lateral condensation technique	30, 75, and 120 days
Paula-Silva et al. [25] 2010 (Brazil)	60	#60	-	15 days	Calen	-	AH Plus JET Mix	Lateral condensation technique	180 days
Tanomaru et al. [26] 2002 (Brazil)	72	#60 and #70	Groups I and II: 5.25% NaOCl and Groups III and IV: 2% chlorhexidine digluconate	15 days	Calen PMCC	27 G long needle attached to ML syringe	Sealapex	Lateral condensation technique	210 days
Leonardo et al. [27] 1995 (Brazil)	40	#70	Group I: 5.25% NaOCl alternating with 3% Hydrogen Peroxide and MIC with Calen PMCC; Group 2: 0.5% NaOCl	07 days	Calen PMCC	27 G long needle attached to ML syringe	Sealapex	Lateral condensation technique	270 days
Katebzadeh et al. [28] 1999 (USA)	72	#45	Saline solution	07 days	Calcium hydroxide (does not describe vehicle details)	Lentulo spiral drill	Roth 811	Lateral condensation technique	180 days

Author	Sample (root canals)	Final diameter	Solution	Time between visits	MIC	Insertion of MIC	Endodontic sealer	Obturation technique	Follow-up
Hidalgo et al. [29] 2016 (Brazil)	96	-	NaOCl 2.5%	15 days	Calen	27 G long needle attached to ML syringe	AH Plus	-	120 (Groups I and II) and 180 days (Groups III and IV)
Cintra, [30] 2008 (Brazil)	110	#40	Saline solution; Natrosol; Sodium hypochlorite 2.5%; 2% Chlorhexidine (gel); 2% Chlorhexidine (fluid)	14 days	Calcium hydroxide + iodoform + saline solution	Lentulo spiral drill	Pulp Canal Sealer - EWT	Lateral condensation technique	270 days
Liévana, [31] 2018 (Brazil)	80	-	NaOCl 2.5%	14 days	Calen	27 G long needle attached to ML syringe	AH Plus	Lateral condensation technique	120 days
Huamán, [32] 2018 (Brazil)	60	Universal Protaper System F5 (#50)	NaOCl 2.5%	14 days	Calen	27 G long needle attached to ML syringe	AH Plus	Lateral condensation technique	120 days
Otoboni Filho [33], 2000 (Brazil)	80	#40	NaOCl 2.5%	07 days (Groups III and IV), 14 days (Groups V and VI)	Calcium Hydroxide + Iodoform + Propylene Glycol	Lentulo spiral drill	Sealapex and Sealer 26	Tagger's hybrid technique	180 days
Silva [34], 2004 (Brazil)	80	#70 (except for mandibular second premolars, in which instruments up to #60 were used).	1% NaOCl (Groups I and II), 2.5% NaOCl (Groups III and IV)	15 days	Calen PMCC	27 G long needle attached to ML syringe	AH Plus	Lateral condensation technique	180 days
César [35], 2003 (Brazil)	60	#70 (lower teeth) and #60 (upper teeth)	NaOCl 5.25%	15 days	Calen PMCC (Group II) and Calapset (Group III)	27 G long needle attached to ML syringe	AH Plus	Lateral condensation technique	180 days
Lopes [36], 2018 (Brazil)	96	Universal Protaper System F5 (#50)	NaOCl 2.5%	15 days	Calen	27 G long needle attached to ML syringe	AH Plus	Lateral condensation technique	120 (Groups I and II) and 180 days (Groups III and IV)

Table 2
Histological characteristics of the studies included in the systematic review.

Author	Histological findings (one-visit)	Histological findings (two-visit)
Holland et al. [9] 2003 (Brazil)	Neoformed cementum in all specimens, with an average thickness of 83 µm. In most cases, the newly formed cementum repaired the areas of resorption and produced closure of the main canal (10 cases). Complete biological closure of all accessory canals was observed in only 4 cases. The average thickness of periodontal ligament was 302 µm. The inflammatory reaction was absent in only two specimens. Gram-negative and Gram-positive microorganisms were present in all but 3 specimens.	**07 days**: Neoformed cementum was observed in all cases, with a mean thickness of 93 µm. The newly formed cementum repaired areas of resorption in almost all cases, and completely sealed the main root canal in 16 specimens. The periodontal ligament was well organized in 05 cases, and there was a partial organization in the other specimens. The average thickness of the apical periodontal ligament was 264 µm. In six cases there were no inflammatory cells. Gram-positive and Gram-negative microorganisms were found in 10 cases in some apical accessory root canals and cementum gaps. **14 days**: Neoformed eosinophilic cementum with a mean thickness of 91 µm was observed in all specimens. This cementum repaired areas of resorption in all but two cases. There was complete closure of the main canal in 14 specimens. The periodontal ligament was well organized in three cases and partially organized in the other specimens. The apical ligament had a mean thickness of 298 µm and showed an inflammatory reaction in seven cases and no inflammation in nine specimens. The Brown and Brenn technique showed Gram-positive microorganisms in four cases and only in some gaps in the apical cementum.
Machado et al. [20] 2009 (Brazil)	**30 days**: All specimens in this subgroup showed intense acute and chronic inflammatory infiltrate, with the presence of neutrophils, macrophages, and plasma cells. **90 days**: Persistence of the inflammatory condition present at 30 days was observed in all specimens. There were areas of cement resorption filled with intense inflammatory infiltrate and cementoclasts and reabsorbing hard tissue.	Neoformation of cementum and/or bone tissue was observed in six roots (three at 30 days and three at 90 days). Areas of resorption associated with neoformation processes were also visualized. Neoformed blood vessels could also be observed. Acute inflammatory infiltrate could be found in two specimens, whereas chronic inflammatory infiltrate occurred in eight specimens.
Silveira et al. [21] 2007 (Brazil)	Success rate of 46% (6 of 13 cases). Histobacteriological analysis revealed that the majority of cases in this group still harbored bacteria on the root canal walls and sometimes within the dentinal tubules. Bacterial cells were also observed in the apical region. Scores: 0 = 31%, 1 = 15%, 2 = 15%, 3 = 39%	Periradicular tissues free of inflammation or with mild inflammation. Areas of root resorption were usually covered by cementum, indicating that these areas had been repaired and resorption had ceased. The vast majority of root canals were free of bacteria. Success rate of 74% (11 of 15 cases). Scores: 0 = 27%, 1 = 47%, 2 = 20%, 3 = 7%
Leonardo et al. [22] 2006 (Brazil)	Inflammatory infiltrate - Absent: 0, Slight: 2, Moderate: 4, Severe: 0 / Periodontal ligament space - Normal: 0, Slightly increased: 3, Moderately increased: 3, Severely increased: 10/ Dentin resorption - Absent: 14, Present: 2/ Cementum resorption - Absent: 4, Present: 12/ Bone resorption - Absent: 0, Present: 16	**15 days**: Inflammatory infiltrate - Absent: 0, Slight: 12, Moderate: 4, Severe: 1/ Periodontal ligament space - Normal: 0, Slightly increased: 4, Moderately increased: 10, Severely increased: 3/ Dentin resorption- Absent: 17, Present: 0/Cementum resorption - Absent: 14, Present: 3 /Bone resorption - Absent: 16, Present: 1. **30 days**: Inflammatory infiltrate- Absent: 0, Slight: 14, Moderate: 2, Severe: 1/ Periodontal ligament space - Normal: 1, Slightly increased: 6, Moderately increased: 8, Severely increased: 2/ Dentin resorption - Absent 17, Present: 0/ Cementum resorption - Absent: 15, Present: 2/ Bone resorption - Absent: 15, Present: 2. **180 days**: Inflammatory infiltrate - Absent: 0, Slight: 13, Moderate: 3, Severe: 0/ Periodontal ligament space - Normal: 0, Slightly increased: 9, Moderately increased: 7, Severely increased: 0/ Dentin resorption - Absent: 16, Present: 0/ Cementum resorption- Absent: 15, Present: 1/ Bone resorption - Absent: 15, Present: 1
Paula-Silva et al. [23] 2009 (Brazil)	Extension of the inflammatory reaction - Absent: 0, Restricted to the apical foramen: 0, Up to half of the apical periodontal ligament: 4 (22.2%), Beyond half of the periodontal ligament: 14 (77.8%) / Tooth resorption - Absent: 3 (16.7%), Cementum resorption – 12 (66.6%), Dentin resorption – 3 (16.7%) / Apical opening sealed with mineralized tissue - Complete sealing: 1 (5.5%), Sealing beyond half: 2 (11.1%), Sealing up to half: 0, Absence of sealing: 15 (83.4%).	Extension of the inflammatory reaction - Absent: 0, Restricted to the apical foramen: 7 (33.3%), Up to half of the apical periodontal ligament: 8 (38.1%), Beyond half of the periodontal ligament: 6 (28,6%) / Tooth resorption - Absent: 9 (42,9%), Cementum resorption – 12 (57.1%), Dentin resorption – 0/ Apical opening sealed with mineralized tissue - Complete sealing: 15 (71.4%), Sealing beyond half: 2 (9.5%), Sealing up to half: 0, Absence of sealing: 4 (19.1%).

Author	Histological findings (one-visit)	Histological findings (two-visit)
De Rossi et al. [24] 2005 (Brazil)	**30, 75 and, 120 days**: Periapical lesion and inflammatory infiltrate composed of neutrophils, histiocytes, xanthomatous histiocytes, plasma cells, and lymphocytes, and marked edema. Surface resorption areas (Howship's lacunae) were evident, similar to those seen on the cementum surface.	**30 and 75 days**: Two different patterns: one mainly showing scarring repair tissue characterized by proliferation of fibroblasts, small vessels, and increased connective tissue, and the other showing an inflammatory infiltrate similar to that of teeth filled in one-visit. **120 days**: Marked reduction in the size of the periapical alterations.
Paula-Silva et al. [25] 2010 (Brazil)	Mean (standard deviation) - Inflammatory infiltrate - Polymorphonuclear: 9.6 (± 6.6); Mononuclear: 59.5 (± 9.1); Fibroblast: 30.9 (± 4.4); Inflammatory activity index – 2.31. Presence of bacteria: 83.3%	Mean (standard deviation) - Inflammatory infiltrate - Polymorphonuclear: 4.3 (± 3.2); Mononuclear: 48.7 (± 9.5); Fibroblast: 46.9 (± 11.2); Inflammatory activity index: 1.23. Presence of bacteria: 42.8%
Tanomaru et al. [26] 2002 (Brazil)	**Group I – 5.25% NaOCl**: No biologic sealing in 16 roots. The surface of cementum was irregular in 15 roots. There was severe inflammatory infiltrate in 12 roots at one or more sites located both near the root apex and distant. The periodontal ligament space was very thick in 12 roots, moderately thickened in 6 roots, and only slightly thickened in 1 root. The alveolar bone showed extensive areas of active resorption and 14 roots had osteoclasts. **Group II - Chlorhexidine**: There was partial apical closure of the root apex in 7 roots and no closure in 11. Diffuse inflammation was mild in 3 specimens, moderate in 11, and severe in 4 roots. The periodontal ligament space was slightly thickened in six roots, moderately thickened in seven, and severely thickened in five roots. The apical alveolar bone had areas of active resorption without medullary spaces and with osteoclasts.	**Group III – 5.25% NaOCl**: There was closure of the root apex in seven roots, partial closure in eight, and no closure in three roots. The periodontal ligament space was moderately thickened in 7 roots, nearly normal in 10, and severely thickened in 1. There were often osteoblasts on the surface of the alveolar bone, and active resorption was seen in only one case. **Group IV – 2% Chlorhexidine digluconate**: There was complete closure of the apex in seven specimens with cemento-mineralized tissue. The apical cementum was regular with newly formed mineralized tissue in previously resorbed areas. The periodontal ligament space was normal or moderately thick in 14 roots and severely thick in 4 roots. There was a severe inflammatory infiltrate in only one specimen. Normal alveolar bone contained osteocytes in its interior and osteoblasts on its surface.
Leonardo et al. [27] 1995 (Brazil)	There was persistence of the lesion in 13 of the 15 specimens analyzed, with virtually no formation of repair cementum in any specimen. The periodontal space width was marked, with group 2 having the greatest periodontal width. The inflammatory reaction was also more severe, with more extensive areas of bone resorption.	The apical root surface was frequently covered with repair cementum. The periodontal space was normal in 3 cases, slightly thickened in 7, and severely thickened in 6. Less intense inflammation was seen near the apical region at the level of the ramifications. Newly formed trabecular bone was frequently present in alveolar bone, surrounded with numerous osteoblasts and occasional osteoclasts. The alveolar process presented wide medullar spaces and a discrete amount of neoformed trabecular bone.
Katebzadeh et al. [28] 1999 (USA)	Overall results: Number of observations: 120, Mean: 1.73, SD: 0.80, Minimum: 1.0, Maximum: 3.0 / Frequency of inflammation scores: 0 = 0%, 1 = 48%, 2 = 30%, 3 = 22%	Overall results: Nº of observations: 105, Mean: 1.38, SD: 0.80, Minimum: 0, Maximum: 3.0 /Frequency of inflammation scores: 0 = 17%, 1 = 32%, 2 = 48%, 3 = 3%

Author	Histological findings (one-visit)	Histological findings (two-visit)
Hidalgo et al. [29] 2016 (Brazil)	**120 days**: Periodontal ligament: 0% with normal ligament, 20% mildly increased, 70% moderately increased and 10% with severely increased ligament. Inflammatory infiltrate: 40% absent, 40% mild, 15% moderate, and 5% severe. Bone and cement resorption: 10% of groups with resorption. Presence of biological sealing: 95% absent sealing, 5% partial sealing, and 0% total sealing. **180 days**: Periodontal ligament: 5% of specimens with normal ligament, 35% with mildly enlarged ligament, 55% moderately increased ligament, and 5% with severely increased ligament. Inflammatory infiltrate: 34% absent, 61% mild, and 5% moderate. Bone and cement resorption: 66% with no resorption and 34% with resorption. Presence of biological sealing: 89% no sealing, 11% partial sealing, and 0% total apical biological sealing.	**120 days**: Periodontal ligament: The ligament was normal in 10% of the specimens, mildly enlarged in 60%, moderately enlarged in 30%, and 0% severely enlarged. Inflammatory infiltrate: 50% of specimens with no infiltrate and 50% with mild. Bone and cement resorption: 100% of the cases did not show resorption. Presence of biological sealing: 50% no sealing, 17% partial sealing, and 33% total sealing. **180 days**: Periodontal ligament: normal in 35% of specimens, mildly increased in 20%, moderately increased in 20%, and severely increased in 25%. Inflammatory infiltrate: 40% no infiltrate, 55% mild, and 5% moderate. Bone and cement resorption: 85% with no resorption and 15% with resorption. Presence of biological sealing: 52% no sealing, 29% with partial sealing, and 19% with total sealing.
Cintra, [30] 2008 (Brazil)	**Group I – Saline solution**: 100% of failure, determined by moderate to severe acute inflammatory infiltrate and/or chronic inflammatory infiltrate associated with the presence of microorganisms in all specimens. **Group II – Natrosol**: 1 specimen without microscopic evidence of bacteria, but isolated focus of acute inflammation was present. 100% of failure was considered in the group. **Group III – 2.5% Sodium hypochlorite**: 44% of endodontic treatment success, determined by the presence of chronic inflammatory infiltrate associated with extrusion of filling material. **Group IV – 2% chlorhexidine gel**: 44% success rate determined by the absence of acute inflammatory infiltrate and chronic inflammation associated with microorganisms. **Group V – 2% chlorhexidine fluid**: 27.3% of success determined by the absence of acute inflammatory infiltrate and chronic inflammation associated with the presence of microorganisms.	**Group VI – Saline solution**: 37.5% of success determined by the absence of acute inflammatory infiltrate and chronic inflammation associated with the presence of microorganisms. **Group VII – Natrosol**: 30% of success determined by the absence of acute inflammatory infiltrate and chronic inflammation associated with the presence of microorganisms. **Group VIII – Sodium hypochlorite 2.5%**: 54.6% of success. **GROUP IX – 2% Chlorhexidine gel**: 60% of success rate determined by the absence of acute inflammatory infiltrate and chronic inflammation associated with the presence of microorganisms. **Group X – 2% Chlorhexidine fluid**: 50% of success rate, determined by the absence of acute inflammatory infiltrate and microorganisms.
Liévana, [31] 2018 (Brazil)	Inflammatory infiltrate - Absent: 0 (0%), Mild: 2 (16.66%), Moderate: 3 (25%), Severe: 7 (58.33%). Periodontal ligament thickness - Normal: 0 (0%), Slightly Increased: 1 (8.33%), Moderately Increased: 5 (41.66%), Severely Increased: 6 (50%). Reabsorption of mineralized tissues - Absent: 2 (16%), Present: 10 (83.33%)	**Group III- Calen**: Inflammatory infiltrate - Absent: 8 (72.72%), Mild: 2 (18.18%), Moderate: 1 (9.09%), Severe: 0 (0%). Periodontal ligament thickness - Normal: 1 (9.09%), Slightly Increased: 5 (45.45%), Moderately Increased: 5 (45.45%), Severely Increased: 0 (0%). Reabsorption of mineralized tissues - Absent: 6 (54.54%), Present: 5 (45.45%)
Huamán, [32] 2018 (Brazil)	**Group III**: Persistence of periapical lesions and absence of repair. Inflammatory infiltrate - Absent: 0 (0%), Mild: 2 (16.7%), Moderate: 4 (33.3%), Severe: 6 (50%). Thickness of the periodontal ligament - Normal: 0 (0%), Slightly Increased: 0 (0%), Moderately Increased: 2 (16.6%), Severely Increased: 10 (83.4%); Cement resorption - Absent: 0 (0%), Present: 12 (100%); Biological Apical Sealing - Complete: 0 (0%), Partial: 0 (0%), Missing: 12 (100%); Bone resorption - Absent: 1 (8.3%), Present: 11 (91.7%); Mean thickness of periodontal ligament (mm): 1.26 (SD 0.45)	**Group II**: Inflammatory infiltrate - Absent: 0 (0%), Mild: 11 (84.6%), Moderate: 2 (15.4%), Severe: 0 (0%); Thickness of the periodontal ligament - Normal: 0 (0%), Slightly Increased: 3 (23.1%), Moderately Increased: 8 (61.53%), Severely Increased: 2 (15.4%); Cement resorption - Absent: 13 (100%), Present: 0 (0%); Apical Sealing - Complete: 4 (30.8%), Partial: 7 (53.84%), Absent: 2 (15.4%); Bone resorption - Absent: 13 (100%), Present: 0 (0%); Mean thickness of the periodontal ligament (mm): 0.31 (SD = 0.19)

Author	Histological findings (one-visit)	Histological findings (two-visit)
Otoboni Filho [33], 2000 (Brazil)	**Group I (Sealapex)** - Medium: Newly formed cementum - Thickness: 1.31; Extension: 1.12; Biological sealing accessory foramina: 1.81; Biological sealing of the main foramina: 1.62; Reabsorption: 1.37; Bone tissue resorption: 1.62; Presence of bacteria: 3.43; Acute inflammation: Internal 1,12; External: 1.06; Chronic inflammation: Internal: 2.87; External: 2.68; Periodontal Ligament: Thickness: 2.37; Organization: 2.12; Giant Cells: 1.43 – General average: 1.81. **Group II (Sealer 26) -** Average: Neoformed cement: Thickness: 1.62; Extension: 1.63; Biological sealing accessory foramina: 2.56; Biological sealing of the main foramina: 2.56; Reabsorption: 2.18; Bone tissue resorption: 1.93; Presence of bacteria: 3.62; Acute inflammation: Internal 1.87; External: 1.68; Chronic inflammation: Internal: 2.62; External: 2.62; Periodontal Ligament: Thickness: 2.75; Organization: 2.50; Giant Cells: 1.50 – Overall Average: 2.16	**Group III (07 days- Sealapex) -** Average: Neoformed cement: Thickness: 1.06; Extension: 1.12; Biological sealing accessory foramina: 1.56; Biological sealing of the main foramina: 1.00; Reabsorption: 1.12; Bone tissue resorption: 1.43; Presence of bacteria: 2.87; Acute inflammation: Internal 1.00; External: 1.00; Chronic inflammation: Internal: 1.81; External: 1.75; Periodontal Ligament: Thickness: 2.18; Organization: 1.93; Giant Cells: 1.25 – General average: 1.5. **Group IV (07 days – Sealer 26) -** Average: Neoformed cement: Thickness: 1.50; Extension: 1.12; Biological sealing accessory foramina: 2.18; Biological sealing of the main foramina: 2.18; Reabsorption: 1.18; Bone tissue resorption: 1.56; Presence of bacteria: 2.50; Acute inflammation: Internal 1.06; External: 1.06; Chronic inflammation: Internal: 2.37; External: 2.56; Periodontal Ligament: Thickness: 2.62; Organization: 2.31; Giant Cells: 1.31 – General average: 1.80 **Group V: (14 days – Sealapex) - Average**: Neoformed cement: Thickness: 1.18; Extension: 1.12; Biological sealing accessory foramina: 1.56; Biological sealing of the main foramina: 1.12; Reabsorption: 1.12; Bone tissue resorption: 1.50; Presence of bacteria: 1.75; Acute inflammation: Internal 1.00; External: 1.00; Chronic inflammation: Internal: 1.75; External: 1.56; Periodontal Ligament: Thickness: 2.50; Organization: 1.93; Giant Cells: 1.06 – General average: 1.43. **Group VI: (14 days – Sealer 26) - Average**: Neoformed cement: Thickness: 1.18; Extension: 1.31; Biological sealing accessory foramina: 2.12; Biological sealing of the main foramina: 2.00; Reabsorption: 1.25; Bone tissue resorption: 1.31; Presence of bacteria: 1.93; Acute inflammation: Internal 1.00; External: 1.00; Chronic inflammation: Internal: 2.18; External: 1.93; Periodontal Ligament: Thickness: 2.75; Organization: 1.93; Giant Cells: 1.25 – Overall Average: 1.64
Silva [34], 2004 (Brazil)	**Group I**: The apical and periapical tissues presented severe inflammatory reaction in most of the analyzed cases. In all 20 cases there was apical inflammatory infiltrate in an extension greater than half of the apical periodontal space. Apical periodontal space was greater than 1.06 mm, corresponding to score 4. Large active areas of bone resorption were found in this group. **Group III**: Inflammatory cells were observed near the apical foramen with diffuse distribution throughout the periapical periodontal space. The level of periapical inflammatory infiltrate had score 4 in almost all cases in this group (n = 18) (located above half of the apical periodontal space). Apical root resorption was present in all cases in this group.	**Group II**: The apical and periapical inflammatory reaction was less intense than that for group I. As for the extent of the apical inflammatory infiltrate, the vast majority of cases (n = 11) were arranged with a score of 2 (restricted to the apical foramen). Apical root resorption was not observed in the vast majority of specimens, and in most cases, there was also bone repair. **Group IV**: The inflammatory reaction in the apical and periapical region was mild. The existing inflammatory cells were concentrated in the region of the apical foramen in most cases. Apical root resorption was present up to half of the cementum thickness in 7 cases and absent in the remaining cases.
César [35], 2003 (Brazil)	**Group I**: Biological Apical Sealing (Average: 1.05) – Absent: 19, Partial: 1, Complete: 0; Inflammatory Infiltrate (Average: 3.75) – Absent: 0, Mild: 0, Moderate: 5, Severe: 15; Thickness of the periodontal ligament (Average: 3.80) – Normal: 0, Mild: 0, Moderate: 4, Severe or Extensive: 16; Cement Resorption (Average: 2.00) – Absent: 0, Present: 20; Dentin Resorption (Average: 1.00) – Absent: 20, Present: 0; Bone Resorption (Average: 2.00) - Absent: 0, Present: 0	**Group II (Calen PMCC)**: Biological Apical Sealing (Average: 2.10) – Absent: 4, Partial: 10, Complete: 16; Inflammatory Infiltrate (Average: 2.55) – Absent: 0, Mild: 12, Moderate: 5, Severe: 3; Thickness of the periodontal ligament (Average: 2.60) – Normal: 0, Mild: 10, Moderate: 8, Severe or Extensive: 2; Cement Resorption (Average: 1.10) – Absent: 18, Present: 2; Dentin Resorption (Average: 1.00) – Absent: 20, Present: 0; Bone Resorption (Average: 1.10) – Absent: 18, Present: 2. **Group III (Calapset)**: Biological Apical Sealing (Average: 1.80) – Absent: 6, Partial: 12, Complete: 2; Inflammatory Infiltrate (Average: 3.25) – Absent: 0, Mild: 3, Moderate: 9, Severe: 8; Thickness of the periodontal ligament (Average: 3.35) – Normal: 0, Mild: 0, Moderate: 13, Severe or Extensive: 7; Cement Resorption (Average: 1.40) – Absent: 12, Present: 8; Dentin Resorption (Average: 1.00) – Absent: 20, Present: 0; Bone Resorption (Average: 1.30) - Absent: 14, Present: 6
Lopes [36], 2018 (Brazil)	**Group I (120 days)**: There were some areas of unrepaired resorption in the apical cementum. The periodontal ligament was moderately enlarged, with few collagen fibers. The inflammatory infiltrate was moderate and predominantly mononuclear. Few collagen fibers were observed, and the alveolar bone was often bare. Average lesion area: 3.29 (± 1.07); **Group III (180 days)**: same pattern, no significant differences; Average lesion area: 120 = 3.29 (± 1.07); 180 = 3.25 (± 2.97)	**Group II (120 days)**: Showed areas of repaired resorption in most specimens. The periodontal ligament was slightly enlarged, with advanced repair and the presence of collagen fibers. There was a scarce and diffuse presence of inflammatory cells. Average lesion area: 120 = 1.18 (± 0.41). **Group IV (180 days)**: same pattern, without significant differences. Periapical lesions were smaller compared to lesions in the groups treated with aPDT and single session. Average lesion area: 180 = 1.41 (± 0.47)

Risk of bias in the studies

Figure 2 summarizes the assessment of the risk of bias of each study.

Characteristics of included studies

Of the included studies, 14 [9, 20–28, 30, 33–35] used manual stainless-steel instruments for biomechanical canal preparation, and 4 [29, 31, 32, 36] used mechanical nickel-titanium instruments. Sodium hypochlorite was the most used solution: 11 studies [9, 21, 23, 24, 29–34, 36] used a concentration of 2.5%, 5 studies [20, 22, 26, 27, 35] of 5.25%, 1 study [34] of 1%, and 1 study of 0.5% [27].

All studies used calcium hydroxide-based intracanal medications, as this was one of the inclusion criteria, but the vehicles and commercial forms varied. The most used commercial forms were Calen [23, 25, 29, 31, 32, 36] and Calen PMCC [22, 26, 27, 34, 35]. Other studies evaluated the association of calcium hydroxide with propylene glycol [9], sterile distilled water [20], camphorated paramonochlorophenol with glycerin [21], 1% chlorhexidine [24], iodophor associated with saline [30], and iodophor [33]. One study used Calapset [35], and another did not describe the vehicle associated with calcium hydroxide [28]. Ten studies used a long 27-gauge needle coupled to a ML endodontic syringe as the method of medication insertion [22, 24, 26, 27, 29, 31, 32, 34–36], six studies used a Lentulo spiral [9, 20 21, 28, 30, 33], and two studies did not report the method of medication insertion [23, 25]. Ten studies used the medication for 15 days [20, 22–26, 29, 34–36], 5 studies used it for 14 days [9, 30–33], and 5 other studies used it for 7 days [9, 21, 27, 28, 33].

Eight studies used AH Plus as the sealing cement [22, 24, 29, 31, 32, 34–36], 4 studies used Sealapex [9, 26, 27, 33], and 2 used AH Plus Jet Mix [23, 25]. Other sealers used in the studies were Endofill1 [9], Sealer 26 [21, 33], Pulp Canal Sealer EWT [30], and Roth 811 [28]. In the vast majority of studies, lateral condensation was the most commonly used obturation technique [9, 20–28, 30–32, 34–36]. In contrast, only one study used Tagger's hybrid technique [33], and one did not report the technique [29].

Follow-up times were 180 days in 11 studies [9, 21–23, 25, 28, 29, 33–36], 120 days in 5 studies [24, 29, 31, 32, 36], and 270 in 2 studies [27, 30]. Other follow-up times were 30 [20, 24], 75 [24], 90 [20], and 210 days [26].

META-ANALYSES

For the meta-analyses, variables were categorized for better interpretation of results. The data were combined to avoid discrepancies and dichotomized into success and failure or absence and presence. Studies that used sodium hypochlorite as the irrigating solution (regardless of concentration) and intracanal medication for two weeks (14 or 15 days) were selected, regardless of the follow-up time. Of the 18 studies, seven [20, 21, 24, 25, 27, 28, 36] were not included in the meta-analysis. The studies by Silveira *et al.* [21] and Katebzadeh *et al.* [28] evaluated the histopathological findings using scores that generalized inflammatory infiltrate, thickening of the periodontal ligament, and destruction of the bone cortices and, therefore, could not be combined with other studies. The other five studies [20, 24, 25, 27, 36] were not included because there was no quantitative data or insufficient information.

Intensity of inflammatory infiltrate

The absence of inflammation or the presence of a mild inflammatory infiltrate was considered a success, while the presence of a moderate or intense inflammatory infiltrate was considered a failure [28]. Five studies [22, 29, 31, 32, 35] with a total of 203 root canals were evaluated in this category. The meta-analysis yielded a data effect size of 5.5% (95% CI: 0.020–0.148; $p < 0.001$). The heterogeneity test yielded values for $I^2 = 0.000$, Tau = 0.000, and Q-value = 1.960 ($p = 0.743$). The forest plot for these subgroups of studies is shown in Fig. 3A.

Periodontal ligament thickness

Six studies were included in this analysis [22, 29–32, 35], with a total sample of 257 root canals. No or slight thickening of the periodontal ligament space were considered successful outcomes, while moderate or severe thickening were considered unsuccessful outcomes [28]. When the data were combined in a meta-analysis, there was a statistically significant difference in favor of the two-visits treatment, with an effect size of 25.6% (95% CI: 0.134–0.487; $p < 0.001$). The test for heterogeneity yielded values for $I^2 = 0.000$, Tau = 0.000, and Q-value = 3.603 ($p = 0.608$). The forest plot of these subgroups of studies is shown in Fig. 3B.

Dentin resorption

Two studies [22, 23] were combined in this analysis, totaling 72 root canals. The presence of dentin resorption in histological sections was considered an unsuccessful outcome and no active areas of dentin resorption was considered as a successful outcome. The meta-analysis resulted in an effect size of 13% (95% CI: 0.015–1.141; $p = 0.066$). The test for heterogeneity indicated values for $I^2 = 0.000$, Tau = 0.000, and Q-value = 0.046 ($p = 0.830$). The forest plot of these subgroups of studies is shown in Fig. 3C.

Cement resorption

Seven studies [22, 23, 30, 32–36] were included in this analysis, with a total sample of 312 root canals. The presence of repair or no resorption areas in the cement was considered a successful outcome and the presence of active areas of cement resorption was categorized as an unsuccessful outcome. The meta-analysis indicated a statistically significant result in favor of the two-visits treatment, with an effect size of 7.1% (95% CI: 0.015–0.325; p = 0.001). The test for heterogeneity resulted in values for I^2= 75.231, Tau = 1.701, and Q-value = 24.224 (p < 0.0001). The forest plot of these subgroups of studies is shown in Fig. 3D.

Bone resorption

Six studies [22, 30, 32–35] were included in this analysis, with a total sample of 273 root canals. The meta-analysis yielded a statistically significant result in favor of a two-sessions endodontic therapy, with an effect size of 1.4% (95% CI: 0.002–0.130; p < 0.001). The heterogeneity test showed values for I^2=71.138, Tau = 2.312, and Q-value = 17.324 (p = 0.004). A forest plot graph comparing groups on bone resorption is presented in Fig. 4E.

Mineralized tissue resorption

The studies included in this analysis considered bone and cementum resorption as mineralized tissue resorption, without separating the two structures in the analysis. Two studies [29, 31] were included in this meta-analysis, totaling 119 root canals. The presence of active resorption areas was categorized as unsuccessful treatment and the presence of repair or absence of active resorption areas as successful treatment. The meta-analysis showed an effect size of 42.8% (95% CI: 0.110–1.671; p = 0.222). The heterogeneity test showed values for I^2= 38.280, Tau = 0.637, and Q-value = 1.620 (p = 0.203). No statistically significant difference was observed between the groups (Fig. 4F).

Apical biological sealing

Eight studies [9, 23, 26, 29, 32–35] were included in this analysis, with a sample size of 424 root canals. Apical biological sealing was categorized as incomplete or unsuccessful (no or partial sealing) and complete or successful. A statistically significant difference was observed in favor of the two-visit treatment, with an effect size of 13.1% (95% CI: 0.055–0.314; p < 0.001). The heterogeneity test showed values for I^2=21.712, Tau = 0.581, and Q-value = 8.941 (p = 0.257). Figure 4G shows the forest plot of the apical biological sealing data.

Presence of microorganisms

The presence of microorganisms in histological sections was considered treatment failure, while their absence was considered treatment success. Three studies [9, 30, 33] were included in this analysis, with a total sample of 116 root canals. The meta-analysis showed a significant difference between the groups, with a higher chance of success for the two-visit treatment (Fig. 4H). The effect size was 10.3% (95% CI: 0.014–0.747; p = 0.025). The heterogeneity values were I^2= 70.033, Tau = 1.441, and Q-value = 6.674 (p = 0.036).

DISCUSSION

The endodontic treatment of teeth with apical periodontitis can be performed in one or multiple visits [41–43], with no consensus in the literature. Systematic reviews of clinical studies comparing the outcomes of teeth treated in one or two visits conclude no difference regarding success rates between the two therapeutic modalities. However, the quality of the available scientific evidence is moderate [13, 44]. It is important to note that these reviews are based on studies that evaluated success based on clinical and radiographic signs, especially periapical and panoramic radiographs. However, these examinations often underestimate the diagnosis of apical periodontitis [45, 46]. Moreover, there is often no control over certain factors, such as the type of restoration performed, the time interval between endodontic treatment and tooth restoration, and the material and technique used for obturation.

Histopathological evaluation is essential for the definitive diagnosis of a periapical condition because conditions with similar clinical and imaging signs may differ histopathologically [23]. However, few consistent reports in the literature correlate the clinical condition with the histopathological finding of persistent periapical pathology [43]. In a previous study by Barthel, Zimmer & Trope [16], a relationship between histological and radiographic findings of endodontically treated cadaver teeth was found. Still, in over 30% of cases with histological signs of inflammation, no periapical radiolucency was seen on the radiograph. Although histological examinations provide the final and definitive diagnosis, this type of examination cannot be routinely performed in clinical practice to diagnose periapical pathologies.

The presence of microorganisms in the root canal system is the main cause of inflammation and persistence of apical periodontitis [2, 47]. The meta-analysis conducted in the present study showed that a two-visit treatment has a higher chance of eliminating microorganisms, with an OR of 10.3%; in other words, there is an 89.7% probability of finding microorganisms more frequently after one-visit endodontic treatment. Three studies [9, 30, 33] were included in this analysis, which showed high heterogeneity but used similar final diameters (#40) in biomechanical preparation, sodium hypochlorite concentration (2.5%), and days between sessions (14 days). Heterogeneity may be due to differences in observation methods and follow-up times. Although our results are based on animal studies, they agree with Vera *et al.* [48], who observed better microbiological status in patients treated in a two-visit protocol than in those treated in a single visit.

The exit of microorganisms and their byproducts inside the root canal system into periapical tissues leads to periradicular lesions, which histologically show numerous inflammatory cells, such as polymorphonuclear neutrophils, lymphocytes, macrophages, and mast cells [49]. Endodontic treatment in teeth with apical periodontitis should reduce the existing contamination to allow the regression of the inflammatory process and restore the health of periapical tissues. Although the absence of inflammation is one of the histological criteria for the success of endodontic treatment [22, 28], radiographic examinations cannot determine the presence of inflammation. In a previous study [50], 44% of endodontically treated teeth without radiographic changes in the periapical region showed inflammation. The present meta-analysis showed favorable results for two-visit endodontic treatments, with a higher chance of no or mild inflammatory infiltrate in periapical tissues. An OR of 5.5% was noted, meaning there is a 94.5% chance of observing a more intense inflammatory infiltrate in teeth treated without using calcium hydroxide-based intracanal medication. The use of calcium hydroxide for intracanal medication reduces the level of pro-inflammatory cytokines in periapical tissues [51], which may have led to better histological responses in cases treated in more than one appointment.

Bacterial components, such as lipopolysaccharides (LPS), induce the production of pro-inflammatory mediators and cytokines that have the potential to stimulate resorption and inhibit bone repair [25, 52]. The present meta-analysis showed significantly better outcomes for mineralized tissue resorption when endodontic therapy was conducted in two visits. As known, biomechanical preparation of the root canal reduces endodontic bacteria. Still, it does not affect LPS [53, 54] and bacterial colonies in intricate areas to which instruments and irrigating solutions cannot access [43]. Calcium hydroxide can neutralize endotoxins in root canals [55]. Moreover, due to its low solubility, calcium hydroxide dissociates into calcium and hydroxyl ions, promoting an alkaline pH in the periapical region [56, 57], which can neutralize the acidic products of clastic cells and stimulate mineralization [9, 39, 58].

Thickening of the periodontal ligament space is one of the pathognomonic signs of apical periodontitis [28]. This meta-analysis demonstrated a 74.4% higher probability of observing an increased thickness of the periodontal ligament after treatment in two visits. The analysis revealed that teeth treated over multiple sessions were more likely to exhibit no or mild thickening of the periodontal ligament space. This could be attributed to the biological and antimicrobial effects of calcium hydroxide, as it reduces microbial load and inflammation in the periapical tissues and facilitates the restoration of physiological conditions [8, 57].

Another histological sign of repair is the invasion of the periodontal ligament into the canal, sometimes associated with the deposition of cementum-like tissue on the canal walls, referred to as biological apical sealing [9, 59]. This meta-analysis indicated that the probability of complete biological sealing was higher in teeth treated in more than one visit, being 86.9% more probable to exhibit the absence of a fully formed biological apical seal after treatment in a single visit. This may be related to the increase in extracellular calcium levels from the calcium hydroxide, which can lead to cementoblastic differentiation of the ligament cells [60]. The release of calcium ions from calcium hydroxide stimulates fibronectin synthesis in dental pulp cells. Fibronectin induces the differentiation of mesenchymal cells into mineralized tissue-forming cells [61].

Although clinical studies are the gold standard for assessing therapeutic interventions, animal application tests, as chosen for this systematic review, are crucial research methodologies for assessing histological repair characteristics after diverse therapeutic interventions. Ethical and legal considerations often limit the use of human samples for such investigations. Animal studies offer advantages, such as better control of the study population and the possibility to perform histological analysis. A meta-analysis of animal studies provides a more reliable summary of evidence and helps avoid unnecessary duplication of research. Moreover, since animal studies are usually more exploratory than clinical trials, meta-analyses of animal data have greater potential to uncover sources of heterogeneity [62].

One limitation of this study was the high risk of bias on several items of the assessment tool. In most studies, a high risk of bias or inconclusive data was observed in selection, performance, detection, attrition, and reporting. The different follow-up times, calcium hydroxide vehicles, medication insertion technique, obturation technique, and sealers used may have influenced heterogeneity. Well-designed studies with standardized methodologies conducted in various centers are needed to improve the transparency and clinical applicability of the results.

CONCLUSION

The results of the meta-analyses showed better biological responses for two-visit endodontic treatment in animals with induced apical periodontitis. Calcium hydroxide-based intracanal medication used between sessions resulted in more teeth with mild or no inflammatory infiltrate, mineralized tissue resorption, periodontal ligament thickening, the presence of microorganisms, and complete biological apical sealing.

Declarations

Author contributions

Marco Antonio Hungaro Duarte and Raimundo Sales de Oliveira Neto contributed to the conceptualization. Raimundo Sales de Oliveira Neto, Thais de Moraes Souza, Heitor Marques Honório and Marco Antonio Hungaro Duarte contributed to the methodology. Raimundo Sales de Oliveira Neto, Marco Antonio Hungaro Duarte, Stefani Jovedi Rosa and Thais de Moraes Souza contributed to the investigation. Murilo Priori Alcalde, Rodrigo Ricci Vivan, and Marco Antonio Hungaro Duarte contributed to the formal analysis. Raimundo Sales de Oliveira Neto and Stefani Jovedi Rosa contributed to data curation. Raimundo Sales de Oliveira Neto, Thais de Moraes Souza, and Marco Antonio Hungaro Duarte wrote and prepared the original draft. Rodrigo Ricci Vivan, Marco Antonio Hungaro Duarte, and Murilo Priori Alcalde contributed to the review and editing of the study report. Marco Antonio Hungaro Duarte and Heitor Marques Honório contributed to supervision. Marco Antonio Hungaro Duarte and Heitor Marques Honório contributed to project administration. Marco Antonio Hungaro Duarte contributed to funding acquisition.

Conflict of Interest The authors declare that they have no conflict of interest.

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References

1. Kakehashi S, Stanley HR, Fitzgerald RJ (1965) The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 20:340–349. https://doi.org/10.1016/0030-4220(65)90166-0
2. Nair PNR (2006) On the causes of persistent apical periodontitis: a review. Int Endod J. 39:249–281. https://doi.org/10.1111/j.1365-2591.2006.01099.x
3. Márton IJ, Kiss C (2000) Protective and destructive immune reactions in apical periodontitis. Oral Microbiol Immunol. 15:139–150. https://doi.org/10.1034/j.1399-302x.2000.150301.x
4. Siqueira JF Jr, Rôças IN (2008) Clinical Implications and Microbiology of Bacterial Persistence after Treatment Procedures. J Endod. 34:1291-1301.e3. https://doi.org/10.1016/j.joen.2008.07.028
5. Byström A, Happonen R, Sjögren U, Sundqvist G (1987) Healing of periapical lesions of pulpless teeth after endodontic treatment with controlled asepsis. Endod Dent Traumatol. 3:58–63. https://doi.org/10.1111/j.1600-9657.1987.tb00543.x
6. Sjögren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J. 30:297–306. https://doi.org/10.1046/j.1365-2591.1997.00092.x
7. Siqueira JF Jr, Magalhães KM, Rôças IN (2007) Bacterial Reduction in Infected Root Canals Treated With 2.5% NaOCl as an Irrigant and Calcium Hydroxide/Camphorated Paramonochlorophenol Paste as an Intracanal Dressing. J Endod. 33:667–672. https://doi.org/10.1016/j.joen.2007.01.004
8. Estrela C, Bammann LL, Pimenta FC, Pécora JD (2001) Control of microorganisms in vitro by calcium hydroxide pastes. Int Endod J. 34:341–345. https://doi.org/10.1046/j.1365-2591.2001.00368.x
9. Holland R, Otobonifilho J, Desouza V, et al (2003) A Comparison of One Versus Two Appointment Endodontic Therapy in Dogs' Teeth with Apical Periodontitis. J Endod. 29:121–124. https://doi.org/10.1097/00004770-200302000-00009
10. Siqueira JF Jr, Paiva SSM, Rôças IN (2007) Reduction in the Cultivable Bacterial Populations in Infected Root Canals by a Chlorhexidine-based Antimicrobial Protocol. J Endod. 33:541–547. https://doi.org/10.1016/j.joen.2007.01.008
11. Zargar N, Marashi MA, Ashraf H, et al (2019) Identification of microorganisms in persistent/secondary endodontic infections with respect to clinical and radiographic findings: bacterial culture and molecular detection. Iran J Microbiol. https://doi.org/10.18502/ijm.v11i2.1073
12. Trope M, Delano EO, Ørstavik D (1999) Endodontic treatment of teeth with apical periodontitis: Single vs. Multivisit treatment. J Endod. 25:345–350. https://doi.org/10.1016/s0099-2399(06)81169-6
13. Su Y, Wang C, Ye L (2011) Healing Rate and Post-obturation Pain of Single- versus Multiple-visit Endodontic Treatment for Infected Root Canals: A Systematic Review. J Endod. 37:125–132. https://doi.org/10.1016/j.joen.2010.09.005
14. Sathorn C, Parashos P, Messer HH (2005) Effectiveness of single-versus multiple-visit endodontic treatment of teeth with apical periodontitis: a systematic review and meta-analysis. Int Endod J. 38:347–355. https://doi.org/10.1111/j.1365-2591.2005.00955.x

15. Manfredi M, Figini L, Gagliani M, Lodi G (2016) Single versus multiple visits for endodontic treatment of permanent teeth. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.cd005296.pub3
16. Barthel C, Zimmer S, Trope M (2004) Relationship of Radiologic and Histologic Signs of Inflammation in Human Root-filled Teeth. J Endod. 30:75–79. https://doi.org/10.1097/00004770-200402000-00003
17. Segura-Egea JJ, Martín-González J, Castellanos-Cosano L (2015) Endodontic medicine: connections between apical periodontitis and systemic diseases. Int Endod J. 48:933–951. https://doi.org/10.1111/iej.12507
18. Page MJ, McKenzie JE, Bossuyt PM, et al (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 372:n71. https://doi.org/10.1136/bmj.n71
19. Hooijmans CR, Rovers MM, de Vries RB, et al (2014) SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 26;14:43. https://doi.org/10.1186/1471-2288-14-43
20. Machado, M. E. de L., Gomes, C. C., Mantesso, A., & Souza, A. D. S. (2009). Avaliação da reparação pós-tratamento endodôntico de dentes de cães em sessão única ou empregando curativos de demora. Rev Assoc Paul Cir Dent. 2009 63(2), 98-102.
21. Silveira AMV, Lopes HP, Siqueira Jr JF, et al (2007) Periradicular repair after two-visit endodontic treatment using two different intracanal medications compared to single-visit endodontic treatment. Braz Dent J. 18:299–304. https://doi.org/10.1590/s0103-64402007000400005
22. Leonardo MR, Hernandez MEFT, Silva LAB, Tanomaru-Filho M (2006) Effect of a calcium hydroxide-based root canal dressing on periapical repair in dogs: a histological study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 102:680–685. https://doi.org/10.1016/j.tripleo.2006.03.021
23. de Paula-Silva FWG, Júnior MS, Leonardo MR, et al (2009) Cone-beam computerized tomographic, radiographic, and histologic evaluation of periapical repair in dogs' post-endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 108:796–805. https://doi.org/10.1016/j.tripleo.2009.06.016
24. De Rossi A, Silva LAB, Leonardo MR, et al (2005) Effect of rotary or manual instrumentation, with or without a calcium hydroxide/1% chlorhexidine intracanal dressing, on the healing of experimentally induced chronic periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 99:628–636. https://doi.org/10.1016/j.tripleo.2004.07.018
25. Paula-Silva FWG, da Silva LAB, Kapila YL (2010) Matrix Metalloproteinase Expression in Teeth with Apical Periodontitis Is Differentially Modulated by the Modality of Root Canal Treatment. J Endod. 36:231–237. https://doi.org/10.1016/j.joen.2009.10.030
26. Tanomarufilho M, Leonardo M, Bezerradasilva L (2002) Effect of Irrigating Solution and Calcium Hydroxide Root Canal Dressing on the Repair of Apical and Periapical Tissues of Teeth with Periapical Lesion. J Endod. 28:295–299. https://doi.org/10.1097/00004770-200204000-00009
27. Leonardo MR, Almeida WA, Bezerra da Silva LA, Utrilla LS (1995) Histopathological observations of periapical repair in teeth with radiolucent areas submitted to two different methods of root canal treatment. J Endod. 21:137–141. https://doi.org/10.1016/s0099-2399(06)80439-5
28. Katebzadeh N, Hupp J, Trope M (1999) Histological periapical repair after obturation of infected root canals in dogs. J Endod. 25:364–368. https://doi.org/10.1016/s0099-2399(06)81173-8
29. Hidalgo LR da C, da Silva LAB, Nelson-Filho P, et al (2016) Comparison between one-session root canal treatment with aPDT and two-session treatment with calcium hydroxide-based antibacterial dressing, in dog's teeth with apical periodontitis. Lasers Med Sci. 31:1481–1491. https://doi.org/10.1007/s10103-016-2014-8
30. Cintra LTA (2008) Análise histológica e radiográfica da influência de substâncias químicas auxiliares e medicação intracanal no processo de reparo periapical em dentes de cães. Dissertation, Universidade Estadual de Campinas.
31. Liévana FS (2018) Efeito do Curativo de Demora com EGCG, Derivada do Chá Verde, na Lesão Periapical em Cães. Dissertation, Universidade de São Paulo.
32. Huamán SAD (2018) Eficácia de um novo protocolo de tratamento endodôntico em sessão única. Estudo radiográfico e histopatológico em dentes de cães com lesões periapicais induzidas. Dissertation, Universidade de São Paulo.
33. Otoboni Filho JA (2000) Processo de reparo de dentes de cães com lesão periapical após tratamento endodôntico em uma ou duas sessões. Influência do tempo de curativo de demora e do tipo de material obturador. Dissertation, Universidade Estadual Paulista.
34. Silva RFd (2004) Influência do curativo de demora à base de hidróxido de cálcio na reparação dos tecidos apicais e periapicais de dentes sem vitalidade pulpar com ou sem lesão periapical visível radiograficamente: estudo histopatológico em dentes de cães. Dissertation, Universidade Estadual Paulista.
35. César CAS (2003) Efeito do curativo de demora à base de hidróxido de cálcio na reparação apical e periapical, pós-tratamento de canais radiculares de dentes de cães com necrose pulpar e reação periapical crônica induzida. Análise histopatológica. Dissertation, Universidade Estadual Paulista.

36. Lopes ZMdS (2018) Terapia fotodinâmica antimicrobiana no tratamento endodôntico em dentes de cães com lesão periapical induzida - Análise histopatológica e imunohistoquímica. Dissertation, Universidade de São Paulo.
37. Holland R, Scares IJ, Scares IM (1992) Influence of irrigation and intracanal dressing on the healing process of dogs' teeth with apical periodontitis. Endod Dent Traumatol. 8:223–229. https://doi.org/10.1111/j.1600-9657.1992.tb00248.x
38. Domingues-Falqueiro LM, Ferreira J, Lopes FM, et al (2007) The effect of timing temporary cements to treat induced pulp necrosis in the teeth of dogs. Pesq. Vet. Bras. 27:85–88. https://doi.org/10.1590/s0100-736x2007000200006
39. Paula-Silva FWG, Arnez MFM, de Campos Chaves Lamarque G, et al (2021) Osteoclast formation, inflammation, and matrix metalloproteinase-9 are downregulated in bone repair following root canal treatment in dogs teeth. Clin Oral Investig. 25:4699–4707. https://doi.org/10.1007/s00784-021-03784-0
40. Pinheiro Junior EC (2017) Análise da eficácia de três protocolos terapêuticos no processo de revitalização de canais radiculares: estudo histológico em dentes de cães com ápices formados, polpa necrosada e lesão periapical. Dissertation, Universidade Estadual de Campinas.
41. de Castro Rizzi-Maia C, Maia-Filho EM, Segato RA, et al (2016) Single vs Two-session Root Canal Treatment: A Preliminary Randomized Clinical Study using Cone Beam Computed Tomography. J Contemp Dent Pract. 17:515–521. https://doi.org/10.5005/jp-journals-10024-1882
42. Schwendicke F, Göstemeyer G (2016) Cost-effectiveness of Single- Versus Multistep Root Canal Treatment. J Endod. 42:1446–1452. https://doi.org/10.1016/j.joen.2016.06.013
43. Ricucci D, Siqueira JF Jr, Bate AL, Pitt Ford TR (2009) Histologic Investigation of Root Canal–treated Teeth with Apical Periodontitis: A Retrospective Study from Twenty-four Patients. J Endod. 35:493–502. https://doi.org/10.1016/j.joen.2008.12.014
44. Wu M, Shemesh H, Wesselink PR (2009) Limitations of previously published systematic reviews evaluating the outcome of endodontic treatment. Int Endod J. 42:656–666. https://doi.org/10.1111/j.1365-2591.2009.01600.x
45. Estrela C, Bueno MR, Leles CR, et al (2008) Accuracy of Cone Beam Computed Tomography and Panoramic and Periapical Radiography for Detection of Apical Periodontitis. J Endod. 34:273–279. https://doi.org/10.1016/j.joen.2007.11.023
46. López FU, Kopper PMP, Cucco C, et al (2014) Accuracy of Cone-beam Computed Tomography and Periapical Radiography in Apical Periodontitis Diagnosis. J Endod. 40:2057–2060. https://doi.org/10.1016/j.joen.2014.09.003
47. Ricucci D, Lin LM, Spångberg LSW (2009) Wound healing of apical tissues after root canal therapy: a long-term clinical, radiographic, and histopathologic observation study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 108:609–621. https://doi.org/10.1016/j.tripleo.2009.05.028
48. Vera J, Siqueira JF Jr, Ricucci D, et al (2012) One- versus Two-visit Endodontic Treatment of Teeth with Apical Periodontitis: A Histobacteriologic Study. J Endod. 38:1040–1052. https://doi.org/10.1016/j.joen.2012.04.010
49. Torabinejad M (1994) Mediators of acute and chronic periradicular lesions. Oral Surg Oral Med Oral Pathol. 78:511–521. https://doi.org/10.1016/0030-4220(94)90046-9
50. Rowe AHR, Binnie WH (1974) Correlation between Radiological and Histological Inflammatory Changes following Root Canal Treatment. Int Endod J. 7:57–63. https://doi.org/10.1111/j.1365-2591.1974.tb01122.x
51. Barbosa-Ribeiro M, Arruda-Vasconcelos R, de-Jesus-Soares A, et al (2018) Effectiveness of calcium hydroxide-based intracanal medication on infectious/inflammatory contents in teeth with post-treatment apical periodontitis. Clin Oral Investig. 23:2759–2766. https://doi.org/10.1007/s00784-018-2719-0
52. Stashenko P (1990) The role of immune cytokines in the pathogenesis of periapical lesions. Endod Dent Traumatol. 6:89–96. https://doi.org/10.1111/j.1600-9657.1990.tb00400.x
53. Buck R, Cai J, Eleazer P, et al (2001) Detoxification of Endotoxin by Endodontic Irrigants and Calcium Hydroxide. J Endod. 27:325–327. https://doi.org/10.1097/00004770-200105000-00003
54. Tanomaru JMG, Leonardo MR, Tanomaru Filho M, et al (2003) Effect of different irrigation solutions and calcium hydroxide on bacterial LPS. Int Endod J. 36:733–739. https://doi.org/10.1046/j.1365-2591.2003.00717.x
55. Sousa ELR, Martinho FC, Nascimento GG, et al (2014) Quantification of Endotoxins in Infected Root Canals and Acute Apical Abscess Exudates: Monitoring the Effectiveness of Root Canal Procedures in the Reduction of Endotoxins. J Endod. 40:177–181. https://doi.org/10.1016/j.joen.2013.10.008
56. Fava LRG, Saunders WP (1999) Calcium hydroxide pastes: classification and clinical indications. Int Endod J. 32:257–282. https://doi.org/10.1046/j.1365-2591.1999.00232.x
57. Farhad A, Mohammadi Z (2005) Calcium hydroxide: a review. Int Dent J. 55:293–301. https://doi.org/10.1111/j.1875-595x.2005.tb00326.x

58. Stamos DG, Haasch GC, Gerstein H (1985) The pH of local anesthetic/calcium hydroxide solutions. J Endod. 11:264–265. https://doi.org/10.1016/s0099-2399(85)80182-5
59. Seltzer S, Soltanoff W, Sinai I, et al (2004) Biologic Aspects of Endodontics Part III. Periapical Tissue Reactions to Root Canal Instrumentation. J Endod. 30:491–499. https://doi.org/10.1097/00004770-200407000-00008
60. Paula-Silva FWG, Ghosh A, Arzate H, et al (2010) Calcium Hydroxide Promotes Cementogenesis and Induces Cementoblastic Differentiation of Mesenchymal Periodontal Ligament Cells in a CEMP1- and ERK-Dependent Manner. Calcif Tissue Int. 87:144–157. https://doi.org/10.1007/s00223-010-9368-x
61. Mizuno M, Banzai Y (2008) Calcium ion release from calcium hydroxide stimulated fibronectin gene expression in dental pulp cells and the differentiation of dental pulp cells to mineralized tissue forming cells by fibronectin. Int Endod J. 41:933–938. https://doi.org/10.1111/j.1365-2591.2008.01420.x
62. Hooijmans CR, IntHout J, Ritskes-Hoitinga M, Rovers MM (2014) Meta-Analyses of Animal Studies: An Introduction of a Valuable Instrument to Further Improve Healthcare. ILAR J. 55:418–426. https://doi.org/10.1093/ilar/ilu042

Figures

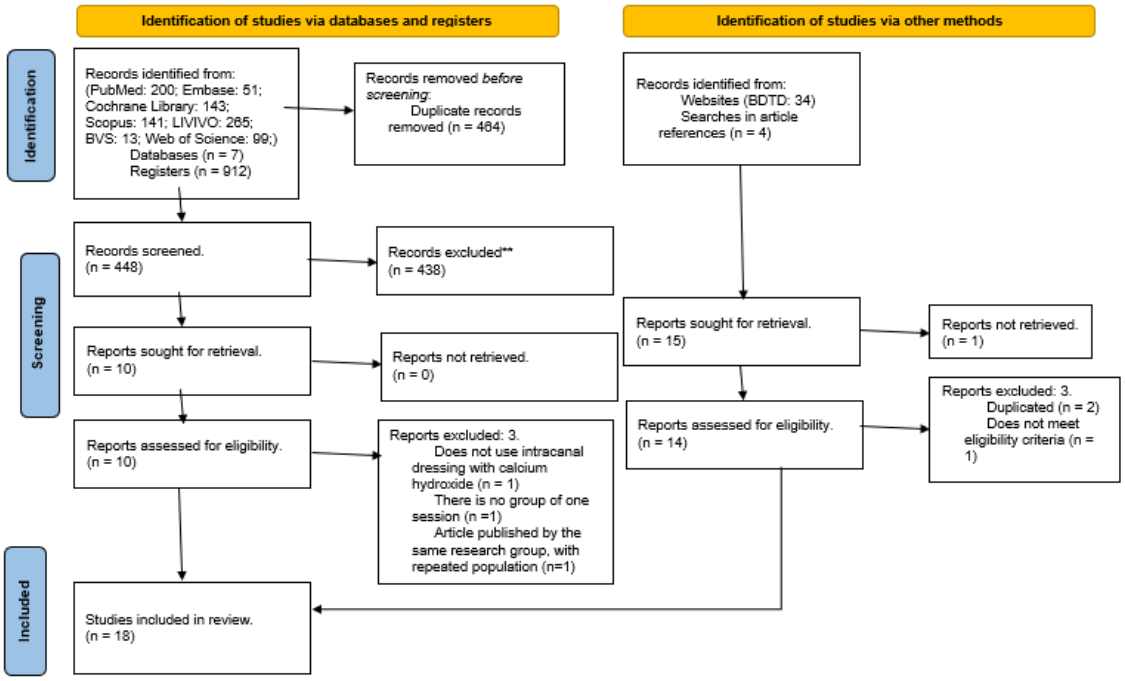

Figure 1

Flowchart of the selection of studies included in the systematic review.

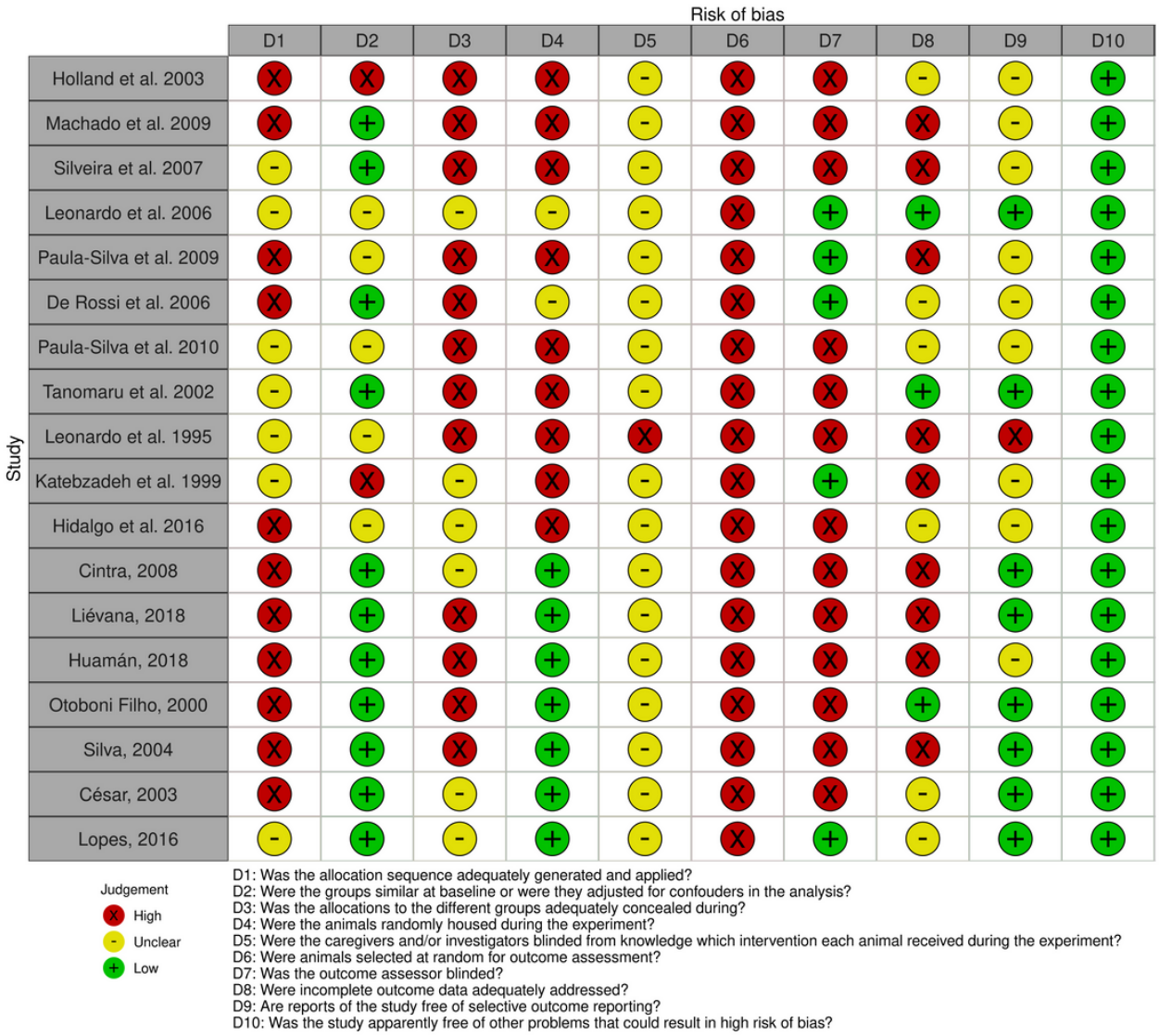

Figure 2

Risk of bias of the included studies (Yes= Indicates low risk of bias; No= Indicates high risk of bias; Unclear= indicates an uncertain risk of bias).

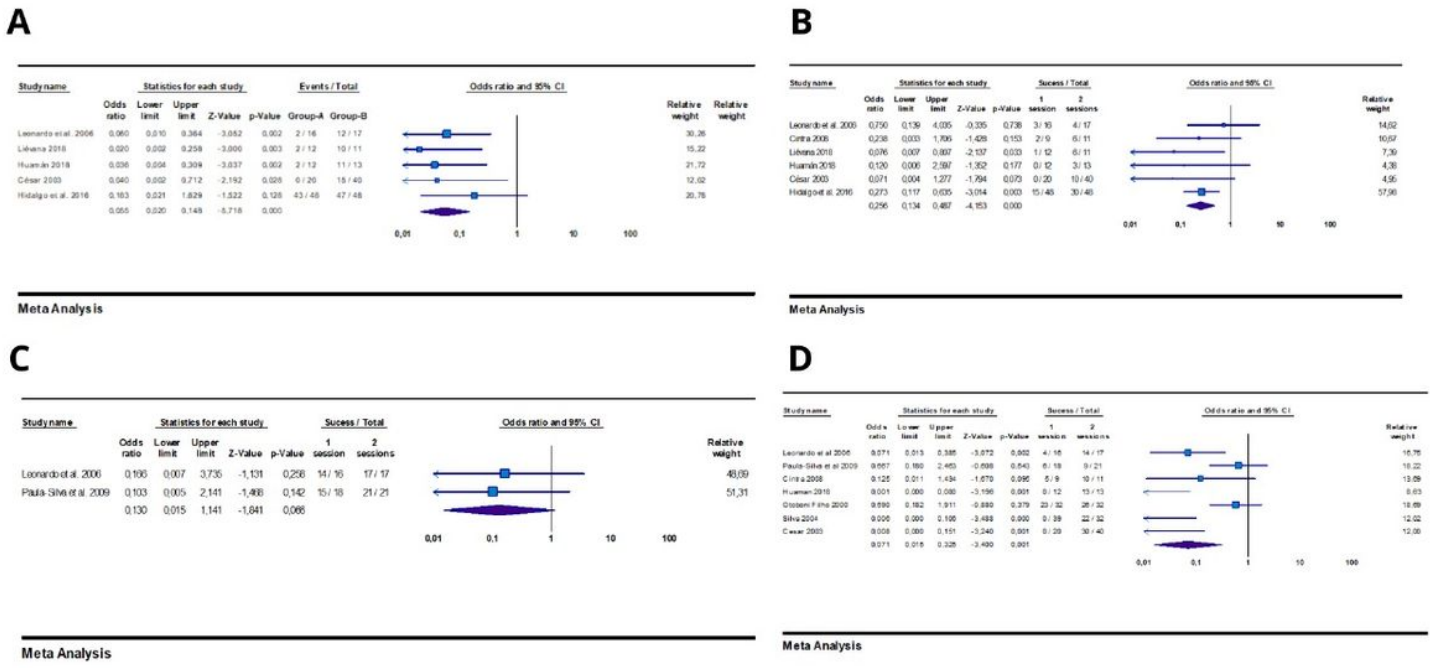

Figure 3

a- Forest plot of data on intensity of inflammatory infiltrate (CI = Confidence interval). **b-**Forest plot of data on periodontal ligament thickness (CI= Confidence interval). **c-** Forest plot comparing groups on dentin resorption incidence data. (CI= Confidence interval). **d-** Forest plot of data on incidence of cement resorption (CI= Confidence interval).

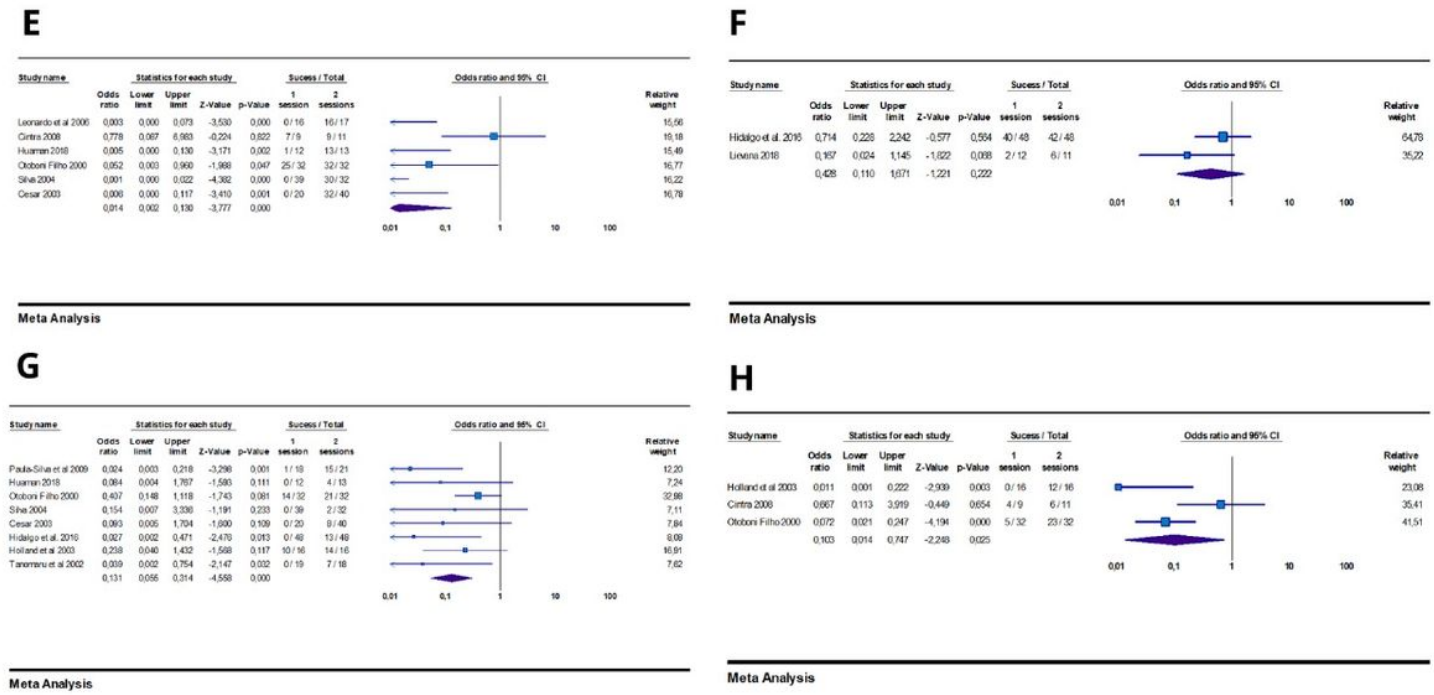

Figure 4

e-Forest plot of data on bone resorption incidence (CI= Confidence interval). **f-**Forest plot graph of data on mineralized tissue resorption incidence (CI = Confidence interval). **g-** Forest plot graph of data on complete apical biological seal incidence (CI= Confidence interval). **h-** Forest plot graph of data on bacteria after treatment (CI= Confidence interval).

Supplementary Files

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